Bioactive Foods and Extracts

Cancer Treatment and Prevention

Edited by

Ronald Ross Watson *and* Victor R. Preedy

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Preface

There is a considerable historical record for the cancer preventative effects of consumption of vegetables, fruits, and herbs. Indeed, the usage of foods and their extracts as therapeutic tools appears in ancient and modern cultures. Recently, however, there has been an increase in the amount of scientific research relating to the effects of plant products in cancer treatment. Clearly information is vital for the researcher, physician, and government regulator with increased availability and media evidence that such agents may have efficacy. Especially in the United States, the use of botanicals and their extracts is widely available and unregulated. Therefore, information from scientific research is critical in helping researchers and healthcare professionals make decisions on the benefits, risks, or value of botanicals and their extracts in the prevention and treatment of cancers.

Ronald Ross Watson

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The work of the editorial assistant, Bethany L. Stevens, in communicating with authors, working with the manuscripts and the publisher was critical to the successful completion of the book and is much appreciated. Her daily responses to queries and collection of manuscripts and documents were extremely helpful. Support for her work was graciously provided by Elwood Richard, treasurer of the National Health Research Institute in support of its mission to communicate to scientists about bioactive foods and dietary supplements was vital (http://www.naturalhealthresearch.org/nhri/). This was part of their efforts to educate scientists and the lay public on the health and economic benefits of nutrients in the diet as well as supplements. Finally, Nguyen T. Nga and Mari Stoddard of the Arizona Health Sciences Library were instrumental in finding the authors and their addresses in the early stages of the book's preparation.

Editors

Ronald Ross Watson, PhD, attended the University of Idaho but graduated from Brigham Young University in Provo, Utah, with a degree in chemistry in 1966. He earned his PhD in biochemistry from Michigan State University in 1971. His postdoctoral schooling in nutrition and microbiology was completed at the Harvard School of Public Health, where he gained two years of postdoctoral research experience in immunology and nutrition.

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Section I

Herbal Medicines and Bioactive Foods and Cancer Treatment

1 Glucosinolates in Brassica and Cancer

Pablo Velasco, Marta Francisco, and María Elena Cartea

CONTENTS

INTRODUCTION

In the last 20 years, a number of epidemiological studies have shown that tumor formation and incidence of cardiovascular diseases are inversely related to the intake of fruit and vegetables (Verhoeven et al., 1996). One group of vegetables that has been widely approved for its beneficial effects on human health are the vegetables from the family *Brassicaceae (= Cruciferae)*. The family *Brassicaceae* is a large group, having about 3000 species in 350 genera, including several types of edible plants, which are sometimes referred to as "the cabbage family." The petals of plants of this family have a distinctive cruciform arrangement, which is the origin of the initial term *Cruciferae*. These plants can be annuals, biennials or perennials. They are well adapted to average temperatures of 16–18°C and are thus grown during the cool season in temperate areas. Crops of *Brassicaceae* are mainly distributed in temperate regions of the northern hemisphere: in the areas of Southwestern and Central Asia, China and Japan, Europe, the Mediterranean region and North America. Brassica production has grown steadily and vegetables represent a major part of the human diet worldwide. Despite the great diversity among the *Brassicaceae*, members of only a few genera are used in human diet (JARC, 2004).

The *Brassica* genus belongs to the *Brassicaceae* family and, economically speaking, it is the most important genus within the tribe *Brassiceae*, containing 37 different species. The taxonomy of this genus is complex. Gómez-Campo (1999) presented a complete classification of the genus

Brassica and its allied genera, indicating subgenera, sections, species and subspecies, later updated by the same author (Gómez-Campo, 2003). The genus includes a group of six interrelated species of worldwide economic importance. U (1935) studied the cytology of the genus and established the relationships among the genomes of the six species. The three diploid *Brassica* species, *Brassica nigra* (L.) Koch ($2n = 16$), *Brassica oleracea* L. ($2n = 18$) and *Brassica rapa* L. ($2n = 20$), form the classic Triangle of U (Figure 1.1). In nature, these species have hybridized in different combinations to give rise to the three amphidiploid species, namely *Brassica carinata* A. Braun ($2n = 4x = 34$), *Brassica juncea* (L.) Czern. ($2n = 4x = 36$) and *Brassica napus* L. ($2n = 4x = 8$).

The genus is categorized into oilseed, forage, condiment, and vegetable crops by using their buds, inflorescences, leaves, roots, seeds, and stems. The same species can be utilized for several uses according to different forms or types. Four species, *B. oleracea*, *B. rapa*, *B. napus*, and *B. juncea*, contain crops that have a horticultural use. The principal vegetable species is *B. oleracea*, which includes vegetable and forage forms, such as kale, cabbage, broccoli, Brussels sprouts, cauliflower, and others; *B. rapa* includes vegetable forms, such as turnip, Chinese cabbage, and pak choi, along with forage and oilseed types; *B. napus* crops are mainly used as oilseed (rapeseed), although forage and vegetable types like leaf rape and "nabicol" are also included; finally, the mustard group, which is formed by three species, *B. carinata*, *B. nigra*, and *B. juncea*, is mainly used as a condiment because of their seeds, although leaves of *B. juncea* are also consumed as vegetables in Asian countries.

Other cruciferous vegetables that are used in human diet belong to other genera of the *Brassicaceae* family. Some of them are used as condiments, as is the case of *Sinapis alba* (white mustard), but most of them are used as salad crops, such as *Nasturtium officinale* (watercress) and *Eruca sativa*, *E. vesicaria*, *Diplotaxis tenuifolia* (rocket salads), *D. muralis* (wall rocket), which is reported to be a component of mixed soups in Italy and *D. erucoides* (white wall rocket), which is used as a raw salad plant in Spain, Sicily, and Malta. The use of edible *D. catholica* flowers has been reported in Spain. *Diplotaxis harra*, an important fodder plant in North Africa, is used as a medicinal plant in Tunisia, but, in Sicily, it is also consumed after being cooked. *Diplotaxis acris*, grazed by animals and used raw in salads in North Africa, is reported to be a component of green salads in Iraq and Jordan as well. *Diplotaxis simplex* is reported to be either a medium-value pasture or an edible species in Egypt (D'Antuono et al., 2009). Finally, other crops can be used because of their roots, as it is the case of *Raphanus sativus* (radish), which is an important vegetable crop worldwide,

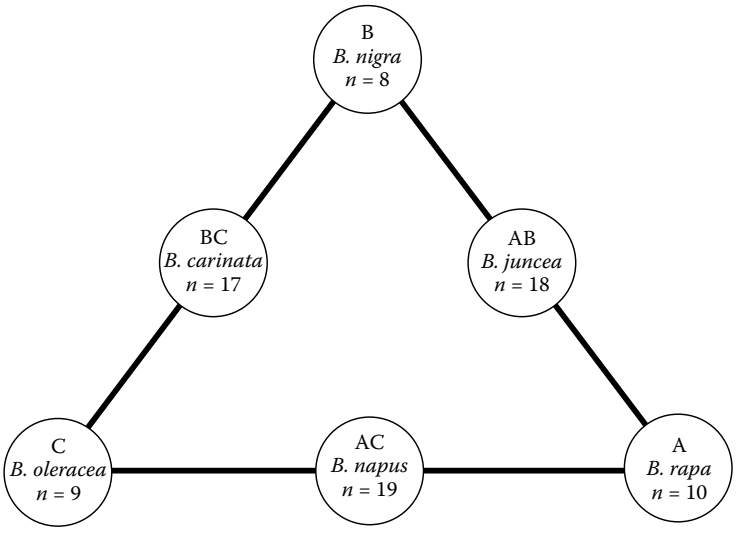

FIGURE 1.1 Triangle of U (Adapted from U, N. 1935. *Jpn. J. Bot.* 7:389–452.)

especially in China, Japan, Korea, and southeastern Asia. Their fresh roots can be cooked or processed by pickling or drying and are used for their nutritive or medicinal uses (Zhao-Liang et al., 2008).

Like most other vegetables, cruciferous vegetables are good sources of a variety of nutrients and phytochemicals that may work synergistically to help prevent cancer (Liu, 2004). These vegetables possess high levels of antioxidants, including vitamin C, vitamin A, folate, soluble fiber, and lignin. Additionally, cruciferous crops have been found to be rich in many minerals, such as calcium, iron, potassium, and acceptable levels of the rest of minerals, including selenium. Besides, they contain other compounds of potential importance to the maintenance of health, such as flavonoids and carotenoids. However, cruciferous vegetables are unique because they are rich sources of glucosinolates.

Glucosinolates are the main class of secondary metabolites found in cruciferous crops. These compounds are largely responsible for the characteristic hot and pungent flavor of crucifers (Mithen, 2001). The potent odour and taste of glucosinolates have resulted in a proposed role of these metabolites in herbivore and microbial defense (Fenwick et al., 1983). There is a wide variety of glucosinolates; to date, more than 120 individual glucosinolates have been isolated from species of the family *Brassicaceae* and allied families (Fahey et al., 2001). All glucosinolates have a common core structure that consists of a β-thioglucoside *N*-hydroxysulfate with a side chain R and a sulphur-linked β-D-glucopyranoside moiety derived from one of the several amino acids (Figure 1.2). Glucosinolates can be grouped into three chemical classes: aliphatic, indole, and aromatic, according to whether their amino acid precursor is methionine, tryptophan, or an aromatic amino acid (tyrosine or phenylalanine), respectively (Giamoustaris and Mithen, 1996). Much of the diversity among glucosinolates arises from the addition of different sized alkyl groups to the side chain of amino acids used in their biosynthesis. This variable elongation of amino acid side chains entails repetitive additions of methyl groups through a series of transamination, condensation, isomerization, and decarboxylation reactions (Graser et al., 2000).

There are 16 families within the order Brassicales, all of which contain glucosinolates (Mithen, 2001). Whilst as many as 15 different glucosinolates have been found in the same plant, usually only 3 to 4 predominate (Rosa et al., 1997). The majority of glucosinolates are found in every plant organ and they can change during plant development. Glucosinolate content in *Brassica* vegetables is about 1% of dry matter, although in flowers and seeds the total amount can be 10 times higher and account for up to 10% of the dry matter. There is a substantial amount of data compiled in several reviews on the occurrence of glucosinolates in representative *Brassica* species (Fenwick et al., 1983; Rosa et al., 1997; Kushad et al., 1999; Rosa, 1999). Each type of cruciferous shows a characteristic glucosinolate profile, differing substantially, even though some of them are part of the same species (Table 1.1). All the different *B. oleracea* types contain glucobrassicin (3-indolylmethyl) and glucoiberin (3-methylsulfinilpropyl) and most contain substantial amounts of sinigrin (2-propenyl). For example, sinigrin, glucobrassicin, and glucoiberin have been identified as the major glucosinolates in kales and cabbages (Cartea et al., 2008b). In broccoli, common glucosinolates are glucoraphanin (4-methylsulfinylbutyl), sinigrin, progoitrin (2-hydroxy-3-butenyl), gluconapin (3-butenyl), and the indole glucosinolates called glucobrassicin and neoglucobrassicin (1-methoxy-3-indolylmethyl) (Kushad et al., 1999). In Brussels sprouts, collards and cauliflower, the predominant glucosinolates are sinigrin, progoitrin, and glucobrassicin (Van Etten et al., 1976; Carlson et al., 1987; Kushad et al., 1999). In *B. rapa* crops, Chinese cabbage accumulates gluconapin and glucobrassicanapin

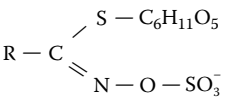

FIGURE 1.2 General structure of glucosinolates.

TABLE 1.1
Principal Glucosinolates Identified in the Six *Brassica* Species of the Triangle of U and Other Important Species of the *Brassicaceae* Family

	Aliphatic Glucosinolates										Indole Glucosinolates				Aromatic
Crop	GIB	PRO/ EPRO	SIN	GAL	GRA	GNA	GBN	GIV	GER	GNL	GBS	NGBS	4HGBS	4MGBS	GNST
Brassica oleracea[a]															
White cabbage	++	+	++	+	+	+	+	+	+	−	++	+	+	+	+
Savoy cabbage	++	+	++	−	+	+	+	+	−	−	++	+	−	+	+
Red cabbage	++	+	++	−	+	+	−	+	−	−	++	+	−	−	−
Kale	++	+	++	−	+	+	−	+	−	−	++	+	+	+	+
Collard	+	++	++	−	−	−	−	+	+	−	++	−	−	−	−
Tronchuda cabbage	++	+	++	+	+	+	+	+	−	−	++	+	+	+	+
Broccoli	+	++	+	+	++	++	+	−	+	+	++	+	+	+	+
Brussels sprouts	+	++	++	−	+	+	−	+	−	−	++	+	−	−	−
Cauliflower	++	++	++	−	+	−	−	+	−	−	++	+	−	−	−
Kohlrabi	+	+	++	+	+	+	−	+	−	−	++	+	+	+	−
Brassica rapa[a]															
Turnip	+	++	−	−	−	++	++	−	+	+	+	+	+	+	++
Turnip greens	+	+	−	+	+	++	++	+	−	+	+	+	+	−	+
Turnip tops	+	+	−	−	−	++	++	+	−	−	+	+	+	−	+
Chinese cabbage	+	+	−	−	−	++	++	−	−	++	+	+	−	+	+

Brassica napus[a]															
Swede	−	+	−	−	+	−	+	−	−	+	+	++	+	++	+
Leaf rape	−	+	−	+	−	+	++	+	−	+	+	+	−	+	+
Brassica carinata[b]	−	+	++	−	−	+	−	−	−	−	+	+	+	+	+
Brassica juncea[b]	−	+	++	−	−	+	−	−	−	−	+	+	+	+	+
Brassica nigra[b]	−	−	++	−	−	+	−	−	−	−	+	−	+	+	+
Crambe maritima[c]	−	++	−	−	+	−	−	−	−	−	−	−	++	−	−
Nasturtium officinalis[c]	−	−	−	−	−	−	−	−	−	−	−	−	+	−	++
Raphanus sativus[c]	−	−	−	−	+	−	−	−	−	−	−	−	++	−	−
Sinapis alba[c]	−	−	−	−	−	−	−	−	−	−	−	−	+	−	++
Diplotaxis tenuifolia[c]	−	−	−	−	+	−	−	−	++	−	−	−	−	−	−
Eruca sativa[c]	−	−	−	−	+	−	−	−	++	−	−	−	−	−	−

Note: GIB: glucoiberin (3-methylsulfinylpropyl); PRO: progoitrin (2-hydroxy-3-butenyl); EPRO: epiprogoitrin (2-hydroxy-3-butenyl); SIN: sinigrin (2-propenyl); GAL: glucoalysiin (5-methylsulphinylpentyl); GRA: glucoraphanin (4-methylsulphinylbutyl); GNA: gluconapin (3-butenyl); GBN: glucobrassicanapin (4-pentenyl); GIV: Glucoiberverin (3-methylthiopropyl); GER: glucoerucin (4-methylthiobutyl); GNL: gluconapoleiferin (2-hydroxy-4-pentenyl); GBS: glucobrassicin (3-indolylmethyl); NGBS: neoglucobrassicin (l-methoxy-3-indolylmethyl); 4HGBS: 4-hydroxyglucobrassicin (4-hydroxy-3-indolylmethyl); 4MGBS: 4-methoxyglucobrassicin (4-methoxy-3-indolylmethyl), GNST: Gluconasturtiin (2-phenylethyl); Major glucosinolates found in each crop are shown with ++ .

[a] Adapted from Cartea, M. E. and P. Velasco. 2008. *Phytochem. Rev*. 7:213–229.

[b] Adapted from Bellostas, N., J. C. Sørensen, and H. Sørensen. 2007. *J. Sci. Food Agric*. 87:1586–1594.

[c] Adapted from Bennett, R. N., F. A. Mellon, and P. A. Kroon. 2004. *J. Agric. Food Chem*. 52:428–438.

(4-pentenyl) and their hydroxylated forms, progoitrin and gluconapoleiferin (2-hydroxy-4-pentenyl), respectively. In turnip roots, the predominant glucosinolates found by Sones et al. (1984) were progoitrin and gluconasturtiin (2-phenylethyl), while gluconapin has been identified as the most abundant glucosinolate in the edible parts of turnip greens (Padilla et al., 2007) and turnip tops (Rosa, 1997). In vegetable crops of *B. napus*, Cartea et al. (2008a) found that glucobrassicanapin, followed by progoitrin and gluconapin, proved to be the most abundant glucosinolates. In swedes, glucobrassicin, progoitrin, and gluconasturtiin have been found to be the major glucosinolates (Carlson et al., 1987). The three species of mustards (*B. juncea, B. carinata*, and *B. nigra*) showed different glucosinolates, but gluconapin was the major glucosinolate in all of them (Bellostas et al., 2007).

The distinctive taste of many minor horticultural cruciferous salad crops is due to their glucosinolate content. For example, rockets (*Eruca* and *Diplotaxis* species) accumulates glucoerucin (4-methylthiobutyl) as the main glucosinolate (Fahey et al., 2001), whereas watercress possesses large amounts of gluconasturtiin, combined with low levels of glucoiberin (7-methylsulfinylheptyl) and glucohirsutin (8-methylsulfinyloctyl) (Rose et al., 2000). Bennett et al. (2004) identified glucosinolates present in more than 60 wild and weed species of the *Brassicaceae* family.

Several reviews demonstrate that glucosinolates occur with a wide biological variation, both quantitatively and qualitatively. Occurrence and concentrations vary according to species and cultivar, tissue type, physiological age, plant health, environmental factors (agronomic practices, climatic conditions), insect attack, and microorganism intrusion (Fenwick and Heaney, 1983; Ciska et al., 2000; Mithen et al., 2000; Velasco et al., 2007, 2008). Glucosinolate and related isothiocyanate contents are also affected by methods of storage and food processing, for example, cutting, chewing, cooking, fermenting, or freezing (Song and Thornalley, 2007). Finally, the myrosinase activity of the intestinal microbial floral may affect the total content and bioavailability of these compounds. For these reasons, it is often difficult to estimate the intake of bioactive components in a population. Nevertheless, there are several studies that report the daily intake of *Brassica* vegetables. The International Agency for Research on Cancer compiled this data in a review that shows a variable consumption of cruciferous intake in Europe, ranging from 5 to 30 g/day (IARC, 2004). In this review, the greatest consumption was reported in China, higher than 100 g per day, whereas other Asian populations and Australia had relatively high daily intakes as well, ranging from 40 to 80 g per day. The average intake in North America was around 25–30 g per day, whereas relatively low daily intakes, 15 g or less, are reported in South Africa and in some countries from South America. On the contrary, very few estimates of glucosinolate intake at the population level have been reported, probably due to the lack of food composition tables. In most cases, they are rough estimates based upon overall amount of cruciferous consumption and limited data on glucosinolate content in some foods. The unique studies based on glucosinolate concentrations of *Brassica* vegetables showed that the average daily consumption of total glucosinolates in the United Kingdom was estimated to be about 50 mg per day (Wattenberg et al., 1986); in the Netherlands it was 22 mg per day (Kistenmaker et al., 1998), whereas in Spain it was 6.5 mg per day (Agudo et al., 2008). The daily intake of glucobrassicin and neoglucobrassicin (the two main dietary indole glucosinolates) was 22.5 mg per day in the United Kingdom 5.5 mg in Denmark, and 2.8 mg in Finland (IARC, 2004). In the United States, the intake of indole glucosinolates was about 22.5 mg per day (Broadbent and Broadbent, 1998). An important consideration when comparing intakes of glucosinolates across countries is the type of cruciferous vegetables that are most commonly consumed, which may vary according to cultural and taste preferences. This may have important impacts on the actual intake of glucosinolates.

GLUCOSINOLATES AND HYDROLYSIS PRODUCTS

Epidemiological data, supported by experimental studies with cell and animal models, suggest that the cancer-protective properties of *Brassica* intake are mediated through glucosinolates. However, glucosinolates are not bioactive as demonstrated in cancer cell toxicity experiments (Musk et al.,

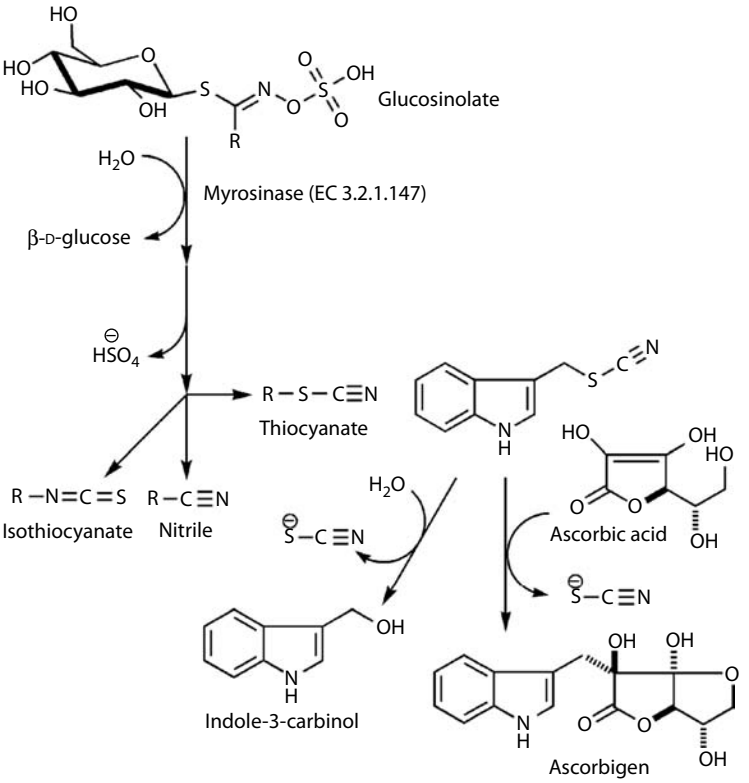

FIGURE 1.3 Degradation and conversion of glucosinolates. Glucosinolates are hydrolyzed by the endogenous enzyme myrosinase to form glucosinolate hydrolysis products: nitriles, isothiocyanates, and thiocyanates. Indol-3-ylglucosinolate (glucobrassicin) is further converted to bioactive products, such as indole-3-carbinol and ascorbigen. (From Suzuki, C., M. Ohnishi-Kameyama, and K. Sasaki. 2006. *J. Agric. Food Chem.* 54:9430–9436. With permission.)

1995). In contrast, their degradation products exhibit protective activities against many types of cancer in humans (Zhang and Talalay, 1994; Fahey et al., 2001; Mithen et al., 2003). Isothiocyanates, nitriles and indoles are the major groups of autolytic breakdown products of glucosinolates (Figure 1.3). Upon cell damage, glucosinolates undergo hydrolysis by myrosinase to yield glucose, sulfate, and aglucones that can undergo fragmentation and/or molecular rearrangement. Therefore, this process will yield isothiocyanates, thiocyanates, oxazolindine-2-thione, and nitriles, depending on the specific glucosinolate substrate, myrosinase isozyme, reaction pH, and presence of certain ions and activity of specific protein factors, such as the epithiospecifier protein (ESP) (Halkier and Du, 1997). Among these, isothiocyanates are of great importance because they have been shown to be highly effective inhibitors of chemically induced tumors. *In vitro* and *in vivo* studies have established that isothiocyanates affect many stages of cancer development, including the induction of detoxification enzymes (phase II enzymes) and the inhibition of activation enzymes (phase I enzymes) (Zhang and Talalay, 1994; Hecht et al., 2000; Fahey et al., 2002; Anilakumar et al., 2006). Isothiocyanates and indoles may regulate cancer cell development by regulating target enzymes, controlling apoptosis, and blocking the cell cycle.

GLUCOSINOLATE-DERIVED ISOTHIOCYANATES

The cancer chemopreventive effect of cruciferous vegetables is mainly attributed to isothiocyanates (ITCs). These compounds have a common basic skeleton but differ in their terminal R-group, which

can be an alkyl, alkenyl, alkylthioalkyl, aryl, beta-hydroxyalkyl, or indolylmethyl group. Although the most studied isothiocyanate compound is sulforaphane (SFN), it is important to mention that other glucosinolates and metabolites found in cruciferous vegetables like allyl isothiocyanate (AITC), phenethyl isothiocyanate (PEITC), and benzyl isothiocyanate (BITC) possess a chemistry, metabolism, and anticancer effects that are similar to those of SFN (Mithen et al., 2003; Stan et al., 2008). Glucosinolate hydrolysis products from glucoiberin, glucoerucin and sinigrin have also been identified to be suppressing agents, protecting human and animal cells against carcinogenesis. These glucosinolates may well exert comparable levels of biological activity to sulforaphane by either inducing phase II detoxification enzymes or by inhibiting phase I enzymes (Fahey et al., 1997b; Nilsson et al., 2006). All of them are shown in Table 1.2 and will be discussed in the next section.

In the last few years, numerous studies have been focused on these compounds. The reason of this increasing interest is due to the strong correlation between the consumption of cruciferous vegetables and the decreased risk of different types of cancer like pancreas, lung, stomach, prostate, or breast, among others. Table 1.3 summarizes different investigations relating glucosinolate degradation products and different types of cancer.

The glucosinolate precursor to SFN is the glucoraphanin, which is present in *B. oleracea* crops like broccoli, cauliflower, cabbage, and kale, finding the highest concentration found in broccoli and broccoli sprouts (Verkerk et al., 2009). SFN was isolated and identified in broccoli by Zhang et al. (1992) and that first study revealed that this compound acts as an antioxidant that detoxifies carcinogens in the body. Since then, scientific papers on SFN have increased 10-fold worldwide. By far it is the best studied isothiocyanate (Talalay and Zhang, 1996; Fahey et al., 2002) and there are complete reviews on its anticarcinogenic activity (Myzak et al., 2006; Juge et al., 2007; Clarke et al., 2008; Traka and Mithen, 2009). A recent review by Juge et al. (2007) summarizes the possible molecular mechanisms of chemoprevention by SFN. There is a large body of research that has examined the effects of SFN on many types of cancers, such as prostate, breast, hepatic, bladder, leukemia, and pancreatic (see Table 1.3).

It has been shown that small quantities of broccoli sprouts reduced the incidence and size of mammary tumors in animals (Zhang et al., 1992; Zhang and Talalay, 1994; Fahey et al., 1997b) and inhibited chemically induced breast cancer in rats. It has also been demonstrated that SFN can inhibit the growth of colon cancer HT29 cells by 50% at a concentration of 15 μM (Gamet-Payrastre

TABLE 1.2
Glucosinolates Related to Cancer Prevention and Single Bioactive Components After Their Hydrolysis Classified into Isothiocyanates, Nitrile and Indole Compounds

	Hydrolysis Products		
Glucosinolate	**Isothiocyanates**	**Nitriles**	**Indoles**
Aliphatic			
Glucoraphanin	Sulforaphane	Sulforaphane nitrile	
Progoitrin/epi-Progoitin		Crambene	
Sinigrin	Allyl isothiocyanate	Allyl nitrile	
Glucoiberin	Iberin		
Glucoerucin	Erucin		
Indolic			
Glucobrassicin			Indole-3-carbinol
Aromatic			
Gluconasturtiin	Phenethyl isothiocyanate		
Glucotropaeolin	Benzyl isothiocyanate		

TABLE 1.3
Bioactive Glucosinolate Derivatives Related to Cancer Prevention and Classified into Isothiocyanates, Indole and Nitrile Compounds. Examples of Recent Studies Relating These Compounds with Different Types of Cancer

Cancer Type	Organism	References
	Isothiocyanates	
Sulforaphane (SFN)		
Bladder	Human, rats	Shan et al. (2006); Tang et al. (2006); Zhang et al. (2006b)
Breast	Human, rats, mice	Wang et al. (2005); Cornblatt et al. (2007); Pledgie-Tracy et al. (2007)
Colon	Human	Traka et al. (2005); Bacon et al. (2007); Nair et al. (2008)
Leukemia	Human	Fimognari et al. (2004a,b)
Liver	Human, mice	Zhang et al. (1992); Bacon et al. (2003)
Lung	Human, rats, mice	Wang et al. (2004); Conaway et al. (2005); Hanlon et al. (2009)
Pancreatic	Human	Chan et al. (2008)
Prostate	Human, rats, mice	Singh et al. (2004); Myzak et al. (2006); Traka et al. (2008)
Skin	Human, mice	Gills et al. (2006); Dinkova-Kostova et al. (2007)
Allyl ITC (AITC)		
Bladder	Rats	Munday and Munday (2002)
Breast	Mice	Kumar et al. (2009)
Colon	Human	Smith et al. (2004)
Liver	Human, mice	Hwang and Lee (2006); Hwang and Kim (2009)
Leukemia	Human	Zhang et al. (2003b)
Prostate	Human	Srivastava et al. (2003); Xiao et al. (2003)
Phenethyl ITC (PEITC)		
Breast	Human	Rose et al. (2005)
Colon	Human	Hu et al. (2003); Visanji et al. (2004)
Esophageal	Rats	Hudson et al. (2005)
Lung	Human, mice	Hecht et al. (2000); Kuang and Chen (2004); Conaway et al. (2005)
Pancreatic	Mice	Nishikawa et al. (1996)
Prostate	Human, mice	Khor et al. (2006); Xiao et al. (2006); Xiao and Shing, (2007)
Benzyl ITC (BITC)		
Breast	Human	Xiao et al. (2008)
Colon	Human	Visanji et al. (2004)
Leukemia	Human	Zhang et al. (2003b); Basu and Haldar, (2008)
Liver	Human	Hwang and Lee (2008)
Lung	Human, mice	Wattenberg (1987); Kuang and Chen (2004)
Pancreatic	Human	Zhang et al. (2006a); Basu and Haldar (2008); Sahu and Srivastava (2009)
Prostate	Human	Basu and Haldar (2008)
Iberin ITC (MSPITC)		
Brain	Human	Jadhav et al. (2007a)
Breast	Human	Wang et al. (2005)
Colon	Human	Jakubikova et al. (2006)
Neuroblastoma	Human	Jadhav et al. (2007b)
Erucin ITC (MTBITC)		
Breast	Human	Wang et al. (2005)
Liver	Human	Lamy and Mersch-Sundermann (2009)
Lung	Rats	Hanlon et al. (2009)

continued

TABLE 1.3 (continued)
Bioactive Glucosinolate Derivatives Related to Cancer Prevention and Classified into Isothiocyanates, Indole and Nitrile Compounds. Examples of Recent Studies Relating These Compounds with Different Types of Cancer

Cancer Type	Organism	References
	Indoles	
Indole-3-Carbinol (I3C)		
Breast	Human	Cover et al. (1998); Xue et al. (2005); Sundar et al. (2006); Brew et al. (2009)
Colon	Human	Zheng et al. (2002)
Endometrial	Human	Leong et al. (2001)
Liver	Rats	Nho and Jeffery (2001)
Lung	Human	Kuang and Chen (2004)
Mammary	Mice	Bradlow et al. (1991)
Prostate	Human	Chinni et al. (2001); Zhang et al. (2003a); Hsu et al. (2005)
	Nitriles	
Allyl Nitrile		
Kidney	Mice	Tanii et al. (2008)
Liver	Mice	Tanii et al. (2008)
Lung	Mice	Tanii et al. (2005)
Rectum	Mice	Tanii et al. (2008)
Small intestines	Mice	Tanii et al. (2008)
Stomach	Mice	Tanii et al. (2005)
Crambene		
Liver	Human, rats, mice	March et al. (1998); Nho and Jeffery (2001); Keck et al. (2002)
Pancreatic	Rat	March et al. (1998)

et al., 2000; Bacon et al., 2007; Nair et al., 2008). Other studies have demonstrated that SFN can induce cell cycle arrest in a variety of cell types, including prostate (Chiao et al., 2002; Fimognari et al., 2004a; Traka et al., 2008), lymphocyte (Fimognari et al., 2004a,b), and mammary (Jackson and Singletary, 2004). On the other hand, Zhang et al. (2006b) found that SFN may be especially effective in protecting against bladder cancer by stimulating phase II enzymes. This compound has also been shown to have a protective effect against other types of cancer as liver, lung, pancreatic and skin (Table 1.3). Moreover, SFN shows potential for treating *Helicobacter pylori*, which causes stomach cancer (Fahey et al., 2002).

Allyl isothiocyanate (AITC) is the degradation product of the aliphatic glucosinolate called sinigrin, which is abundant in several *Brassica* crops, especially *B. oleracea* and in mustard oils. Xiao et al. (2003) proved the antiproliferative activity of this compound against human prostate cancer cells. These authors demonstrated the ability of AITC to arrest cells in G2/M phase and induce apoptosis. Using human leukemia HL60/S as model cells, and focusing on AITC and BITC, Zhang et al. (2003a) found that both compounds modulated multiple cellular targets involved in proliferation, including the disruption of mitochondrial membrane potential, activation of multiple caspases, arrest of cell cycle progression, and induction of differentiation. The positive effect of this compound on other types of cancer like the hepatic has also been demonstrated (Hwang and Lee, 2006; Hwang and Kim, 2009). Based on *in vitro* results, these authors concluded that AITC might be potentially useful in suppressing tumor cell migration in SK-Hep1 human hepatoma cells. Kumar et al. (2009) explore the mechanism of action of AITC on Ehrlich

ascites tumor (EAT) cells and concluded that AITC inhibits tumor growth by both antiangiogenic and proapoptotic mechanisms. Munday and Munday (2004) studied the ability of six plant-derived isothiocyanates (allyl isothiocyanate, iberverin, erucin, sulforaphane, iberin, and cheirolin) to increase tissue levels of the phase II detoxification enzymes called quinone reductase (QR) and glutathione-*S*-transferase (GST) in a variety of rat tissues. Little difference was observed in the inductive activity of these various isothiocyanates. With the exception of cheirolin, all of the other isothiocyanates increased GST and QR activities in the duodenum, forestomach and/or the urinary bladder of the animals, with the greatest effects being seen in the urinary bladder. The anticancer properties of AITC on other cancer cells as bladder and colon have also been reported in different papers (Table 1.3).

Phenethyl isothiocyanate (PEITC) is the degradation product of the aromatic glucosinolate called gluconasturtiin, which occurs in large quantities in some minor crops of *Brassicaceae* family such as watercress and radish. This compound may inhibit phase I enzymes that are related to the activation of carcinogens. A protective effect of PEITC has been reported against lung tumorigenesis in mice (Hecht et al., 2000; Conaway et al., 2005) and rats (Chung et al., 1996) and against lung and pancreatic tumors in hamsters (Nishikawa et al., 1996). On the other hand, PEITC administration was shown to be ineffective in the prevention of lung tumorigenesis (Adam-Rodwell et al., 1993). The effect of PEITC on human leukemia cells *in vitro* has been proved as well as its role to inhibit tobacco smoke-induced lung tumors in mice. Rose et al. (2005) showed that extracts of broccoli and watercress inhibit the invasive potential of the human breast cancer cell line *in vitro* and suggested that their phytochemical constituents, isothiocyanates, are a new class of invasion inhibitors. PEITC has been shown to induce apoptosis specifically in different human cancer cells as leukemia, prostate, and pancreatic (Basu and Haldar, 2008). The chemoprotective effect of PEITC against different types of cancer is summarized in Table 1.3.

Benzyl isothiocyanate (BITC) is the degradation product of the aromatic glucosinolate called glucotropaeolin. This compound has also been reported to have anticancer properties and it may be responsible for the selective induction of cancer cells to apoptosis, supporting the potential preventive and/or therapeutic benefit of the glucosinolate hydrolysis products against different type of cancers (Zhang and Talalay, 1994; Fahey et al., 1997a). Kuang and Chen (2004) showed the effects of BITC on the induction of apoptosis in human cell lung cancer. The results indicated that this compound is able to inhibit the growth of A549 cells by inducing apoptosis at low concentrations and necrosis at high concentrations. BITC also induces apoptosis in some types of pancreatic cancer cells and the mechanism whereby it inhibits the growth of human pancreatic cancer cells has been recently elucidated (Sahu and Srivastava, 2009). BITC has been shown to have a protective effect against lung and forestomach tumors in mice but not on inhibition of lung tumors in mice (Wattenberg, 1987) or esophageal tumors in rats (Wilkinson et al., 1995). A recent study indicates that the BITC-induced apoptosis in human breast cancer cells is initiated by mitochondria-derived ROS (Xiao et al., 2008). Other studies have been published in the last years relating the chemoprotective effect of this compound against different types of cancer (see Table 1.3).

Iberin (4-methylsulfinilpropyl isothiocyanate, MSPITC) is the isothiocyanate derived from a glucosinolate called glucoiberin and is present in *B. oleracea* crops in large quantities. Jakubikova et al. (2006) studied the effect of iberin in human colon carcinoma Caco-2 cells and concluded that iberin represents the effective member of the natural chemopreventive isothiocyanate family with which apoptotic potential can be employed to eliminate tumor cells. Recently, the antiproliferative and proapoptotic effects of this isothiocyanate were evaluated in human glioblastoma (Jadhav et al., 2007a) and neuroblastoma cells (Jadhav et al., 2007b). The authors demonstrated the antigrowth, cell cycle modulation, and proapoptotic effects of iberin. Findings from these studies could provide a basis for potential usefulness of the diet-derived isothiocyanate called iberin as a promising therapeutic micronutrient in the prevention and intervention of brain tumors.

Erucin (4-methylthiobutyl isothiocyanate, MTBITC) is the isothiocyanate derived from glucoerucin. It was first characterized in steam volatile oils of *Brassica* vegetables by Buttery et al. (1976)

and it is present in a large number of *Brassica* vegetables in considerable amounts. However, it is only recently that its chemopreventive properties have been addressed. MTBITC is of particular interest, not only because of its quantitative presence in plants, but because it has been shown that SFN, which has been extensively studied and is one of the most potent chemopreventive ITC known to date, is oxidized *in vivo* to its structure analogue to MTBITC.

Lamy and Mersch-Sundermann (2009) showed for the first time the inhibitory potency of this compound on the proliferation of human hepatoma HepG2 cells. MTBITC induced apoptosis in HepG2 cells after 6-hour exposure and this effect was accompanied by a time-dependent arrest of HepG2 cells at the G2/M phase of the cell cycle. The results of this study also suggest that although ITCs are only present at maximum concentrations in a living system for a rather short time, this might be sufficient to exert their therapeutic effects. The anticancer properties of this compound against breast cancer in human cells were demonstrated by Wang et al. (2005). On the other hand, the potential of erucin and SFN to modulate the enzyme systems metabolizing a pulmonary carcinogen has been recently evaluated by Hanlon et al. (2009) in a rat lung. The authors concluded that these compounds have the potential to antagonize the carcinogenicity of pulmonary carcinogens.

GLUCOSINOLATE-DERIVED INDOLES

Indole-3-carbinol (I3C) is another glucosinolate breakdown product found in vegetables of the *Brassica* genus (cabbage, broccoli sprouts, Brussels sprouts, cauliflower, bok choy, and kale). I3C is the degradation product of the indole glucosinolate called glucobrassicin. Isothiocyanates formed from indole glucosinolates are unstable and they separate spontaneously into I3C. The association between broccoli consumption, cancer risk, and glucosinolate genotype suggests that indole compounds may be less important than isothiocyanates in modulating cancer risks. In contrast with SFN, there is little scientific literature that studies indolyl glucosinolate metabolites and the anticancer effects of I3C. Recent reviews include Aggarwal and Ichikawa (2005), Higdon et al. (2007), Weng et al. (2008), and Agerbirk et al. (2009). Literature is mixed in its results. Different clinical studies find I3C to be safe and to have anticancer properties (Wong et al., 1997; Weng et al., 2008), although there remains a considerable concern about whether dietary I3C prevents or enhances carcinogenesis. This compound can induce either phase I (activation) or phase II (detoxification) enzymes, depending on the metabolic pathway it takes, and it can, therefore, either activate or deactivate some carcinogens.

Some research indicates this compound may be a promising anticancer agent against prostate cancer (Chinni et al., 2001; Zhang et al., 2003b) and reduce the incidence and multiplicity of mammary tumors in human cells (Rahman and Sarkar, 2005). Coinciding with these studies, oral administration of I3C has been shown to have a possible beneficial effect on estrogen metabolism in humans, and epidemiological studies support the claim that high intakes of I3C may have a broad chemopreventive effect (Brignall, 2001). I3C has an interesting anticarcinogenic potential, acting *via* different metabolic and hormonal pathways (Hanf and Gonder, 2005). The beneficial effect of I3C in human cancer cells has been proved in different cancer cells as breast, hepatic, colon and lung (see Table 1.3). Its beneficial effect to reduce the incidence of tumors in reproductive organs, the growth of breast cancer cells (Telang et al., 1997; Staub et al., 2002; Sundar et al., 2006; Brew et al., 2009) and respiratory papilloma (Rosen et al., 1998) has been proved. In addition to these cancer preventative effects, there is compelling evidence that I3C has a direct antiproliferative response in cultured human reproductive cancer cell lines (Cover et al., 1998; Zhang et al., 2003a; Aggarwal and Ichikawa, 2005; Kim and Milner, 2005). Furthermore, oral I3C administration to breast cancer patients alters estrogen metabolism, leading to a reduction in breast tumor growth without detectable side effects, suggesting that this natural indole could potentially be utilized in anticancer therapeutic strategies that target indole-responsive cancers.

GLUCOSINOLATE-DERIVED NITRILES

Compared with isothiocyanates and I3C, there have been few studies that evaluated the ability of nitriles to induce the phase II antioxidant and detoxification enzymes. The benzyl-, phenethyl-, allyl-isothiocyanate and sulforaphane are formed through the hydrolysis of their naturally occurring corresponding precursor glucosinolates by the endogenous plant myrosinase activity. However, glucosinolate aglycones may yield a nitrile rather than an isothiocyanate under certain conditions, like a low pH. Upon crushing of the plant tissue and subsequent hydrolysis by the myrosinase, nitrile compounds may be formed in similar or even greater concentrations than ITCs. Besides pH, the production of nitriles as opposed to ITCs is likely to be due to the greater activity of a protein similar to the epithiospecifier protein (ESP) that has been characterized in *B. napus* and *Arabidopsis thaliana* (Lambrix et al., 2001; Matusheski et al., 2004). This protein does not catalyze glucosinolate hydrolysis by itself, but instead directs the products of glucosinolate hydrolysis toward epithionitriles, rather than isothiocyanates. The epithiospecifier protein requires iron for its activity. A study in *A. thaliana* suggests that ESP may regulate nitrile formation in addition to epithionitrile formation (Lambrix et al., 2001). Nitrile formation *in vitro*, however, does not require ESP but only the presence of Fe(II) and myrosinase.

Nitriles such as crambene (*S*-l-cyano-2-hydroxy-3-butene), one of the hydrolisis products of progoitrin and 1-cyano-2-hydroxy-3,4-epithiobutane are the most toxic of the normal glucosinolate hydrolysis products, with a human lethal dose of 170 and 178 mg/kg, respectively (Fenwick and Heaney, 1983). Although toxic effects can result from nitrile products, as far as we know, no toxic effects have been identified in humans.

The production of nitriles as opposed to ITC impacts negatively on the anticancer potential of these vegetables. It appears that isothiocyanates are more potent inducers compared to allyl nitriles and their activity is expressed in a wider range of tissues (Tanii et al., 2005). However, relatively few studies have been conducted with regard to glucosinolate-derived nitriles and, consequently, little is known about whether glucosinolate-derived nitriles are involved in the chemopreventive effect of cruciferous vegetables. Among the known cruciferous nitriles, the most studied are the allyl nitrile, one of the hydrolysis product of sinigrin, which is distributed widely throughout the *Brassicaceae* family, and the sulforaphane nitrile, the hydrolysis product of glucoraphanin. The biological activity of other cruciferous nitriles is not known.

Little work has been done to determine positive or negative health effects derived from sulforaphane nitrile [5-(methylsulfinyl) pentane nitrile]. This compound was evaluated for the first time because of its chemoprotective properties by Matusheski and Jeffery (2001). They demonstrated that SFN nitrile is substantially less potent than SFN as an inducing agent of phase II detoxification enzymes. Therefore, glucoraphanin hydrolysis directed toward the production of SFN rather than SFN nitrile could increase the potential chemoprotective effects of broccoli. Thus, the potential health benefit of broccoli as a result of sulforaphane formation is compromised by the alternative formation of an inactive nitrile when broccoli is crushed.

Among glucosinolate-derived nitriles, allyl nitrile appears to be a more active inducer of phase II enzymes compared with other inducers. Although allyl nitrile is neurotoxic at high doses, low doses such as through the intake of vegetables can be beneficial for health. It has been demonstrated that at a dose level of 50 μmol/kg/day, this compound has the ability to induce the phase II antioxidant and detoxification enzymes in the liver, kidneys, rectum, and small intestine, and these results suggest an involvement of allyl nitrile in the antioxidant defense in the body (Tanii et al., 2008), except for colon cancer. These authors studied the inductive ability of allyl nitrile by measuring the activities of GST, QR, and GSH in different tissues (stomach, small intestine, colon, rectum, urinary bladder, kidneys, lungs, and liver) of mice and found that allyl nitrile at subtoxic doses has the ability to increase GST, QR, and GSH. Therefore, they demonstrate that this compound displays an inductive effect in several tissues, especially in stomach and lungs.

In vitro digestion of sinigrin by *Bifidobacterium* sp. showed allyl nitrile to be the major product (Cheng et al., 2004), suggesting a possible generation in the large intestine under *Bifidobacteria*

preponderant conditions. Accordingly, consumption of cruciferous vegetables containing sinigrin implies that we are exposed to the allyl nitrile, which, although not toxic when consumed in vegetables (Tanii et al., 2005), is a neurotoxicant when administered at high doses.

Nitrile crambene (1-cyano-2-hydroxy-3-butene) formed through the hydrolysis of progoitrin and epi-progoitrin, is an aliphatic nitrile occurring naturally in cruciferous vegetables including Brussels sprouts, broccoli, and cauliflower. Seeds from the plant *Crambe abyssinica* are the richest source of crambene (Fenwick et al., 1983). Crambene is known to upregulate the synthesis of phase II detoxification enzymes, including QR and GST in liver and other organs (March et al., 1998; Nho and Jeffery, 2001). Moreover, the induction in hepatic QR activity was found to be similar to SFN (Keck et al., 2002). Crambene (5 mM) induced QR activity and caused cell cycle arrest in the G(2)/M phase in mouse Hepa 1c1c7 cells, rat H4IIEC3 cells, and human Hep G2 cells. From these studies two findings were relevant. First, doses of crambene needed for the induction of QR in cell culture were similar to 100-fold greater than effective doses of SFN, and second, the potential chemoprevention provided by crambene may differ between tissues because of differences in the degree and pattern of induction (March et al., 1998).

MECHANISMS OF CANCER PROTECTION BY GLUCOSINOLATE DEGRADATION PRODUCTS

A number of mechanisms appear to contribute to the anticarcinogenic activity of glucosinolates, including impairment of the bioactivation of carcinogens and increased detoxification of their reactive intermediates, suppressed cellular proliferation and increased apoptosis (Hanlon et al., 2008).

Glucosinolate-Derived Isothiocyanates

Initial research about the mechanisms of glucosinolate degradation products was focused on phase II enzyme induction, as well as the inhibition of enzymes involved in carcinogen activation. However, there has been a growing interest in other mechanisms of chemoprotection, mainly based on the effects of SFN, as was stated in the excellent review of Clarke et al. (2008). In this review, the authors depict the main mechanisms by which SFN protects against different types of cancer. These mechanisms can also be useful for other ITCs, as we will briefly show. Postinitiation, ITCs can act to suppress cancer development through various molecular targets that are involved in controlling cell proliferation, differentiation, apoptosis, or cell cycle.

Blocking Mechanisms: Phase I and Phase II Enzymes

The anticarcinogenic properties of isothiocyanates have been attributed to their ability to alter detoxification pathways, leading to a decreased activation of procarcinogens and an increased excretion of carcinogens. Some isothiocyanates appear to increase both phase I and phase II enzymes; that is, they act as bifunctional inducers that activate both the antioxidant response element (ARE) and the xenobiotic response element (XRE) in the gene promotor region. Other isothiocyanates may upregulate only phase II enzymes, thus functioning as monofunctional inducers through the ARE (Keck and Finley, 2004).

Phase I enzymes usually involve oxidation, reduction, or hydrolysis and generally lead to detoxification, but are also involved in converting procarcinogens into carcinogens. Inhibition of phase I enzymes is thought to be an important step in blocking chemically induced carcinogenesis.

Phase II enzymes such as QR, GST, UDP-glucuronyl transferase and NADPH reductase, are able to conjugate with activated carcinogens and turn them into inactive water soluble compounds. They can be excreted through the urine, which results in the neutralization of potential carcinogens from mammalian cells. Inductions of phase II cellular enzymes are largely mediated by the antioxidant responsive element (ARE), which is regulated by the transcriptional factor, Nrf2. The most powerful inducers of the phase II enzymes are the isothiocyanates sulforaphane, iberin, and erucin, which

are the hydrolysis products of glucoraphanin, glucoiberin, and glucoerucin, respectively, as it was already explained in the previous section (Nilsson et al., 2006).

There is *in vitro* and *in vivo* evidence that dietary isothiocyanates regulate phase I and II enzyme activities. Isothiocyanates such as PEITC and SFN can modulate phase I metabolism through direct interactions with cytochrome P450 or regulation of their transcript levels within the cell (Clarke et al., 2008). In cell culture and animals, SFN increases the activity of phase II enzymes, such as QR and GST. Preliminary QR activity data using rodents suggest that SFN present in whole broccoli is more beneficial than purified SFN. In rodents, PEITC increases hepatic GSH concentrations, QR and GST activity. Rats given the parent compound of AITC (24 mg/day) for 11 days had significantly increased the total GST activity, and Brussels sprouts high in the parent GS of AITC increased the hepatic and intestinal GST. I3C is a unique isothiocyanate because it can increase phase I isozymes including cytochrome P450 1A, increases phase II enzymes and acts as a phytoestrogen (Keck and Finley, 2004).

Suppression via Antiproliferative Mechanisms

Several ITCs, including PEITC, BITC, SFN, and AITC, have been shown to inhibit cell growth and cell cycle progression and cause apoptosis in various cell types (Stan et al., 2008). Significant progress has been made in our understanding of the mechanism by which ITCs cause cell death. First, ITCs seem selective toward cancer cells since normal epithelial cells display a significant resistance toward ITC-induced apoptosis. Second, they produce reactive oxygen species (ROS) to initiate apoptotic signal transduction in various cell types. The ITC-mediated apoptosis is significantly attenuated by antioxidants and overexpression of catalase (Stan et al., 2008). Third, the ITC mediated apoptosis is caspase-dependent and appears to involve both intrinsic and extrinsic caspase cascades (Singh et al., 2004; Xiao et al., 2005). Fourth, the ITC-mediated apoptosis correlates with changes in Bcl-2 family protein levels and Bax activation, and deficiency of some proteins of this family (Bax and Bak) confers a significant protection against ITC-mediated cell death (Choi and Singh, 2005).

Cell Cycle Arrest

One characteristic of cancer is hyperproliferation due to the loss of cell cycle regulatory mechanisms. The key regulators of cell cycle progression are the cyclin-dependent kinases (CDKs), cyclins, and CDK inhibitors. The regulation of CDK complexes is dependent upon the phosphorylation status of the various components of the complex. The cyclin/CDK complexes promote cell cycle progression, while the CDK inhibitors promote cell cycle arrest. Research suggests that the action of ITCs on various CDKs, cyclins, and CDK inhibitors is complex and the regulation is likely affected by the cell type, dose of treatment, and time of exposure (Clarke et al., 2008).

In general, cell cycle arrest occurs at the G(2)/M phase, as was reviewed by Stan et al. (2008) for PEITC, BITC and AITC. Remarkably, SFN has been shown to arrest the cell cycle at all three phases-G(1), G(2)/M, and S phase (Traka and Mithen, 2009).

Apoptosis

Apoptosis, or programmed cell death, is a highly regulated process that occurs under a range of physiological and pathological conditions as part of the cellular mechanism. Apoptosis plays important roles in the development and maintenance of homeostasis and in the elimination of cells that are damaged or no longer necessary for the organism (Traka and Mithen, 2009). Regulation of the apoptosis can be accomplished either through the death-receptor caspase cascades or the mitochondria caspase cascades. Caspases are the effectors of apoptosis and some of the hallmarks of apoptosis are cytoplasmic histone associated DNA fragments, poly(ADP-ribose) polymerase (PARP) cleavage, changes in Bcl-2 protein family ratios (increased proapoptotic proteins and decreased antiapoptotic proteins) and cytochrome *c* release from the mitochondrial membrane (Clarke et al., 2008).

Different experiments have demonstrated that SFN, PEITC, BITC, and AITC induced apoptosis by different mechanisms including activation of signaling kinases (like p38 kinase), which resulted in cytochrome *c* release from the mitochondria and caspase-3, -8, and -9 activation; downregulation of antiapoptotic proteins Bcl-2 and Bcl-XL; and increase of the proapoptotic protein Bax (Traka and Mithen, 2009).

Histone Deacetylase Inhibition

Histone deacetylase (HDAC) inhibition is emerging as a promising field in cancer chemoprevention and therapy. HDACs affect the histone acetylation status and the transcription factor access to DNA, thereby depressing epigenetically silenced genes in cancer cells, and resulting in a deregulation of differentiation, cell cycle arrest, and/or apoptosis. SFN was first reported to inhibit HDAC activity in human colon cancer cells and then in various human prostate lines, with evidence for an increase in both global and local histone acetylation status, such as on the promoter regions of *P21* and *bax* genes (Dashwood and Ho, 2008).

Additional work with the synthetic isothiocyanate called phenylhexyl isothiocyanate (PHITC) provided evidence for HDAC inhibition and chromatin remodeling in human leukemia cells, leading to growth arrest (Ma et al., 2006). The inhibition of the HDAC activity was associated with changes in multiple histone "marks." Specifically, there was a dose-dependent increase in acetylated histones H3 and H4, as well as methylated H3K4, with concomitant loss of the "repressive" histone mark methylated H3K9. Induction of p2l WAFl was seen coincident with the cell growth arrest.

Mitogen-Activated Protein Kinases

Mitogen-activated protein kinases (MAPKs) belong to the superfamily of serine/threonine kinases including the extracellular signal-regulated kinases (ERK), c-Jun NH2-terminal kinases (JNK), and p38. They are believed to play a role in carcinogenesis and cancer development. An important downstream effector protein of MAPKs is the activator protein-1 (AP-1), a dimeric basic protein that is activated by different MAPKs. Modulation of AP-1 members may have effects on both promoting and inhibiting carcinogenesis. These divergent responses observed are likely dependent on the genetic background, cell type, tumor state, and signaling networks that are affected in response to specific agents (Clarke et al., 2008). SFN is implicated in the activation of AP-1, which plays an important role in the regulation of cell death. This activation occurs at low concentrations of SFN, while AP-1 is inhibited at a high concentration.

Nuclear Factor Kappa-B

Inflammation is a well-recognized risk factor in carcinogenesis. Isothiocyanates possess anti-inflammatory activity through inhibiting the nuclear factor kappa-B (NF-κB) (Hayes et al., 2008). NFκB is a heterodimeric transcription factor that consists of a p50 and p65 subunit and, when active, promotes inflammatory gene expression, cell proliferation and cell survival. The ability of SFN and PEITC to inhibit the transcriptional activity of NF-κB is a consequence of the phytochemicals antagonizing phosphorylation of IκB, the inhibitor of NF-κB, which is carried out by IκB kinases. Constitutive activation of NFκB is common in various human malignancies, including colon and prostate cancer, and leads to upregulation of genes encoding adhesion molecules, inflammatory cytokines, growth factors and antiapoptotic genes (Clarke et al., 2008). Inhibition of NF-κB prevents transcriptional activation of genes such as cyclin D1, VEGF, Bcl-XL, COX2, and MMP-9. Although these gene products influence various biological processes, the inhibition of NF-κB generally appears to make cells more sensitive to apoptosis (Hayes et al., 2008).

Reactive Oxygen Species

Oxidative stress, a cellular difference between production and elimination of reactive oxygen species (ROS), is thought to underlie the pathogenesis of various diseases. Protection from ROS comes either from direct scavenging or by increasing protective mechanisms that result in an improved defence

against ROS (Traka and Mithen, 2009). Different evidences indicate that the treatment of cells with ITCs results in a generation and production of reactive oxygen species (ROS), which seem to signal cellular responses to this class of dietary phytochemicals (Antosiewicz et al., 2008). The ROS generation in response to ITC treatment was first documented about BITC in rat liver epithelial RL34 cells, which suggested that the antioxidant effect of ITCs may be in part related to ROS generation (Nakamura et al., 2000, 2002). The same authors reported a strong correlation between ROS generation and apoptosis induction by BITC. SFN administration to PC3 prostate cancer cells resulted in ROS generation, which was accompanied by the disruption of mitochondrial membrane potential, cytosolic release of cytochrome *c*, and apoptosis (Clarke et al., 2008). Besides, a positive correlation between ROS generation and apoptosis induction has been observed for PEITC in human prostate cancer cells and for BITC in human breast cancer cells (Antosiewicz et al., 2008). These authors concluded that different studies provide experimental evidence to implicate ROS in signal transduction leading to programmed cell death by several ITCs. The aromatic ITCs (PEITC and BITC) are relatively more potent inducers of ROS production and apoptosis than SFN (Antosiewicz et al., 2008).

Glucosinolate-Derived Indoles

Indole-3-carbinol is the main degradation product of the indole glucosinolates like glucobrassicin. Several authors have shown the chemopreventive effect of this compound against different types of cancer and then proposed mechanisms of action similar to those of isothiocyanates.

- Attenuation of carcinogen bioactivation *in vivo* by modulating the expression of phase I and II metabolic enzymes (Bell et al., 2000).
- Upregulation of cell cycle inhibitors including p21 and p27 to repress proliferation of cancer cells (Chinni et al., 2001).
- Upregulation of proapoptotic Bax protein and increase of the Bax/Bcl-2 ratio to promote apoptosis of cancer cells (Nachshon-Kedmi et al., 2004).
- Reduction of DNA damage caused by various carcinogens by inducing DNA repair enzymes to block DNA strand breakage (Bonnesen et al., 2001).
- Inhibition of angiogenesis by suppressing proliferation of blood endothelial cells (Wu et al., 2005).
- Altered estrogen metabolism caused by I3C may lower the risk of hormone-dependent cancers. Plant estrogen agonists (phytoestrogens) lower the growth-promoting activities of estrogens by increasing the 2-hydroxylation of estrogen. Hydroxylation on the 2-position lowers the concentration of 16-hydroxylation estrogen products, which are stronger estrogen receptor agonists (Keck and Finley, 2004).

Glucosinolate-Derived Nitriles

Relatively little research has been conducted with glucosinolate derived nitriles, perhaps because only a few bioactive nitriles have been discovered (Keck and Finley, 2004). The nitrile crambene is a hydrolysis product of progoitrin and is abundant in Brussels sprouts, cabbage and crambe (an oilseed); sulforaphane nitrile is a hydrolysis product of glucoraphanin, and allyl nitrile is one of the hydrolysis products of sinigrin. The main effects attributed to nitriles are delineated in the following sections.

Detoxification

Nitriles may increase phase II detoxification enzymes *in vitro* and *in vivo*. Rats given crambene had a significant increase in QR, GST, and GSH. The amount of crambene needed to induce hepatic QR activity in rats was similar to that reported for SFN, but in cell culture, crambene was only 1% as effective as SFN. Sulforaphane nitrile bioactivity was similar to crambene in cell culture, but did

not increase phase II enzyme activities in rats. This suggests that some nitriles may play a role in the ability of crucifers to protect against carcinogenesis.

Allyl nitrile increased the activity of the enzymes thioredoxin reductase (TR) in the liver, kidneys, and rectum, and glutathione peroxidase (GPx) in the kidneys and small intestine, while it lowered the activities of glutathione reductase (GR) and catalase in the colon alone, suggesting that allyl nitrile is involved in the antioxidant defense in the body, except for the colon. Besides, allyl nitrile has been shown to be an inducer of the phase II enzymes GST, QR, and GSH in several tissues (Tanii et al., 2005).

Tumor Growth Inhibition

Nitriles may also prevent tumor growth. Crambene-induced cell cycle arrest without affecting cell viability (95%) in the G2/M phase in mouse Hepa 1c1c7 cells, rat H4IIEC3 cells, and human HepG2 cells. The mechanism of cell cycle arrest is not clear, but crambene arrested the cells in the same phase as cells treated with SFN (Keck and Finley, 2004).

ENHANCING GLUCOSINOLATES IN HORTICULTURAL CRUCIFERS: BREEDING AND BIOTECHNOLOGY

The growing interest in a healthy human diet along with the development of new strategies has led to different methods to increase the benefits of plants in the food supply and to prevent diseases. The two major strategies are conventional breeding and genetic engineering. The terms "biotechnology" and "genetically modified/enhanced" have been used to describe various strategies that implement some form of plant biochemistry modification. Most crops were modified primarily for insect resistance or to improve tolerance to herbicides. However, crops are being increasingly modified to enhance the nutritional profile to promote health, to enhance taste, and to decrease nutritional deficiencies. Enhancing the level of glucosinolates in cruciferous vegetables through conventional breeding or genetic engineering has been achieved in the last years. The aim is to improve the chemopreventive properties of these vegetables. Conventional breeding is exemplified by the production of broccoli with enhanced glucosinolate content.

The advances in our understanding of the genetic and environmental factors that lead to glucosinolate accumulation in crops and to isothiocyanate delivery upon consumption may facilitate the development of varieties of *Brassica* crops with an enhanced health-promoting activity. The most promising varieties for future breeding purposes would be those with the highest total glucosinolate content and, particularly, glucosinolates with beneficial effects related to human health. Isothiocyanates and some indole and aromatic compounds derived from glucosinolates have, as it was previously explained, a chemoprotective effect related to a reduction of the risk of certain cancers in humans. Enhancing glucosinolates in broccoli is attractive due to the substantial body of epidemiological evidence that relate health benefits of broccoli and biological activity of sulforaphane. The development of enhanced *Brassica* hybrids with a high glucosinolate content is possible for aliphatic glucosinolates such as glucoraphanin. Hybrids between commercial broccoli cultivars and two wild *Brassica* species showed enhanced levels of glucoraphanin and its associated anticarcinogenic activity (Faulkner et al., 1998; Mithen et al., 2003). Faulkner et al. (1998) showed that hybrids formed by crossing inbreds and wild relatives express a higher induction potential of phase II enzymes than the broccoli inbreds themselves. Later, Mithen et al. (2003) reported an enhanced isothiocyanate production in broccoli after the introgression of two genomic segments from *Brassica villosa* L., a member of the *B. oleracea* ($n = 9$) species through several breeding cycles. In addition to enhanced levels of glucosinolates, these genotypes have enhanced conversion of glucosinolates to isothiocyanates through a reduction in nitrile production.

The application of genomic techniques has considerably improved the knowledge of the genes and physiological processes that modify phytochemical formation, leading to improved food crops.

Genes necessary to alter glucosinolate profiles have been found within *Brassica* genus and *A. thaliana*. Qualitative differences observed among aliphatic composition may be due to allelic variation in a few genes encoding key regulatory enzymes at key points in the glucosinolate pathway (Li and Quirós, 2003). Besides, production of glucosinolates in noncruciferous plants has been achieved in *Nicotiana bethamiana* by genetic engineering (Geu-Flores et al., 2009) which opens the field of glucosinolate enriched foods in different vegetable products, aimed at use in cancer prevention.

The use of *Brassica* vegetables to improve human health and the interpretation of epidemiological data require an understanding of glucosinolate chemistry and metabolism across the whole food chain, from production and processing to the consumer. Environmental conditions and physiological factors may modify the amounts of these compounds present in *Brassica* vegetables, but also crop management strategies could increase the production of these phytochemicals. Considering the chemical classes, previous studies have shown that the synthesis of indole glucosinolates is regulated in a way very differentfrom that of the aliphatic glucosinolates (Brown et al., 2002; Kim et al., 2003). Aliphatic glucosinolate content is highly heritable and varies among *Brassica* crops and varieties of the same crop (Kushad et al., 1999). On the other hand, indole glucosinolates are common in *Brassica* vegetables, although their levels are not only subject to environmental fluctuations but to conditions during harvest and processing. Nevertheless, it may be necessary to determine the mechanisms whereby environment and processing cause upregulation of indole glucosinolates before developing hybrids with a specific content of this last class of glucosinolates.

Besides plant glucosinolate content, it seems important to modify the conditions for glucosinolate degradation to obtain the most interesting metabolites for different uses, isothiocyanates or nitriles. Zabala et al. (2005) provide proofs that, by using a targeted transgenic approach, it is possible to alter the chemistry of plants without any noticeable phenotypic effect on the plant. They suggest that it is possible to manipulate ESP in *Arabidopsis* and therefore, it will be possible to use this approach to alter the bioactive components of glucosinolate profiles that will be of importance in investigating the importance of nitriles versus isothiocyanates in plant–insect interactions and in studies examining biological interactions.

As a result of the vast information relating glucosinolates and healthy properties, there is an increasing market of foods and products enriched with glucosinolates, and several patents with methods to increase beneficial glucosinolates have been registered. The use of glucosinolates and their derivatives in functional food products and pharmaceuticals may provide additional routes for the exploitation of these attractive natural plant products. In the last few years, pharmaceutical forms (pills, powders, capsules, vials, etc.) containing glucosinolates as food bioactive compounds (especially broccoli extracts that provide sulforaphane and other phytochemicals) have appeared in the market. Among them it is possible to find Brassica teas, broccoli sprouts, capsules with sulforaphane, with indole-3-carbinol and capsules made from a cruciferous mix. The U.S. Broccosprouts® patent (broccoli sprouts obtained from glucosinolate-rich genotypes) is an early application of these studies (Fahey et al., 1997b). More recently, Mithen et al. (2003) developed a high-glucosinolate broccoli ("Superbroccoli") in Europe, which is also being considered by the commercial sector. In Italy, the health-promoting concept has been exploited to promote frozen *Brassica* products.

The diversity in glucosinolate levels reported in several studies suggests that the potential health benefits from crucifer vegetables are greatly dependent on the crop and accession selected. Most research on *Brassica* vegetable crops has been focused on *B. oleracea* crops, especially, in broccoli. In contrast, there is relatively little information on the glucosinolate pattern in green tissues of *B. rapa*. With the increased interest in diet and health, it is necessary to have information about profiles and levels of glucosinolates in other *Brassica* species. Our research group at Misión Biológica de Galicia (MBG—Spanish Council for Scientific Research) kept a collection of *B. rapa* (including turnip greens and turnip tops varieties) and *B. napus* (leaf rape), which is maintained as part of the *Brassica* genus germplasm bank. This collection has been evaluated for its glucosinolate and phenolic content (Padilla et al., 2007; Cartea et al., 2008a,b; Francisco et al., 2009). As a result, varieties differed greatly in their glucosinolate content, being gluconapin, glucobrassicanapin, and

progoitrin the major glucosinolates found in their leaves. Gluconapin and glucobrassicanapin have been associated with the bitter taste characteristic of these crops but, unlike other aliphatic glucosinolates, nothing is known about their anticancer properties. Therefore, an attractive objective is to know the possible biological effects of glucosinolates and hydrolysis products of *B. rapa* and *B. napus* varieties kept at the germplasm collection at the MBG.

Biosynthesis of gluconapin requires a functional allele at the Gsl-alk locus that converts glucoraphanin into its alkenyl homolog, gluconapin. The manipulation of the *BoGSL-ALK* gene and, consequently, the alteration of aliphatic glucosinolate profiles in specific plant genotypes were demonstrated by Li and Quirós (2003) in *A. thaliana*. The authors obtained transformed *Arabidopsis* plants with a reduced concentration of glucoraphanin which was converted into gluconapin through cloning of *BoGSL-ALK* gene. This key finding provides the opportunity to engineer *Brassica* crops with specific glucosinolate content. For example, downregulation or silencing of *BoGSL-ALK* could produce *B. rapa* varieties lacking gluconapin and would simultaneously produce plants accumulating glucoraphanin as a source of anticarcinogens. Previous results obtained by our group suggest that the genes necessary for altering glucosinolate profiles can be found within *B. rapa* germplasm. This information should be useful for developing new cultivars with an appropriate glucosinolate profile, from which high quality added value products can be produced.

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2 MK615, an Extract of the Japanese Apricot (*ume*)
A Promising Anticancer and Anti-Inflammatory Compound

Tokihiko Sawada and Keiichi Kubota

CONTENTS

INTRODUCTION

In the nineteenth century, Philipp Franz Balthazar von Siebold, a German doctor who lived in Japan and taught modern Western medicine in Nagasaki, was fond of a Japanese tree that produced attractive flowers in early spring and was found all over the country. He subsequently described this tree as *Prunus mume* Siebold et Zuccarini (known as *ume* in Japanese) in his textbook *Flora Japonica* (Figure 2.1). In Japanese society, *ume* has been used as food as well as a supplement. Because fresh *ume* plums have a high cyanide content, they cannot be eaten directly, and instead the fruit have been traditionally pickled.

MK615, an extract from *ume*, contains several triterpenoids (Figure 2.2) and other unknown components. Recently, it has been widely recognized that MK615 has diverse biological effects, including anticancer and anti-inflammatory actions.

MK615

MK615 was developed by the bio-company, AdaBio Co., Ltd. (Gunma, Japan). Gunma prefecture is the second largest area for *ume* cultivation in Japan, and the company therefore developed the idea of using *ume* for the production of a new bioactive drug.

MK615 is extracted from fresh *ume* fruit. Briefly, the preparation procedure involves extraction of the plum of the Japanese apricot juice using a press, and this is then heated and concentrated. The concentrate is dissolved in diethylether, which is then completely removed from the extract by a rotary evaporator. The dried hydrophobic extract, MK615, is dissolved in DMSO. MK615 prepared

FIGURE 2.1 *Prunus mume* Siebold et Zuccarini (*ume* in Japanese).

by this procedure has a low pH. Recently, MK615 with a neutral pH has been developed and used in experimental assays.

It has been shown that MK615 contains several triterpenoids, but its other biological components remain unknown.

Anticancer Effect

Apoptosis

The anticancer effect of MK615 was first reported by Adachi et al. (2007), using a pancreatic cancer and dog fibrosarcoma cell-based assay. Adachi, who was a founder of AdaBio Co. and his colleagues observed a dose-dependent growth inhibition of cancer cells by MK615, and demonstrated induction of apoptotic cell death by MK615 using electron microscopy. The typical appearance of cancer cell apoptosis is shown in Figure 2.3. MK615 showed dose-dependent growth inhibition of the breast cancer cell lines, HTB132 and MCF7 *in vitro* (Nakagawa et al., 2007). Breast cancer cell line, CRL-2314 exposed to MK615 also developed intracytosolic vacuolation and underwent apoptotic cell death (Figure 2.3a–c). The precise linkage between apoptosis and cytosolic vacuolation remains unknown.

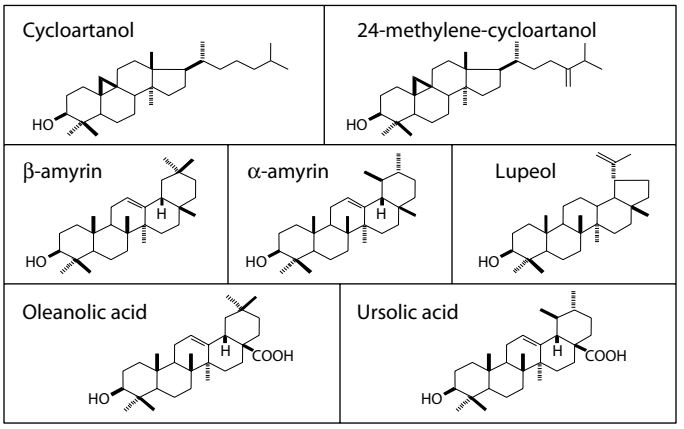

FIGURE 2.2 MK615, an extract from *ume* contains several triterpenoids.

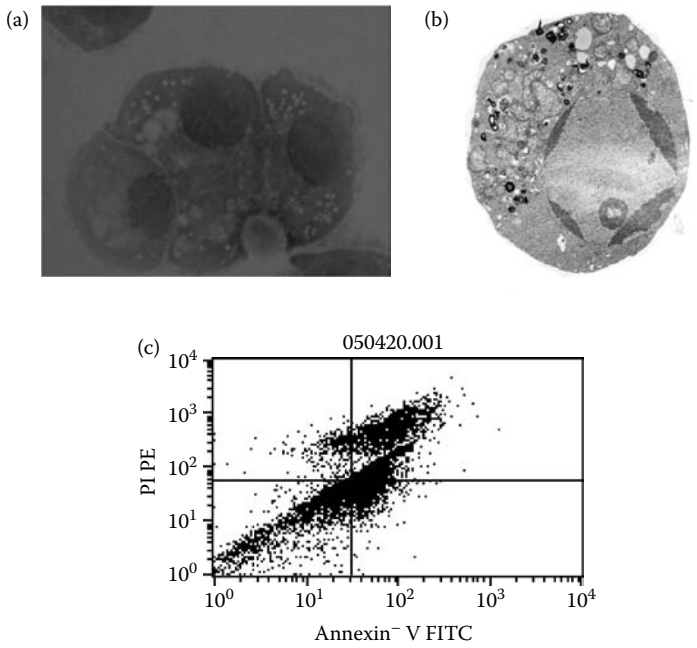

FIGURE 2.3 Anticancer effect of MK615 on breast cancer cells (a) Massive vacuolation was evident in CRL-2314 from 6 hours after the start of incubation with MK615. (b) Electron microscopy showed that the nuclei of CRL-2314 were condensed and fragmented, with typical features of apoptosis. The cytoplasm contained massive vacuoles. (c) MK615 induced apoptosis in CRL2314 after 12 hours of incubation. Flow cytometry was performed with Annexin V-FITC (*x*-axis) and povidium iodide (*y*-axis).

Cell Cycle Modification

We have analyzed changes in the cell cycle induced by MK615 in many cell lines, including those derived from esophageal cancer, colon cancer, hepatocellular carcinoma, pancreatic cancer, and breast cancer. In all cases, we found that MK615 increased the frequency of cells at the G2 checkpoint. Cancer cells can be detected at the cell-division checkpoints (G1 or G2) and are found to die as a result of apoptosis. In general, however, disruption of these checkpoints is often observed in cancer, and thus cancer cells can bypass these checkpoints and proliferate.

MK615 accumulates in cells at the G2 checkpoint and induces apoptosis. One mechanism of this effect is the inhibition of Aurora kinases in cancer cells (Okada et al., 2007, 2008). Aurora kinases act as key mediators of cell division by controlling chromatid segregation. There are three isoforms of Aurora kinases in mammals: Aurora A, Aurora B, and Aurora C. Despite their sequence similarities, their functions and cellular localizations differ. Aurora A is localized at the centrosome and forms the mitotic spindle apparatus that plays a crucial role in segregating chromosomes into daughter cells. Aurora B is localized in inner centromeric chromatin during metaphase-anaphase transition, and relocalizes to microtubules in the spindle midzone during telophase, being required for accurate cytokinesis. Unlike Aurora A and Aurora B kinases, the function of Aurora C still remains unclear. Overexpression of Aurora A and Aurora B is frequently observed in various human cancers, and MK615 accumulates in cancer cells at G2 phase by inhibiting the expression of Aurora A and B kinases.

Autophagy

Apoptosis is a well-known form of programmed cell death (PCD), and is widely accepted as the main mechanism of cancer cell death. Currently, apoptosis is classified as type I PCD, whereas autophagic cell death is classified as type II PCD (Bursch et al., 2000a,b). Autophagic cell death

differs in several ways from type I PCD. Type II PCD is a form of caspase-independent cell death, displays no DNA-laddering, and is typically characterized by formation of cytoplasmic vacuoles. Autophagy is an evolutionarily conserved pathway that delivers and recycles cytoplasmic components, such as mitochondria and Golgi apparatus. Some studies have indicated that cancer cells show less autophagy than normal cells (Kirkegaard et al., 2004; Otsuka et al., 1978), suggesting that induction of autophagy could be an attractive mode of anticancer therapy. MK615 has been shown to induce cytoplasmic vacuoles after six hours of incubation (Figure 2.4) (Mori et al., 2007). Electron microscopy has demonstrated that these cytoplasmic vacuoles are autophagosomes, containing cell components such as mitochondria and Golgi apparatus. MK615 may exert its anticancer effect through induction of autophagy-related programmed cell death in colon cancer cells.

Anti-Inflammatory Effect

Recently, an anti-inflammatory effect of MK615 has been reported. High mobility group box-1 (HMGB1) is a nuclear protein that plays a pivotal role in nuclear function (Lu et al., 1996). However, once HMGB1 is released into the extracellular space, it induces pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, resulting in a severe inflammatory state, including systemic inflammatory syndrome. Kawahara et al. (2009) have reported that MK615 inhibits the LPS-stimulated release of HMGB1 from the macrophage-like cell line, RAW264.7. This inhibition is due to the activation of the transcription factor Nrf2 and heme oxygenase (HO-1).

Interestingly, it is known that the cell surface receptor for HMGB1 is a receptor for advanced glycation end-products (AGEs) (Clynes et al., 2007). AGEs are formed by nonenzymatic, irreversible binding of glucose and proteins, and there is considerable evidence to suggest that AGEs are potent inducers of inflammation. AGEs and HMGB1 share the cell surface receptor RAGE, and both are strong inducers of inflammation. Although further investigations are needed, the fact that MK615 inhibits the activation of HMGB1 suggests that MK615 would be a potent inhibitor of AGEs.

CONCLUDING REMARKS

Ume is a fruit with a long history of use by the Japanese people, and is widely believed to confer health benefits when consumed as pickled plum. Now the scientific evidence suggests that this may indeed be the case. *Ume* is a rich source of anticancer and anti-inflammatory substances. MK615 may therefore be a promising new avenue for the treatment of cancer and inflammation.

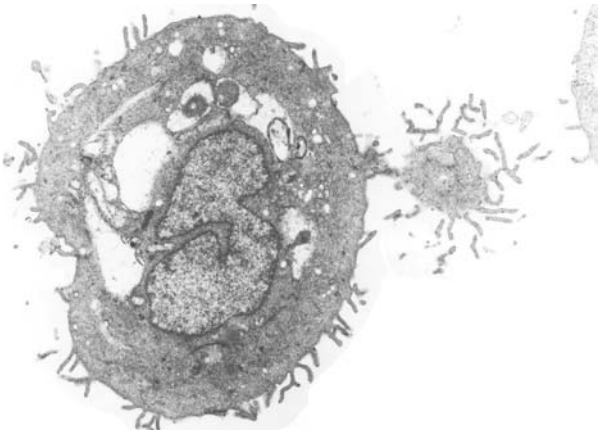

FIGURE 2.4 MK615 induces autophagy. Electron microscopy shows autophagosomes containing cytoplasmic components in COLO cells.

SUMMARY

1. MK615 is an extract of the Japanese apricot, *ume*.
2. It exerts an anticancer effect by inducing apoptosis in cancer cells.
3. It also exerts an anticancer effect by inducing autophagy in cancer cells.
4. It accumulates in cancer cells at G2 phase by inhibiting Aurora kinases A and B.
5. It exerts an anti-inflammatory effect by inhibiting HMGB1.

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3 *Rhodiola* and Related Plants
A Role in Cancer Prevention and Therapy

Kelly J. Gauger and Sallie Smith Schneider

CONTENTS

INTRODUCTION

There are approximately 200 different species of *Rhodiola* that grow in Europe and Asia, and a handful of these plants have been used in traditional eastern medicine. Examples of the different species include *Rhodiola rosea*, *R. crenulata*, *R. sachalinensis*, *R. imbricata*, *R. quadrifida*, *R. algida*, *R. kirilowii*, and *R. sacra*. Typically, these plants are found growing at high altitudes in barren soils, and it is believed that the adaptation to grow under these conditions contributes to some of the protective and medicinal nature of *Rhodiola*.

The rhizomes and roots of *Rhodiola* are traditionally harvested in the late summer and fall for the medicinal purposes of the plant. Depending on the property being studied, both aqueous and hydro-alcoholic extracts are used since they have slightly different activities. Chemical analyses of the genus *Rhodiola* indicate that each species has common as well as unique components that add to the distinct nature of the various species. In recent years, increasingly more attention has been paid to standardizing the methods employed for identifying the diversity among the different *Rhodiola* species. A common component frequently discussed in the literature for its possible anti-cancer and anti-inflammatory activity is the phenolic glycoside, salidroside, and its aglycone form, *p*-tyrosol. It is clear though that other phenolic acids, monoterpenes, triterpenes, phenol propanoids, organic acids, and flavonoids (rosavin, crenulatin, rosin, gallic acid, caffeic acid, catechins, proanthcyanidins) unique to the different plants may also be involved in *Rhodiola's* beneficial effects.

An assortment of *Rhodiola* species have been prescribed for a variety of symptoms and *R. rosea* is perhaps the best studied of the group. *R. rosea* has been shown to facilitate adaptation to stressful conditions (fatigue, heavy exercise, high altitudes, and radiation) in a variety of organisms with little

to no toxicity except at exceptionally high doses. For this reason, it has been given the title of an "adaptogen." It has also been suggested to act as an antidepressant as well as a cardioprotective agent and several species of *Rhodiola* have been shown to exert anticancer properties against bladder cancer, lung cancer, breast cancer, and melanoma. This chapter will review these studies as well as the collection of *in vitro* and *in vivo* data supporting *Rhodiola's* role in different aspects of cell functioning and general health as it pertains to cancer prevention and therapy.

THE ROLE *RHODIOLA* SPECIES PLAY IN CANCER TREATMENT

Rhodiola Inhibits Proliferation and Promotes Cell Death

It is well established that uncontrolled cellular proliferation contributes to the pathogenesis of cancer. Interestingly, numerous reports have demonstrated that several different *Rhodiola* extracts are able to block cancer cell growth *in vitro* and *in vivo*. Most of the literature focuses on the antitumor effects elicited by *R. rosea*. First, Dement'eva and Iaremenko (1987) demonstrated the antitumor and antimetastatic effects of an extract of *R. rosea in vivo* by treating mice and rats with transplantable NK/Ly tumor, Ehrlich's adenocarcinoma (EAC), melanoma B16, and Lewis lung carcinoma. These animals all exhibited a significant increase in survival. Then, in experiments with transplanted tumors and partial hepatectomy, Udintsev and Shakhov (1989) showed that *R. rosea* extracts, or their combination with anticancer drugs, inhibited the rate of EAC and Pliss' lymphosarcoma (PLS) growth as well as dissemination of the latter. A follow-up study by the same group transplanted EAC as well as metastasizing PLS in rats and showed that when 0.5 mL/kg *R. rosea* was administered orally in the morning hours beginning from the fourth day after transplantation, there was a significant inhibition of tumor growth and metastases in rats that were given partial hepatectomy, *R. Rosea* extract, or subjected to both partial hepatectomy and *R. Rosea* extract. The tumor growth was inhibited by 37%, 39%, and 59%, respectively, and metastases were reduced by 42%, 50%, and 75%, respectively. Furthermore, based on ^{3}H-Thymidine assays, the proliferative activity of the tumors and metastases was markedly decreased. Using the method of diffusion chambers as well as double-layer agar systems, these researchers also found that both partial hepatectomy as well as treatment with a *R. rosea* extract resulted in decreased clonogenic activity of PLS (Udintsev and Shakhov, 1991). A *R. rosea* extract has also been shown to exert anticancer effects on promyelocytic leukemia cells (HL-60). Analysis of the cell cycle after treatment with *R. rosea* extract showed that, during incubation in the extract, the proportion of G1 and G2/M phase cells was reduced while there was an increase in the number of S phase cells. These effects were highly dependent upon both treatment dose as well as duration. The mitotic index was investigated by staining the cells with DAPI and counting the number of indices with a fluorescent microscope. Each dose of *R. rosea* extract reduced the mitotic index of HL-60 cells and at the highest concentrations (180 and 225 μg/mL), led to a total inhibition of cell division. Finally, treatment with the highest concentrations (225 and 450 μg/mL) reduced cell viability to nearly zero after 48 and 72 hours of incubation. Moreover, at all concentrations, flow cytometry revealed that the decrease in the percentage of live cells was associated with a concomitant increase in the percentage of apoptotic and necrotic cells (Majewska et al., 2006).

The effects of *Rhodiola imbricata* on the proliferation of human erythroleukemic cells (K-562) has been studied by Mishra et al. by incubating the cells in the presence of a wide concentration range (0–500 μg/mL) of *R. imbricata*. Utilizing a MTT assay, these researchers demonstrated that *R. imbricata* could inhibit proliferation of K-562 cells in a dose-dependent manner and that there was a significant reduction in proliferation at 100 and 200 μg/mL. Analysis of the cell cycle following *R. imbricata* treatment showed that the percentage of G2/M phase cells was increased while the percentage of cells at the S phase of the cell cycle was decreased. A concentration of 200 μg/μl caused a significant increase in the percentage of G2/M phase cells within 24, 48, and 72 hours of incubation which indicates that *R. imbricata* arrests cell cycle progression in the G2/M phase (Mishra et al., 2008).

Rhodiola crenulata extract has been shown by Tu et al. (2008) to reduce the proliferation of several types of breast epithelial cells ranging from malignant to normal. The malignant cell lines analyzed were the human MDA-MB-231 cell line and the V14 mouse tumor cell line. The effects of *R. crenulata* extract on human immortalized mammary cell line (76N TERT) and true normal epithelial cells (HMECs) were also investigated. ^{3}H-Thymidine assays revealed that the V14 cell line is highly sensitive to the growth-suppressive effects of *R. crenulata* since there was a significant dose-dependent inhibition of thymidine uptake at doses as low as 25 μg/mL. However, MDA-MB-231 cells growth arrested at only higher doses were not used in that paper. Interestingly, HMECs were sensitive to the growth-arresting properties of *R. crenulata*, while 76N TERT cells were resistant. Next, these researchers chose to study the effectiveness of *R. crenulata* at inducing death in these cell lines. They showed that when V14 cells were treated with 50 μg/mL *Rhodiola* there was a decrease in cell confluence and an increase death. Moreover, flow cytometry revealed that after 48 hours in the absence of serum, 75 μg/mL *R. crenulata* induced cell death in V14 cells and 100 μg/mL *R. crenulata* induced cell death in MDA-MB-231 cells, but did not alter cell death in HMECs or 76N TERT cells.

Tu et al. (2008) also studied the inhibitory effects of *R. crenulata* on breast cancer *in vivo*. Mice injected with an estrogen receptor negative breast tumor line derived from a p53 heterozygous mouse were administered 0.5 mg/kg/day or 20 mg/kg/day *R. crenulata* in their drinking water. A delay in the growth of tumors was observed and the occurrence of tumors as prevented in half of the mice treated with *R. crenulata*.

To study the anticancer activity of *Rhodiola sachalinensis in vitro* and *in vivo*, Zhao et al. (2008) used various concentrations of the extract (50–250 μg/mL) on T241 fibrosarcoma cells in culture as well as on T241 fibrosarcoma tumor bearing C57Bl/J mice. *R. sachalinensis* extracts inhibited T241 tumor cell growth in a dose-dependent manner. Moreover, the daily administration of *R. sachalinensis* resulted in a significant suppression of the growth of primary tumors (70.9%) when compared with the control group.

The chemical components of *Rhodiola* can be subdivided into several distinct categories. Relevant to anticancer activity, the phenylethanol derivative salidroside has been shown by Li et al. (2008b) to inhibit the proliferation of adenoid cystic carcinoma cells (SACC-2) with an IC_{50} value of 4.99 ± 0.23 μg/mL. Growth curves showed that the number of salidroside treated SACC-2 cells decreased with extending culture time and immunohistochemistry staining revealed that the number of cells expressing proliferating cell nuclear antigen (PCNA) was lower upon salidroside treatment. In addition, Ming et al. (2005) demonstrated that 1 μg/mL of the flavonoid glycosides found in *R. rosea* (gossypetin-7-*O*-L-rhamnopyranoside and rhodioflavonoside) could inhibit the proliferation of a human prostate cancer cell line (LNCaP).

Rhodiola Blocks Angiogenesis

Angiogenesis is the process of new blood vessel formation and while it occurs under certain normal physiological conditions (including menstrual cycle, pregnancy, and embryogenesis) the pathological process of neovascularization is one of the prominent processes required for cancer growth and metastasis. Skopinska-Rozewska et al. (2008a) were the first group to determine whether the antitumor effects of *R. rosea* are in part due to an angioinhibitory effect. Doses of *R. rosea* ranged from 0 to 400 μg and were administered to mice for three consecutive days following the transplantation of L-1 sarcoma cells. A cutaneous angiogenesis assay revealed that when compared with vehicle treated mice, aqueous extracts of all tested doses significantly decreased the neovascular reaction induced in the skin of syngeneic mice. A similar inhibitory effect was observed when mice were treated with higher concentrations (100–400 μg) of a 50% hydro-alcoholic extract. In addition, the antiangiogenic properties of rosavin, a phenylpropanoid specific to the *rosea* species, were tested in a similar manner and the authors found that at the highest dose (8 μg), rosavin could inhibit neovascularization. To determine whether other *Rhodiola* species and chemical components could also

inhibit angiogenesis, Skopinska-Rozewska et al. (2008b) administered a range of either 0–400 μg *Rhodiola quadrifida* 50% hydro-alcoholic extract or 0–4 μg salidroside to mice for three successive days following L-1 sarcoma cell transplantation. The extract in all tested doses significantly decreased neovascular reaction induced in the skin of syngeneic mice three days after the cells were grafted. A similar inhibitory effect was observed for all concentrations of salidroside.

Rhodiola may prevent angiogenesis by inhibiting the angiogenic factors that are secreted by transplanted L-1 sarcoma cells or by blocking angiogenic signaling for endothelial cells. In order to elucidate the mechanisms by which *Rhodiola* may impede angiogenesis, Skopinska-Rozewska et al. (2008c) repeated their previous experiments and in addition to injecting living murine L-1 sarcoma cells, the researchers injected a separate group of mice with a sonicated homogenate of human kidney cancer tissue which contains a variety of angiogenic cytokines. In both types of experiments, the authors used 400 μg daily does of *Rhodiola* extracts. When the homogenate of human kidney cancer cells were injected into the mice, *R. quadrifida* extracts were unable to block an angiogenic response. These experiments indicate that the attenuation of a neovascular reaction in mice grafted with L-1 sarcoma living cells and fed *R. quadrifida* extracts is likely dependent upon suppressing the production of and/or the release of pro-angiogenic factors by transplanted cells. Interestingly, hydro-alcoholic *Rhodiola kirilowii*, but not aqueous *R. kirilowii* and *R. rosea*, was effective at inhibiting angiogenesis in both types of experiments these findings were attributed to the different chemical compositions of various these particular *Rhodiola* extracts.

THE ROLE *RHODIOLA* SPECIES PLAY IN CANCER PREVENTION

Rhodiola and the Immune System

The immune system plays a paradoxical role in both the progression as well as the prevention of cancer. The basis for this paradox seems to lie in the difference between cancers that can be eliminated easily by immunosurveillance and those that arise in part due to chronic inflammation. Immunosurveillance is vital for the detection and elimination of malignant cells. This process requires both the innate immune system as an early response, including macrophages, mast cells, dendritic cells and natural killer (NK) cells, as well as the more specific second line of defense, the adaptive immune response, made up of T and B lymphocyte populations. Cancers can adapt to and resist the immunosurveillance efforts by a number of mechanisms. For example, cancer cells may downregulate the major histocompatibility complex molecules as well as the secretion of TGF-β which drives the conversion of CD4+ T helper cells to a T regulatory (Treg) or suppressor phenotype. The Treg cells are important in the general immune cell population because they are responsible for controlling autoimmune reactions. However, patients with tumors containing high levels of these Treg cells often have a poorer prognosis due to reduced immune function. Therapeutics aimed at increasing or recovering the immunosurveillance efforts through vaccinations, dendritic cell therapy or interleukin therapy designed to enhance the T cells responses have shown promise in recent years. The various roles the immune system plays in cancer will be discussed below with respect to the published studies on the various *Rhodiola* species. Many species of *Rhodiola* have been tested for their ability to regulate the immune system using both *in vitro* and *in vivo* techniques. The most comprehensive *in vivo* study examined *R. rosea* in the context of three models for inflammation including carragenen-induced paw oedema, formaldehyde induced arthritis, and nystatin-induced paw oedema (Bawa and Khanum, 2009). In these studies, *R. rosea* (250–500 mg/kg body weight delivered intraperitoneally) was shown to reduce the oedema formation as assessed by paw volume and reduce arthritis as determined by serum aspartate amino transferase (AST) and alanine aminotransferase (ALT) values. Moreover, cyclo-oxygenase 2 (COX-2) activity, an inflammatory mediator, was significantly reduced in response to *Rhodiola* treatment. The effect on COX-2 activity is interesting with regards to the role of *Rhodiola* extracts in cancer prevention since transgenic COX-2 expression has been shown to initiate mammary tumor development (Liu et al., 2001)

and inhibitors of COX-2, such as NSAIDs and Celecoxib, have been shown to be protective against certain types of cancer (Harris, 2009). This reduction in COX-2 activity is in agreement with our unpublished *in vitro* studies which suggest that *R. crenulata* can decrease cyclooxygenase activity in breast cancer cells. COX-2 expression is involved in inflammation and is thought to be involved in promoting malignancy by activating both motility and pro-survival pathways.

Rhodiola sp. have also been shown to increase cytokine secretion and immune receptor modulation in several *in vitro* studies. Human peripheral blood mononuclear cells (PBMCs) are easy to access and treatment with *R. imbricata* and *R. algida* have been shown to increase NF-κB, IL-6, IL-10, TNF-α, IL-2, IL-4, IL-6, and IL-10, respectively (Li et al., 2009; Mishra et al., 2009). In addition, analysis of mouse splenocytes treated with *R. imbricata* revealed that the expression of Toll-like receptor-4 (TLR4) and granzyme-B are upregulated. Together, these data indicate that in the absence of antigen stimulation, these two species of *Rhodiola* (at 50 μg/mL) have immunostimulatory effects. Interestingly, at higher concentrations, *Rhodiola* lost its ability to induce these cytokines. Similarly, *R. quadrifida* at low concentrations (5–10 μg/mL) enhanced the *in vitro* bacteriocidal activity of pig leukocyte cultures, but this activity was lost when the levels of *R. quadrifida* were increased to 50 μg/mL. In an *in vivo* study, this same group showed that mice fed aqueous or 50% hydro-alcoholic *R. quadrifida* extracts (400 μg daily dose) for seven days had increased granulocyte activity and enhanced graft versus host rejection (Skopinska-Rozewska et al., 2008d). Supporting a role for increased humoral immunity in cancer patients, AdMax, which includes *R. rosea* as one of the four components, was given to women with stage III/IV ovarian cancer undergoing chemotherapy. The women who took the plant preparation exhibited enhanced T lymphocyte counts and increased IgG as well as IgM when compared with women who did not receive the AdMax (Kormosh et al., 2006). Taken together, these findings suggest that *Rhodiola* extracts might help tumor immunosurveillance.

Nitric oxide is an important immuno-modulator released from macrophages. Macrophages can be activated to become M1 or "killer" macrophages, which are capable of destroying tumor cells through the secretion of nitric oxide. Alternatively, macrophages can also be polarized to the M2 phenotype by exposure to IL-4 or IL-13. These M2 macrophages are known as "healer" macrophages because they play an important role in scavenging, wound repair, and angiogenesis. However, M2 macrophages are frequently found associated with tumors and have been shown to promote tumorigenesis (O'Brien and Schedin, 2009). The M2 macrophages express high levels of arginase I which serves to feed back and lower the secretion of nitric oxide. Thus, the ratio between arginase and inducible nitric oxide synthase (iNOS) expression in macrophages is critical for the balance between the tumor suppressive and tumor promoting properties of macrophages. The expression of iNOS has been shown to be upregulated by *R. sachalinensis* and *R. imbricata* in both RAW264.7 macrophage cells stimulated with LPS and hepatocytes primed with IFN-γ (Mishra et al., 2006; Pae et al., 2001; Seo et al., 2001). It is highly likely that this increased expression of iNOS is critical for the immunomodulatory effects of *Rhodiola* and possibly the cancer preventive effects of *Rhodiola in vivo*.

Further research is needed to determine the effects of the different *Rhodiola* species on tumor immunology. *Rhodiola* may act as an anti-inflammatory agent by reducing chronic inflammation and COX-2 activity which would decrease the chances of tumor progression while increasing immunosurveillance. Several papers have also indicated that *Rhodiola*, or its constituents, can inhibit the secretion of the cytokine TGF-β under different circumstances (Wu et al., 2003; Yin et al., 2009). This activity may also play a role in the immune system and tumor progression, as TGF-β secreted from tumor cells or immune cells is known to polarize the T cells toward the Treg phenotype which leads to a decrease in immunosurveillance.

The Antioxidant Activity of *Rhodiola*

Reactive oxygen species (ROS) are important signaling cues which elicit several different cellular responses. ROS can alter intracellular signaling pathways through modifications of phosphatases,

kinases, and transcription factors, all of which control cell fate and proliferation (Mates et al., 2008). However, excess free radicals are also capable of causing damage to membrane lipids, proteins, and DNA. For this reason, antioxidants have been suggested to play a pivotal role in cancer prevention. Oxidative stress, which is defined as an imbalance between the production and control of ROS, occurs in response to a variety of stimuli and is associated with increased cancer risk. Such stimuli may be environmental, such as exposure to radiation or toxicants, or internal due to chronic inflammation or obesity. Aging and disease states are also often associated with increased levels of ROS by means of decreased scavenging defenses and chronic inflammation, respectively (Khansari et al., 2009; Roberts and Sindhu, 2009).

Exposure to external antioxidants, such as vitamin C, can help to reduce ROS-induced damage but preliminary nontoxic exposure to an agent that induces minimal oxidative or nitrosative stress can be equally effective at reducing cellular damage (Dickinson et al., 2003). This prior exposure causes cells to upregulate their own internal antioxidant defense system eliciting an "adaptive" response to oxidative stress. The classic system involves the regulation of glutathione synthesis, which controls free radicals, or chaperones that can bind misfolded proteins during situations of stress (Panossian et al., 2009). *Rhodiola* has been frequently cited for its antioxidant activity and may be able to both directly scavenge dangerous free radicals as well as upregulate the glutathione synthesis mechanism (De Sanctis et al., 2004; Gupta et al., 2007; Kanupriya et al., 2005; Kim et al., 2006). Whether or not *Rhodiola* extracts accomplish this by acting as an initial stressor, is up for debate. In a *C. elegans* model, 10–25 μg/mL *R. rosea* increased the life span of the animals after an initial stress (Wiegant et al., 2009). This phenomenon was also observed in a *Drosophila* model, where 30 mg/mL *Rhodiola* improved oxidative stress which correlated with increased lifespan (Jafari et al., 2007). Studies in diabetic rodents illustrated that treatment with *R. rosea* at 200 mg/kg/day led to increased liver levels of catalase and superoxide dismutase, as well as glutathione reductase, glutathione peroxidase, and glutathione-*S*-transferase (Kim et al., 2006). Experiments using a rat excision model revealed that *R. imbricata* improved wound healing which was associated with an increase in glutathione and a decrease in lipid peroxide levels in the granulation tissue after the eighth day (Gupta et al., 2007). Histological analysis of the wounded tissue also suggested less edema and fewer polymorphonuclear leukocytes infiltrating in the region which agrees with the anti-inflammatory activity of *Rhodiola*.

In vitro experiments on erythrocytes, macrophages, and neurons treated with oxidants such as hypochlorous acid, *tert*-butyl hydroperoxide, or hydrogen peroxide respectively, have shown that the extracts of various *Rhodiola* species imparts protection against ROS-induced death (Battistelli et al., 2005; De Sanctis et al., 2004; Kanupriya et al., 2005; Mook-Jung et al., 2002). When the mechanism of this protection was examined, they found that the intracellular glutathione levels were rescued in *Rhodiola* treated cultures (De Sanctis et al., 2004; Kanupriya et al., 2005). *R. rosea* was shown to have a high capacity to scavenge singlet oxygen and hydrogen peroxide, as well as the ability to reduce and chelate ferric, and protect protein thiols (Chen et al., 2008). The antioxidative capacity of *Rhodiola* appears to be a conserved effect for the family since a number of different *Rhodiola* species, as well as salidroside, can protect cells against oxidative damage (Yin et al., 2009).

Only a few studies have examined the *in vivo* antioxidant potential of *Rhodiola* in humans. One such study analyzed low-level photon emission as a noninvasive means of indirectly measuring oxidative status. This type of photon emission is related to the electron excitation states in many organisms and correlates with UV-induced stress in humans (Hagens et al., 2008; Laager et al., 2008). Photon emission from the dorsal side of hands was recorded in 10 students before and after treatment with two tablets/day (containing 144 mg of a root extract of *R. rosea*) for seven consecutive days. The findings of this small study indicated that the *Rhodiola* extract caused a significant reduction in photon emission which suggests that *Rhodiola* may be capable of reducing oxidative stress in humans (Schutgens et al., 2009).

In several Russian studies, it has been suggested that *Rhodiola* is able to prevent off-target toxicity elicited by chemotherapeutic agents, including anthracycline antibiotics (adriamycin, rubicin)

(Borovskaia et al., 1988; Udintsev et al., 1992). This is interesting because anthracyclines are known to cause toxicity by increasing the generation of reactive oxygen species (Injac and Strukelj, 2008). Taken together, it seems likely that *Rhodiola* may impart its protective action against the anthracycline-induced toxicity through its ability to regulate antioxidant enzymes and prevent damage in normal tissues.

Rhodiola Protects against the Effects of Radiation

Damage due to irradiation may in part be due to oxidative stress, but may also be the result of complex interactions involved in tissue remodeling and the immune system. Inflammation caused by radiation has been shown to play a role in the induction of signaling pathways which lead to fibrosis, particularly in the lung, and also participates in mortality ratios. Several studies have suggested that *Rhodiola* can act as a radioprotectant. For instance, mice treated with whole body irradiation and treated with *R. imbricata* at 400 mg/kg body weight had an 83% survival rate by 30 days, whereas all of the placebo treated mice had a 100% mortality rate by day 12 post-irradiation (Arora et al., 2005). In the study described above, they also found that irradiated liver homogenates had much lower levels of lipid peroxidation suggesting that the antioxidant properties of *Rhodiola* may be contributing to the increase in survival rate. In addition, these studies showed that there was a dose-dependent increase in superoxide scavenging activity as well as an increased ability to chelate iron. Similar survival protection against radiation effects was observed in a second study examining *R. imbricata* in rodents (Goel et al., 2006). In this study, they showed that the 350 mg/kg body weight of *Rhodiola* given 30 minutes prior to radiation exposure was optimal with both the aqueous as well as the aqua-alcoholic extracts. While neither of these radiation studies have looked closely at radiation-induced fibrosis or immune regulation, a separate study examining the effects of *R. sachalinensis* on liver fibrosis induced by CCL(4) treatment in rats found that this extract had a striking antifibrotic effect (Wu et al., 2003). As in other immune studies examining *Rhodiola*, this extract was shown to reduce ALT and AST levels, as well as TGF-β expression. Moreover, procollagen III and collagen IV levels were reduced and the expression of the Na^+/Ca^{2+} exchanger was reduced. The net effect of this is likely due to the inactivation of TGF-β and is consistent with a more controlled influx of calcium and survival. *R. rosea* may also protect against radiation through its ability to prevent mutations on the basis several Russian and Ukranian studies propose that *R. rosea* has antimutagenic properties (Duhan et al., 1999; Salikhova et al., 1997).

THE ROLE OF *RHODIOLA* IN THE MAINTENANCE OF ENERGY BALANCE AND SURVIVAL PATHWAYS

Rhodiola sp. may be protective in normal tissues through the upregulation of proteins involved in cell survival and bioenergetics. Studies using isoproteranol, which is a drug that induces myocardial toxicity, showed that *R. rosea* could decrease the size of infarction and reduce the levels of 99mTc-pyrophosphate in the heart (Maslov and Lishmanov Iu, 2007). Furthermore, the rate of death was also reduced. This particular study was also able to show an increase in glucose uptake, a decrease in intracellular Ca^{2+}, and an increase ATP synthesis in response to *Rhodiola* treatment. These observations are consistent with the theory that *Rhodiola* may be able to protect nonmalignant cells against death through its ability to increase ATP production, which is an essential process required to drive the ion pumps and generate more glutathione when cells are under assault. Reactive oxygen species have been shown to lower the spare respiratory capacity needed to maintain ATP (Nicholls, 2008). In fact, SOD2 null mice are exceptionally unhealthy due to their inability to deal with stress (Liang and Patel, 2004).

Several papers have shown that *Rhodiola* will increase glucose uptake and increase ATP content in various contexts and cell types. Rats that were stressed as well as exhausted by exercise and subsequently administered *R. rosea* at 50 mg/kg, were able to maintain ATP levels in muscle

mitochondria and increase the duration they were able to exercise (Abidov et al., 2003). Furthermore, salidroside (80 μM) can also increase glucose uptake via AMP-activated protein kinase activation in skeletal muscle cells (Li et al., 2008a). The ability of *Rhodiola* to maintain ATP production under stress may be responsible for many of the effects that we have discussed in the sections depicting the protective aspects of these extracts. This mechanism may also help explain the antiaging effects of *Rhodiola*. Aging is believed to be a significant risk factor for neurodegenerative diseases because with age comes decreased antioxidative capabilities and an associated decrease in the respiratory capacity of mitochondria (Wei et al., 2009).

SAFETY CONCERNS

R. rosea is available at many nutrition stores and through the Internet. It is frequently cited as having minimal side effects. However, a word of caution should be interjected here, as increased dosages are associated with increased side effects. In rodent studies, *R. imbricata* was tolerated up to 1400 mg/kg body weight when injected intraperitonealy (Arora et al., 2005) and 1–2 g/kg was tolerated orally for 14 consecutive days without significant changes in bodyweight, liver, or kidney function (Gupta et al., 2007). In humans, a mild-to-moderate dose (100–300 mg) of *R. rosea* is recommended to relieve daily anxiety and stress with little to no side effects. A dose of 1.5–2 g *R. rosea* standardized for 2% rosavin has been given to humans in clinical trials; however, the risk of side effects increased with dosage. Side effects include drowsiness or difficulty sleeping, irritability, headache, and gastrointestinal discomfort.

A second point of possible concern is that preliminary *in vitro* analyses of estrogen receptor positive breast cancer cell lines have indicated a possible estrogenic or pro-proliferative effect in *R. rosea* and *R. crenulata* extracts. Further research is being performed in this area to evaluate the safety of this plant for this type of breast cancer.

SUMMARY

- Various species of Rhodiola (*R. rosea*, *R. sachalinensis*, *R. imbricata*, and *R. crenulata*) have shown promise in *in vitro* studies on human cells and in xenograft experiments for treating cancer.
- Multiple tumor cell types have been tested and have been found to be sensitive to *Rhodiola* sp. [NK/Ly tumor, Ehrlich's adenocarcinoma (EAC), melanoma (B16), and Lewis lung carcinoma, promyelocytic leukemia cells (HL-60), erythroleukemic cells (K-562), breast cancer cells (MDA-MB-231), fibrosarcoma cells (T241).]
- *Rhodiola* sp. appear to have both growth inhibitory, as well as pro-death properties.
- Salidroside, rosavin, gossypetin-7-*O*-L-rhamnopyranoside and rhodioflavonoside are components which might contribute to the antiproliferative and antiangiogenic properties.
- *Rhodiola* sp. may be useful in cancer prevention through their ability to modulate the immune system, to enhance the antioxidant response, to protect against radiation damage, and to alter the bioenergetics of the cells.
- Multiple *Rhodiola* sp. appear to be able to enhance acute immune responses *in vitro. R. rosea* in particular, has been demonstrated to inhibit chronic immune responses *in vivo.*
- *Rhodiola* sp. has been shown to increase anti-oxidant defense proteins (catalase and superoxide dismutase, glutathione reductase, glutathione peroxidase, and glutathione-*S*-transferase).
- *R. rosea* reduced low-level photon emission in human studies.
- *R. rosea* and *R. imbricata* have been shown to have radio-protective effects.
- Up to 1.5–2 g of *Rhodiola* sp. has been given in human clinical trials, but in general ranges of 100–300 mg are recommended for daily use.
- Side effects include drowsiness or difficulty sleeping, irritability, headache, and gastrointestinal discomfort.

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4 Greco-Arab and Islamic Herbal Medicine and Cancer Treatment/Prevention

Omar Said, Hilal Zaid, and Bashar Saad

CONTENTS

INTRODUCTION

Herbs have been used for thousands of years, either in their crude forms or as herbal teas, syrups, infusions, and powders in the treatment and prevention of all known diseases. Owing to a statement (Hadith) by Prophet Mohammad Peace Be Upon Him (PBUH), "The one who sent down the disease sent down the remedy," and "For every disease, God has given a cure," every Muslim is encouraged to search for those remedies and use them with skill and compassion. With the high prevalence of tumors in modern times, it is worthwhile looking back in history to the views of famous Arab-Muslim scholars in medicine, such as Al Razi (known as Rhazes, AD 860–930), Al Zaharawi (known as Alucasis, AD 936–1013), and Ibn Sina (known as Avicenna, AD 980–1037). Razes described most types of cancers known at his time and suggested several treatments based on his belief that cancer was a result of excess of burned black bile in the affected tissue. Therefore, he recommended evacuation of the organ from black bile by excessive vomiting and laxatives and using cold medications and food. The Andalusian scholar Albucasis was the first who conducted classic removal of the cancerous breast. He recognized that cancer can be treated surgically only in its early stages when complete removal is possible. Like Rhazes and the ancient Greeks, Avicenna understood cancer to be an

extremely difficult disease to treat that was caused by an excess of burned black bile, which created excessive heat in the body. Avicenna believed that if one of the body's humors was out of balance, then all four of them were unbalanced. He distinguished benign tumor from a cancerous one by certain symptoms such as pain, throbbing, and rapid growth. He also noted that cancerous tumors send out "crablike tracks" and occurred more often in "hollow" organs, which is why they were more common in women. Avicenna also stated that cancers often strike muscles, tendons, and lymph nodes. Like Albucasis, he recognized that a "cure was most likely if the cancer was caught at its earliest stage. The first goal should be to halt the cancerous growth."

This chapter presents a systematic review on cancer chemoprevention and treatment in Greco-Arab and Islamic herbal medicine, including historical background, medical innovations introduced by Arab physicians, and commonly used Arab herbal medicines.

HISTORICAL BACKGROUND

Development of Greco-Arab and Islamic Medicine and Pharmacology

The history of Greco-Arab and Islamic medicine can be divided into two phases (Figure 4.1): Greek-to-Arab phase and Arab-Muslim phase. The first phase started in the eighth century when Islam

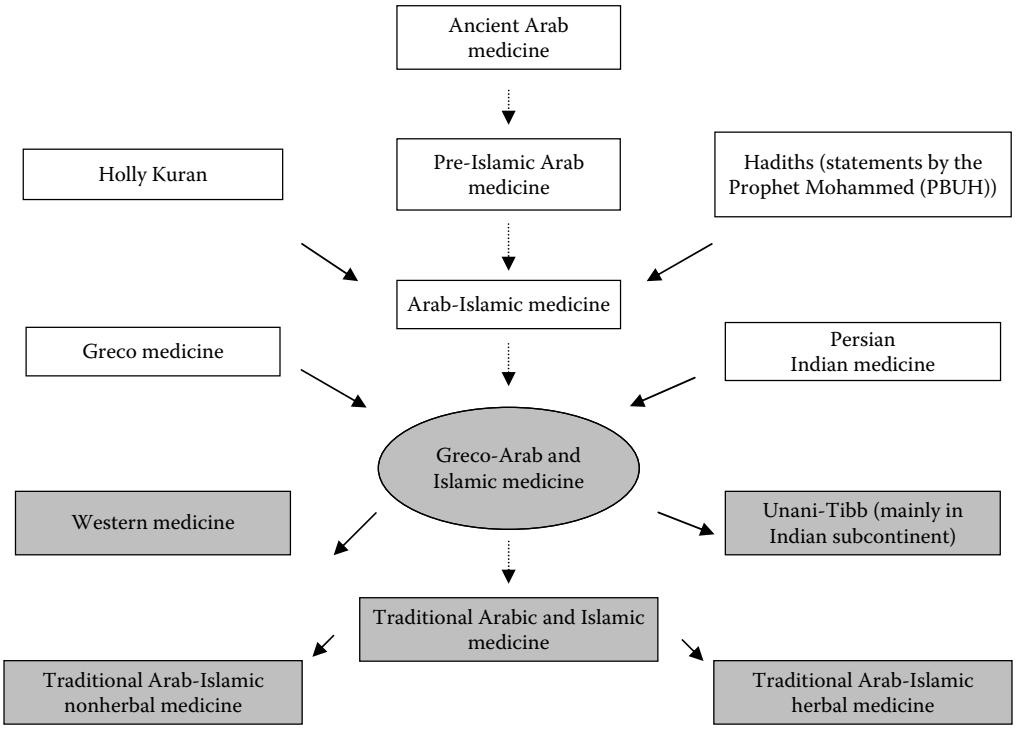

FIGURE 4.1 Development of Greco-Arab and Islamic medicine. The impressive development of the herbal medicine in the Arab-Islamic empire was the result of several facts including: (i) Islam as a religion was openly admiring the field of science and the Prophet (PBUH) demand Muslims to "Seek knowledge from the cradle to the grave … even as far as China." Arab caliphs generously supported the researchers in their endeavors and their activities; (ii) the systematic and massive translation of ancient scientific scripts resulted in the absorption of new concepts of medicine into the Islamic medical heritage; (iii) the conquered new lands gave the Arab-Muslim scholars the opportunity to conduct field research among people from different ethnic groups; (iv) The strong "healthy" competitions between the eastern center (Baghdad in Iraq) and western center (Andalusia in Spain) of the of the Islamic world; (v) the development of chemistry to a level that guaranteed a high level of herbal extract quality or even fraction to treat a certain disease, besides the establishment of new quality control methods.

covered nearly two-thirds of the known world. Greek scientific and philosophical manuscripts as well as Indian and Persian scripts were translated into Arabic. The Khalif Al-Ma'mun in Baghdad became aware of what was to be learned from other civilizations, and therefore, he founded for this purpose, "The House of Wisdom." The most famous of all the translators was Hunayn Ibn-Is'haq. He and his colleagues translated a large number of medical manuscripts by Hippocrates and Galen, philosophical works by Plato and Aristotle, and mathematical works by Euclid and Archimedes.

During the second phase, Arabic became the scientific language. Arabs and Muslims established and promoted their own medical sciences in theories and practices that became highly influential in Western science and teaching. Physicians, whether they were Muslims, Christians, or Jews, under the umbrella of Islam raised the dignity and caliber of the medical profession. During this period, known as the Arab-Islamic golden age, medical sciences rose in esteem from that of a menial calling to the rank of a learned profession. Greco-Arab and Islamic medicine had advanced from ephemeral talisman and theology to tangible hospital wards, mandatory testing for doctors and the use of technical terminology. Baghdad and Cairo had hospitals open to both male and female patients, staffed by attendants of both sexes. These medical centers contained libraries, pharmacies, intern systems, externs, and nurses. There were mobile clinics to reach the disabled, the disadvantaged, and those in remote areas. There were regulations to maintain quality control on drugs. Pharmacists became licensed professionals and were pledged to follow the physician's prescriptions. Furthermore, numerous scientific and medical innovations were introduced by Arab-Muslim physicians during the golden age of the Arab-Islamic civilization. Medical innovations introduced by Arab-Muslim physicians included the discovering of the immune system, the introduction of microbiological science, the separation of medicine from pharmacological science, the introduction of scientific methods to medicine, including animal tests, clinical trials, and quantification. Furthermore, hospitals in the Arab-Islamic world featured the first drug tests, drug purity regulations, and competency tests for physicians. The earliest known medical experiment was carried out by Rhazes (AD 865–925) (Figure 4.2). In order to locate the most hygienic place to build a hospital, he hung pieces of meat in places

FIGURE 4.2 Al Razi, known as Rhazes (AD 860–930), the most free-thinking of the major philosophers of Islam, was born in Rayy (in Persia), where he was well trained in the Greek sciences. He was the chief physician at the Baghdad hospital. Rhazes is well known for his description of smallpox and measles, the first authentic account of these diseases. He developed many chemical apparatus used up to the beginning of the twentieth century, and he wrote a manual that classified chemicals and described procedures for their mixing and preparation that were later adopted by the Western world. He was also the first to distinguish smallpox and measles from each other and to propose the theory of acquired immunity by recognizing that individuals surviving smallpox never get it again. Other contributions to the field of medicine include several innovations in pharmacy and chemistry.

throughout Baghdad and built the hospital where the meat decomposition was the least. Rhazes described in his *Comprehensive Book of Medicine* clinical cases of his own experience and provided very useful recordings of various diseases. He also introduced stool tests and urinalysis. Avicenna's (Ibn Sina, AD 980–1037) treatise *The Canon of Medicine*, was the first book dealing with evidence-based medicine, efficacy tests, and randomized controlled trials (Figure 4.3). Avicenna stresses that only clinical studies in humans can provide the final proof of the efficacy and toxicity (e.g., possible side effects) in man, "The experimentation must be done with the human body, for testing a drug on a lion or a horse might not prove anything about its effect on man." He also discovered the contagious nature of diseases, and described many medical treatments, including clinical trials, risk factor analysis, and the idea of a syndrome in the diagnosis of specific diseases. Avicenna discussed in his second book (on simple drugs, or *Materia Medica*) the nature and quality of drugs (they were each assigned a pair of qualities, cold or warm, dry or moist), and was of the oipinion that the way of mixing them influenced their effectiveness. With respect to the potency of drugs he stated, "You can tell the potency of drugs in two ways, by analogy (qiyas) and by experiment (tajribah). We say experimenting leads to knowledge of the potency of a medicine with certainty after taking into consideration certain conditions." Furthermore, Avicenna was the first to describe the surgical procedure of intubation in order to facilitate breathing, and he also described the "soporific sponge," an anasthetic imbued with aromatics and narcotics, which was to be placed under a patient's nose during surgical operations. Other Arab and Muslim scientists introduced many new ideas, upgraded the knowledge

FIGURE 4.3 Abu Ali al-Husayn ibn Abd Allah ibn Sina (AD 980–1037), known as Avicenna, began his studies in Bukhara under the guidance of several well-known scholars of the time. He studied logic, philosophy, metaphysics, and natural sciences, and gradually developed an interest in medicine. Ibn Sina is remembered in Western medical history as a major historical figure who made fundamental contributions to medicine and the European reawakening. About 100 treatises were ascribed to Avicenna. The best known among them is his 14 volume *The Canon of Medicine*, which was a standard medical text in Western Europe for seven centuries. One of the most important citations from Avicenna concerning psychological and organ diseases: "We have to understand that the best and effective remedy for the treatment of patients should be through the improvement of the power of the human body in order to increase its immune system, which is based on the beauty of the surroundings and letting him listen to the best music and allow his best friends to be with him."

about herbs and their potential medical efficacy and safety, and developed theoretical and practical knowledge on various preparation techniques and administration forms of medicinal plants. Numerous encyclopedias on botany were written, with highly accurate details of medicinal plants (Table 4.1) (Rhazes, 925; Avi Senna, 1037; Ibn AlBitar, 1874; Hitti, 1970; Bin Murad, 1991; Shams Aldeen, 1991; AlTurkimany, 1993; Munke, 1993; Oumeish, 1998; Esposito, 2000; Saad et al., 2005; Pormann and Savage-Smith, 2007; Saad et al., 2008).

Development of Four Humor Theory

The main stream of Islamic physicians and pharmacologists (represented by Al-Razi and Ibn Sina) adopted the medical methodologies of the Greeks, which were based on the four humor theory, whereas other scholars (represented by Al-Zahrawi) adopted a nonphilosophical but practical ones; however, both contributed deeply to the development of the Greco-Arabic medicine.

The establishment of the pharmacologic foundations of the Arabic medicine started during the ninth century in Baghdad (capital city of the Abbasids). In his book *al-Masail*, Hunayn bin Ishaq al-lbadi (AD 809–874) mentions several methods for testing the pharmacological effects of plant extracts on patients. Correct prognosis and diagnosis of the diseases was an extremely important tool for choosing candidate plants for new treatments. For starting research on new plants, earlier Arab scientists first tried to collect ethnobotanical information, along with those from the ancient books, and when they couldn't meet reference criteria they implemented the "humor theory" as a tool for discovering new materials. Hippocrates was the first who applied this idea to medicine, which became strongly accepted as a medical theory through the influence of the writings of Galen. The "humors theory" or the "Doctrine of the Four Temperaments" presumes the presence of four humors in the body: blood, phlegm, yellow bile, and black bile. Each humor has a specific temperament: blood is hot and moist, phlegm cold and moist, yellow bile hot and dry, and black bile cold and dry. They are held in balance when a person is healthy. According to the preponderance of these humors, the temperaments of persons are expressed by the words sanguine, phlegmatic, choleric,

TABLE 4.1
Some of Arab-Muslim Scholars and Their Innovations

Arab-Muslim Scholar	Innovations
Al-kindi (Alkindus) (AD 800–873)	Was the first scholar in history who developed a scale to define the meaning of drug "degrees" in order to allow physicians to quantify the potency of their prescriptions.
Al-Dinawari (AD 828–896)	Is considered as the founder of Arabic botany for his *Book of Plants*, in which he described about 640 plants and described the phases of plant growth and the production of flowers and fruits.
El-Zahrawi (Albucasis) (AD 930–1013)	Is also called "the father of surgery" and considered as one of the greatest physician-surgeon of all times. His book *At-Tassrif* appeared as medical encyclopaedia of 30 volumes.
Ibn Zuhr (Avenzoar) (AD 1091–1161)	Wrote *Al Kitab Al Jami*, about liquids and creams. This book includes 230 herbal medications and gives a full description of the uses of herbs, whether they are roots, seeds, or leaves.
Abu al-Abbas al-Nabati (early thirteenth century)	This Andalusian–Arabian biologist published several books and dictionaries on the use of medicinal plants describing each plant species, the plant parts used, the preparation procedure used for each remedy, and the treatment procedure of certain diseases.
Ibn al-Baitar (AD 1197–1241)	Published *The Book on Drinks and Foods*, which is a collection of different drinks and foods. It is the most prestigious book in the Arabian pharmacopeia; it contains 260 references. The medications were classified in alphabetical order.

and melancholic, respectively. Every person is supposed to have a unique humoral constitution, which represents his healthy state. Self-preservation power called "medicatrix naturae" is responsible for maintaining the healthy humoral balance. Diseases are supposed to result due to an imbalance in the humor composition. Medicines and correct diet are helpful for the body to regain the preserving power to restore normal humor balance.

The drugs themselves, according to the "humor theory," have characteristic temperaments derived from the base elements they are composed of, whereby cure can be achieved by using drugs with antagonistic temperaments against the preponderant humor related to the specific disease. The public understood these terms in a simple way, and the herbs were classified according to their spicy qualities, touch, and temperature (hot, cold, dry, and moist), paralleling the four elements—fire, air, earth, and water. The ancient Greek scientists discussed the qualities and temperaments of only simple drugs (single plants) but not of compound drugs, which play a major and important role in medicine. The concept of "temperament," in the minds of Hippocrates and Galen, was not clear or defined or of the term "degree" which appeared as the sensational effect of the drugs that could not be measured and controlled on the basis of agreed scales of scientific methods. The four degrees of temperament of each drug, which represented the strength of the drug, or maybe, the pharmacological and toxicological effects, were understood as mathematical sum of each degree; so a second degree meant double that of the first degree and a third degree meant three times that of the first degree, and so on, They could not determine the main temperament of the drug mixture with complicated factors, and these resulted in no real development of scientifically based pharmacology.

Al-Kindi (known as Alkindus, AD 803–873), was the first to distinguish and quantify the "drug degrees" concept and developed a mathematical method to determine the strength and quality of compound drugs. This crucial contribution by an extraordinary creative mind was achieved due to the original development in Arab pharmacognosy and chemistry (Jaber Ibn Hayan). In addition, new mathematical concepts (Al Khawarizmi—Algebra and logarithms) allowed pharmacology to stand on scientific foundations and to start a "modern" revolution in pharmacy. Al-Kindi was a pioneer in linking theoretical concepts and applied sciences and deeply influenced the development of biomedical system in the middle ages. The drugs' temperament, according to Al-Kindi, means the chemical properties, toxicity, drug interactions, mechanism of action, and efficacy. The temperaments were determined following their physiological action, examined and observed by physicians in *in vivo* studies and clinical trials using single as well as compound drugs. Correct diagnosis is the ultimate condition for successful treatment and choosing the right medication, and was achieved mainly through examination of urine and stool, pulse reading, naked eye and through the other conventional methods, that is, auscultation, palpation, percussion with the help of some modern tools. Thus, the spot diagnosis was made very easy.

"Hot drugs," for instance, indicate drugs which can stimulate and enhance body temperature in the first and second degrees while enhancing basic metabolic rates. The third "hot" degree is quite toxic and the fourth one is highly toxic with severe side effects and should be controlled using ingredients with antidotal or antagonistic effects based on Al-Kindi methods. Later on, Avicenna developed the humor theory further by adding new concepts such as "secondary humors" (which represent in modern biology intercellular and extracellular fluids) and clarified their roles in pathogenic and healing processes. Development of the theory became an urgent need so as to follow the huge developments in the medical field; and at the same time, he criticized many of Galen's statements and medical concepts regarding theoretical and applied methods.

Wile Dioscorides (first century AD), was the first to initiate a strong base for medicine. He mentioned approximately 500 medicinal substances in his famous book *Materia Medica*. The Arab scholar Ibn al-Baitar (AD 1197–1241) described in his book, *The Comprehensive Book on Materia Medica and Foodstuffs*, more than 1400 medicinal plants, out of which more than 250 species were completely new and never documented before. This great expandsion of knowledge was not, for sure, a result of translation of books from past cultural heritages, but the result of intensive rational researches and developments (Rhazes, 925; Avi Senna, 1037; Ibn AlBitar, 1874; Hitti, 1970; Eltorai,

1979; Bin Murad, 1991; Shams Aldeen, 1991; AlTurkimany, 1993; Munke, 1993; Oumeish, 1998; Esposito, 2000; Saad et al., 2005; Pormann and Savage-Smith, 2007; Saad et al., 2008).

CANCER IN THE GRECO-ARAB AND ISLAMIC MEDICINE

Arab and Muslim physicians identified many types of cancer. These included eye cancer, nasal cancer, tongue cancer, stomach (gastric) cancer, liver tumor and cancer, spleen tumor, nerve tumor, urinary system cancer, kidney cancer, testis cancer, and breast cancer. Kidney cancer was mentioned clearly, for the first time, by Albucasis who had distinguished between acute kidney inflammation and kidney cancer. Both Rhazis and Avicenna described cancer as a tumor that is extremely difficult to treat. They noted that a "cancerous tumor progressively increases in size, is destructive and spreads roots which insinuate themselves among the tissue elements." Avicenna noted that cancers were caused by an excess of black bile, which caused excessive heat in the body. He recognized that if one of the body's humors was out of balance, then all four of them were unbalanced. He said a benign tumor could be distinguished from a cancerous one by certain symptoms such as pain, throbbing, and rapid growth. Avicenna also stated that cancers often strike muscles, tendons, and lymph nodes. He also noted that cancerous tumors send out "crablike tracks" and occurred more often in "hollow" organs, which is why they were more common in women.

Rhazis, Albuqasis, Avicenna, and all the earlier Arab and Muslim scholars realized that a cure is most likely if the cancer was identified at its earliest stage. The first goal of a treatment strategy should be to halt the cancerous growth. They suggested surgical removal if the tumor was small and accessible, and not close to major organs. Avicenna noted, "can be arrested with anything, it can be so by vigorous excision … including all the [blood] vessels supplying the tumor so that nothing of these will be left." Other citation by Avicenna "… and it was told by one of the predecessors that a physician had excised a cancerous breast radically then cancer developed in the other breast. My opinion is that the second breast might have been on its way to cancerization (a dormant cancer) which fits this case and it is possible to be a spread of the material (cancerous from the first breast) and this is more evident (opinion) …." He also recommended that surgery be preceded by purifying the body of excess black bile. This could be achieved by providing a nutritious and balanced diet to the patient to maintain purity and strengthen of his or her organs and immune system. Avicenna most often treated cancer patients with drug remedies. He also advised cancer patients to change their diets. Avicenna also attempted the earliest known treatments for cancer. One method he discovered was the "Hindiba" (*chicorium intybus*), a herbal compound drug which Ibn al-Baitar later identified as having "anticancer" properties and which could also treat other tumors and neoplastic disorders. Another method for treating cancer first described in the *Canon of Medicine* was a surgical treatment. It stated that the excision should be radical and that all diseased tissue should be removed, which included the use of amputation or the removal of veins running in the direction of the tumor (Rhazes, 925; Avi Senna, 1037; Ibn AlBitar, 1874; Hitti, 1970; Eltorai, 1979; Bin Murad, 1991; Shams Aldeen, 1991; AlTurkimany, 1993; Munke, 1993; Oumeish, 1998; Esposito, 2000; Saad et al., 2005; Khan, 2006; Pormann and Savage-Smith, 2007; Saad et al., 2008).

PREVENTION AND TREATMENT OF CANCER IN THE GRECO-ARAB AND ISLAMIC MEDICINE

The holy Koran and Hadith by the Prophet Mohammad (PBUH) have provided Moslems with many ideas of foods that should be included in the ideal diet. As outlined in Chapter 17, the Koran mentions many fruits and vegetables as well as meat, milk, and many spices among the foods Muslims can enjoy and benefit from their nutritional and health values. Among some of the fruits (fresh or dried) and vegetables mentioned in the Koran and Hadith are grapes, citrus, squash, figs and dates. The Prophet (PBUH) mentioned figs and then stated, "If I had to mention a fruit that descended from paradise I would say this is it because the paradisiacal fruits do not have pits … eat

from these fruits for they prevent hemorrhoids, prevent piles, and help gout." Figs are a top source of fiber, as well as potassium and vitamin B6. Fiber results in bulkier stools, which lessen the incidence of constipation, hemorrhoids, and colon cancer. Fiber also lowers cholesterol and the risk of heart disease. Al-Bukhari (AD 810–870) states that melon was one of the fruits most often eaten by the Prophet (PBUH). In fact, melon is one of the best recommendations for health the Prophet (PBUH) has given us. Melon is one of the few fruits and vegetables rich in vitamin C, beta-carotene, and potassium. The foods favored by the Prophet (PBUH) were dates, honey, olive oil, and black seeds. Concerning olive oil, he said "Eat olive oil and massage it over your bodies since it is a holy (mubarak) tree." Black seeds were regarded as a medicine that cures all types of diseases. The Prophet (PBUH) once stated, "The black seed can heal every disease, except death." Dates are mentioned in 20 places in the Koran. The Prophet (PBUH) is reported to have said: "if anyone of you is fasting, let him break his fast with dates. In case he does not have them, then with water. Verily water is a purifier."

More than 100 medicinal plants, tens of animal- and mineral-derived materials were applied in treating cancer. As mentioned above, the main medical stream (including Rhazis and Avicenna) within the Arab-Islamic medical system was restricted to the four humors theory which explained cancer as a result of burned black bile diffusion to the body organs which lead to "boiling" of the black bile in that cancerous site. The first step in cancer treatment was to take the black bile out the body using methods and medications to induce vomiting and diarrhea, besides removal of veins running in the direction of the tumor. As a logical outcome of that theory, all the plant-based medications were plants with cold temperaments in order to antagonize the cancer hotness. Rhazis and all previous scholars had recommended cold plants in the third and fourth degree like *Lactuca seriolla*, *Lactuca sativa*, *Bryonia syriaca*, *Linum usitatissimum*, *Arum palastinum*, *Brassica oleracea*, *Portulaca oleracea,* and *Malva sativa*. The recommended food also was belonging to cold foods like milk, beans, *Hordeum vulgare*, *Portulaca oleracea and Malva sativa*, and chicory. The usage of mineral-derived materials was made mainly to stop and prevent cancer development, in a manner which may remind us of modern chemotherapy. Minerals (zinc, blue vitriol, iron) were applied onto the developed cancer or immediately after removal by surgical operation.

According to Avicenna cancer medications have four purposes: "(1) total arrest of cancer and this is difficult; (2) preventing its progress; (3) preventing ulceration; and (4) treatment of ulceration. And those (medications) aiming at arrest of cancer are those aiming at reversal of what happened through the bad material (atrabile) and preventing its effect on the organ (involved)" Avicenna recommended that these measures should not be of much strength and mobilization (stimulation) "since strong medications increase cancer evil." He indicated that "one should avoid irritant medications. And for this, good medications are: washed (pure) minerals like washed pure tutty mixed with greases (oils) like rose grease (oil) and the oil of yellow gillyflower mixed with it."

For cancer prevention he mentioned "it can be reached by controlling the material (atrabile), improving the diet and reinforcing the involved organ by the known effective medicines, and by using mineral smears like those containing millstone dust and whet-stone dust and from smears taken from a mixture between the stone pounder for aromatics and black head stone moisturized with rose oil and coriander water poured on pounder. And also a dressing with well pounded verjuice is good and useful."

Medication used in the prevention of ulceration were described as "smears that prevent its (cancer) progress provided they will not be irritant; all of them are useful, especially if mixed with the mixture mentioned from lead stone and stone pounder of aromatics. And if added to the total sealing clay or Arminian bole or underground oil (mineral oil?) or houseleek water, ceruse and lettuce juice, or the mucilage of fleawart or ceruse of lead (all) constitute a good preparation. And of great benefit is the dressing with raw (soft) fluvial cancer (crab)."

It is worth mentioning that our ethnobotanical findings show that many of the main herbs currently used in the Arab communities are completely new herbs and do not belong to the herbs used

by classic Arab-Islamic medicine. Some of these new anticancer medicinal plants are not in direct line with the four humors theory (with hot temperaments and not cold like *eryngium creticum* and *ziziphus spina-chrsti*). This may indicate the importance of the nonphilosophic public wisdom and knowledge as a source for potential treatments of cancer.

Due to space limitations we will focus on three widely used herbal products, namely, black seeds, pomegranate, and olive leaf and fruits. Other commonly used medicinal plants and wild edible plants are listed in Table 4.2.

Black Seeds (*Nigella sativa*)

Black seeds are known to have many medicinal properties and have been widely used in Greco-Arab and Islamic medicine for centuries as a protective and curative remedy for numerous diseases. The seeds, known as black seed, black cumin or "Habatul-Barakah" in Arabic (meaning the *seed of blessing*) have long been prescribed in Greco-Arab and Islamic medicine as well as in Indian and Chinese traditional medicine for prevention and treatment of a wide range of diseases, including bronchial asthma, headache, dysentery, infections, obesity, back pain, hypertension, and gastrointestinal problems. It is the black seed referred to by the Prophet Mohammad (PBUH), who once stated, "The black seed can heal every disease, except death." Avicenna refers to the black seed in his *Canon of Medicine*, as the seed that stimulates the body's energy and helps recovery from fatigue and dispiritedness. In the Unani Tibb system of medicine, these seeds are regarded as valuable source of remedy for a number of diseases. The oil from the seeds has been used to treat skin conditions such as eczema and boils and to treat cold symptoms.

Recent research has indicated that black seed contains the ability to significantly boost the human immune system—if taken over time. The prophetic phrase, "hold onto the use of the seed" also emphasizes consistent usage of the seed. Therefore, one important fact is that the black seed should be regarded as part of an overall holistic approach to health and should be incorporated into one's everyday diet. The black seed is traditionally used in eastern Mediterranean as an enhancer of milk production during breastfeeding. This has also been substantiated by studies (Gali-Muhtasib et al., 2004; Salem, 2005; Salem et al., 2005a,b). Black seed (black seeds mixed with toasted flour, toasted sesame, and honey and prepared as cakes) is an excellent form of added nutrition for both mother and the growing child while its immune system boosting properties serve as a natural, safe way to build resistance against illness.

Anticancer Properties of Black Seeds

Black seeds contain pharmacologically active compounds, namely, thymoquinone, dithymquinone, thymohydroquinone, and thymol. These compounds are the main active compounds responsible for the therapeutic effects of black seeds. Numerous preclinical *in vitro* tests indicate that both the oil and the active ingredients of black seeds exhibit anticancer properties. The oil expressed significant cytotoxic properties against cells from various types of human cancer cell lines. Many of the published *in vitro* anticancer evidences of the active ingredients have also been confirmed in different animal cancer models. For example, the growth of Ehrlich ascites carcinoma and Dalton's lymphoma ascites cells was completely inhibited by the active principle fatty acids derived from *Nigella sativa*. In addition, skin carcinogenesis induced in mice by 7,12-dimethylbenz[*a*]anthracene in mice was inhibited by topical application of *Nigella sativa* oil. Furthermore, the onset of papilloma formation was delayed and the mean number of papillomas was reduced. Moreover, α-hederin, another active ingredient of black seeds oil, was also found to show *in vivo* antitumor activity against leukemia and Lewis lung carcinoma, prolonging the lifespan of the tumor-bearing mice. In addition, hepatic tumor in rat induced by diethylnitrosamine or by partial hepatectomy was suppressed by oral feeding with *Nigella sativa* extract. Furthermore, colon carcinogenesis induced by methylnitrosourea or by 1,2-dimethylhydrazine was suppressed *by Nigella sativa* oil. According to recent preclinical studies, the antitumor effects of *Nigella sativa* oil is mediated by

TABLE 4.2
Medicinal Plants Used to Treat Cancer Based on Traditional Arab Medicine[a]

Plant Species	Preparation	Additional Uses
Allium cepa L.	Bulb juice	Diabetes, loss of appetite, liver disease, coughing, external infection
Anetheum graveolens	Seeds	Intestine gas, digestive system, eye inflammations
Artemisia absinthium	Seeds	Intestinal parasite
Arum palaestinum	Leaf	Urinary system
Arum palaestinum Boiss.	Foliage decoction	Internal bacterial infections, poisoning, circulatory system
Astoma seselifolium	Bulb decoction	General tonic, aphrodisiac, increasing appetite
Brassica oleracea L.	Whole plant juice	Respiratory system, asthma, joint inflammation, bacterial infection
Ceterach officinarum	Seeds	Constipation, internal bleeding
Chrysthanemum coronarium	Flower decoction	Fever
Crataegus azarolus L.	Fruit and flower decoction	Cardiovascular diseases, sexual weakness, diabetes
Crocus sativus	Fiber	Constipation, liver diseases, eye inflammations.
Cuminum cyminum	Seeds	Coughing, urinary infections, kidney stones, liver, digestion problems, intestinal gas
Cuscuta campestris	Stem	Urinary system problems
Eryngium creticum	Leaf, seeds	Ulcer, gallbladder and kidney stones
Ficus sycomorus	Fiber, stem sap	Psoriasis, warts
Glycyrrhiza glabra	Roots	Ulcer, cough ing, liver problems, constipation.
Juglans regia	Leaf, bark	Diabetes, asthma, sexual weakness, tooth whitening, fungi
Lens culinaris	Seeds	Inflammation in mouth, skin
Lilium candidum	Flowering parts	Headache
Matricaria aurea	Flower decoction	Stomach, intestine pain, coughing, Anti inflammatory Urinary system
Narcissus tazetta	Bulb, flowers	Lung inflammation, coldness
Nigella sativa	Seeds	Diarrhea, fever, intestinal parasites, vomiting, general tonic, skin diseases
Opium (Papaver somniferum)	Fruit	Insomnia, pain, diarrhea
Pistacia Lentisaues	Seed and leaf	Sexual weakness, jaundice, respiratory problems
Punica granatum	Bark	Diarrhea, dysentery, ulcer, wounds
Quercus calliprinos	Bark, stem and fruit	Fever, ulcer, high blood pressure
Quercus calliprinos Decne	Fruit and bark decoction	Bed wetting, ulcer, diabetes, skin diseases
Quercus ithaburensis Webb.	Stem, bark and fruit decoction	Fever, bed wetting, high blood pressure, ulcer
Sinapis arvensis	Seed, leaf	General tonic, back pain, rheumatism.
Triticum aestivum L.	Shoot decoction	Anaemia, skin disease (seed decoction)
Urtica pilulifera L.	Foliage decoction	Stomach, intestine pain and inflammation, liver disease, bed wetting (seeds)
Vicia faba	Seeds	Skin diseases, whitening
Vinca rosa	Leaf	Diabetes
Viscum cruciatum	Seeds, fruit, foliage decoction	Constipation, rheumatism, back ache
Withania somnifera	Seeds	Wounds
Zea mays L.	Kernel and fibre decoction	Urinary system and stones in kidneys, blood pressure, joint inflammation and weight loss
Ziziphus spina-christi	Seeds, bark, roots	Stomach and intestine pain, hemorrhoids, tooth pain, cholesterol problems, skin diseases

Source: Adapted from Saad, B. et al. (2006). *Evid Based Complement Alternat Med 3*, 433–439; Said, O. et al. (2002). *J Ethnopharmacol 83*, 251–265.

[a] Based on an old literature, field surveys, and traditional medicine.

TABLE 4.3
Effects of Food and Herbal-Derived Compounds in Cancer Chemoprevention

Source	Active Principle	Antioxidant/ Anti-Inflammatory Property	Antimetastasis Effect	Apoptosis Induction	Antiangiogenesis
Olives	Oleuropein	+	+	+	+
Black seeds	Thymoquinone	+	+	+	+
Onion	Quercetin	+		+	+
Garlic	Diallyl sulfide	+		+	+
Tumeric	Curcumin	+		+	+
Figs	Several flavonoids	+	+		
Pomegranate	Several polyphenols	+	+	+	+
Honey	Several active compounds	+	+		
Milk thistle	Silymarin	+		+	+
Chicory	Inulin-type fructans [beta(2,1) fructans]	+	+		+
Bread wheat	Fibers, lignans, isoflavones, and phenolic acids			+	

thymoquinone. Administration of this molecule in drinking water significantly suppressed the benzo[*a*]pyrene-induced forestomach tumor. Using the fibrosarcoma tumor model thymoquinone significantly inhibited the tumor incidence and tumor formation of 2-methyclonathrene-induced soft tissue fibrosarcoma. Using the same tumor model, treatment with black seeds extract 30 days after subcutaneous administration of methyclonathrene reduced fibrosarcoma tumor incidence by about 67%, compared with control tumor-bearing mice, indicating therapeutic potentials of *Nigella sativa*. In addition, oral administration of thymoquinone to mice bearing Ehrlich ascites carcinoma xenograft significantly enhanced the antitumor effect of ifosfamide, coincided with less body weight loss, and mortality rate. Other preclinical observations suggest that suppression of immune cell function associated with chemotherapy, radiotherapy, and late stages in tumor-bearing hosts is mediated, at least in part, by nitric oxide (NO) produced by immature granulocytes that are massively generated under these conditions. Therefore, it is possible that the antitumor effects reported for *Nigella sativa* oil and thymoquinone are mediated by their abilities in scavenging the NO produced by immature granulocytes that are massively generated under these conditions. The impact of black seeds' active compounds, in particular thymoquinone on immature granulocytes under these conditions in the tumor-bearing hosts needs to be explored. In addition, since chemotherapy induces massive expansion of the NO producing immature granulocytes, it might be feasible to follow chemotherapy with thymoquinone treatment that might alleviate the suppressive effects on the immune responses by chemotherapy-induced NO. In addition to the possible antioxidant-mediating antitumor effects of thymoquinone, it is also possible that its antitumor effects is mediated by the ability to suppress leukotriens. Higher levels of these inflammatory mediators have been reported to correlate with tumor progression *in vivo*, and several drugs that are able to block the eicosanoid signaling, both COX-1 and COX-2 pathways, are being tested now in clinical trials. However, the possibility that both the antioxidant and anti-inflammatory effects of thymoquinone mediate its antitumor effects needs to be directly tested by using mice that are knock-out for these mediators.

Taken together, the findings of these studies indicate to the potential of the active ingredients of black seeds oil, in particular thymoquinone, as a powerful chemopreventive agents against several experimental cancer, including forestomach, fibrosarcoma, colon, skin, and hepatic tumors. It

still remains to be elucidated if antitumor effects of black seeds oil and thymoquinone are immune-mediated through modulation of antitumor immune responses (Gali-Muhtasib et al., 2004; Salem, 2005; Salem et al., 2005a,b).

The Pomegranate, *Punica granatum*

The pomegranate, *Punica granatum*, is a fruit-bearing deciduous shrub or small tree native to the region from Persia to northern India and has been cultivated and naturalized over the whole Mediterranean region as well as in other regions since ancient times. The fruit, commonly known as Rumman in the Arab world, can be divided into three structural compartments: seed, juice, and peel.

The pomegranate has long been used in traditional Greco-Arab and Islamic medicine to treat a variety of ailments, including sort throat, inflammation, and rheumatism. The fruit is also used for treating bladder disturbances, strengthening gums, and soothing mouth ulcers. Pomegranates feature prominently in all religions, Judaism, Christianity, Islam, Buddhism, and Zoroastrianism. According to the Qur'an, pomegranates grow in the gardens of paradise. Pomegranates, along with dates and olives, are also mentioned in the following verse from the Holy Qur'an, which speaks of the dues that have to be paid upon each harvest, as well as the evil of wastefulness.

> "And it is He Who produces gardens trellised and untrellised, And date-palms, and crops of different shape and taste and olives, and pomegranates, similar (in kind) and different (in taste). Eat of their fruits when they ripen, but pay the due thereof on the day of its harvest, And waste not by extravagance. Verily, He likes not those who waste by extravagance."

The most abundant polyphenols in pomegranate juice are the hydrolyzable tannins called punicalagins, which are powerful antioxidants. Punicalagins have dietary value as antioxidants; other phytochemicals include beta-carotene and polyphenols catechins, gallocatechins, and anthocyanins. The pharmacological uses of the pomegranate, as was seen with the two other plants of the Qur'an, dates and olives, are numerous. These include use as antioxidants, in hormone replacement therapy, resolution of allergic symptoms, cardiovascular protection, oral hygiene, as ophthalmic ointment, weight loss soap, and as an adjunct therapy to increase bioavailability of radioactive dyes during diagnostic imaging. Pomegranate-mediated antioxidant activity can be considered a means of lowering the threshold for inflammation. Antioxidant activity, as well as suppression of inflammation, may contribute to chemotherapeutic and chemopreventive utility against cancer (Salem et al., 2005a).

Antitumor Properties of *Punica granatum*

Evidences of antitumor properties of *Punica granatum* were found at proliferation, apoptosis, tumor cell invasion, and angiogenesis.

Proliferation

Selective inhibition of tumor cell proliferation but not normal cells is the most desired anticancer effects. In this regard, pomegranate peel extracts have been shown to reduce proliferation of cells from different human cancer cell lines. For instance, significant antiproliferative effects of fermented pomegranate juice and pomegranate peel extract were observed in cells from human breast cancer cells, compared with immortalized normal breast epithelial cells. Furthermore, treatment of cells from androgen-independent PC-3 cell line with acetone extract of whole pomegranate fruits dose-dependently inhibited proliferation, corresponding to changes in the cyclin-dependent kinase (cdk) inhibitor network. In addition, the acetone extract of whole pomegranate fruits in treatment of nude mice implanted with androgen-sensitive human prostate cancer cells resulted in suppression of growth and a significant decrease in serum prostate-specific antigen. Other studies indicate that whole, complex pomegranate products possess potential antiproliferative activity against tumor

cells superior to that of their principle active compounds, again suggesting therapeutic strategies that may depart from the traditional preference for pure single compound.

Despite the aforementioned impressive preclinical work indicating cancer chemopreventive and therapeutic efficacy with limited toxicity, there still remain few well-designed clinical trials measuring the anticancer and other health benefits of pomegranate.

Tumor Cell Invasion

The vast majority cancer deaths arise from the spread of primary tumors. Local invasion and the formation of metastases are clinically the most relevant, but most difficult to target since they are the least well understood. Nonetheless, recent investigations have indicated that pomegranate-derived-components may be capable of suppressing metastasis. For instance, cold-pressed pomegranate seed oil inhibited invasion of estrogen sensitive MCF-7 human breast cancer cells *in vitro* across an artificial Matrigel-TM membrane. In addition, pomegranate seed oil, pomegranate peel extract, and fermented pomegranate juice each resulted in 60% suppression of invasion in Matrigel-TM of human PC-3 androgen negative prostate cancer cells. Synergistic effects were seen when equal amounts of any two of pomegranate seed oil, pomegranate peel extract, or fermented pomegranate juice were combined, such that the combination resulted in a 90% suppression of invasion.

Angiogenesis

The initiation and development of new blood vessels (angiogenesis) are essential to supply oxygen and nutrients for tumor growth and metastasis. Inhibition of tumor blood vessel formation, first suggested by Avicenna who noted that cancer "can be arrested with anything, it can be so by vigorous excision … including all the [blood] vessels supplying the tumor so that nothing of these will be left," is still a promising therapeutic approach for treating solid tumor afflicted patients. Interestingly, recent studies indicate that *Punica granatum* possess antiangiogenic properties. Thus, angiogenesis in chicken chorioallantoic membrane was significantly suppressed by fermented pomegranate juice. Pro-angiogenic vascular endothelial growth factor was potently downregulated in estrogen-dependent breast cancer cells, estrogen-negative breast cancer cells, and immortalized normal breast epithelial cells by fermented pomegranate juice and pomegranate seed oil (Salem et al., 2005a).

Apoptosis

Apoptosis is an early and a useful marker for predicting anticarcinogenesis effects of potential drugs. Aqueous pomegranate peel extract induced DNA fragmentation and suppression of growth of cells from two human Burkitt's lymphoma cell lines. Pomegranate seed oil increased the occurrence of apoptosis by 54% in estrogen receptor negative, metastatic human breast cancer cells, compared to tocopherol, a known apoptotosis-inducing compound. Fermented pomegranate juice and peel extract have also been shown to induce apoptosis in two androgen receptor negative human prostate cancer cell lines PC-3 (highly metastatic cell line) DU-145 (slower growing cell line). These effects were at least partially mediated by capsase enzyme, suggesting involvement of inflammatory processes in executing the apoptotic cascades. Capsase activation in cells from the PC-3 cell line by acetone extract of whole pomegranate fruits correlates with downregulation of proapoptotic factors Bax and Bak and downregulation of antiapoptotic factors Bcl-XL and Bcl-2. Similarly, acetone extract of whole pomegranate fruits reduced expression of cyclins (D1, D2, and E) and cyclin-dependent kinase (CDK). Taken together, both the hydrophobic and hydrophilic pomegranate fractions appear to possess selective apoptotic potential with respect to different hormone-independent cancer cell lines, suggesting chemotherapeutic potential for compounds originating from each of these pomegranate compartments.

Chemopreventive Properties of *Punica granatum*

In mouse mammary organ culture, cold-pressed pomegranate seed oil reduced the tumor occurrence in 7,12-dimethyl-benz[*a*]anthracene treated cells by about 87%. In addition, topical exposure

to pomegranate seed oil significantly decreased tumor incidence and multiplicity in female CD-1 mice with skin tumors induced by 7,12-dimethyl-benz[*a*]anthracene and subsequently promoted by 12-*O*-tetradecanoylphorbol 13-acetate. Similarly, topical pre-treatment with acetone extract of pomegranate fruits prior to 12-*O*-tetradecanoylphorbol 13-acetate applications in 7,12-dimethyl-benz[*a*]anthracene-treated CD-1 mice reduced the tumor incidence by about 70% and increased the latency of tumor development by five weeks. Pomegranate seed oil has also been shown to reduce both the incidence and multiplicity of colon tumors in rats treated with carcinogen azoxymethane. In addition, various preclinical *in vitro* studies reported that the anticancer effects of extracts and active compounds may be mediated through modulation of cell differentiation, cell cycle, and intracellular enzymes. For example, cell cycle changes were seen after treatment of human Burkitt's lymphoma cells to pomegranate peel extract and human monocytic leukemia cells to pomegranate seed oil. Mechanisms for these effects likely involve modulation of cell signaling molecules in the cell cycle machinery. Other studies showed that differentiation of cells from the promyelocytic human leukemia cell line (HL-60) is potently promoted by pomegranate peel extract and fermented pomegranate juice, whereas the ethyl acetate extract of fresh, unfermented pomegranate juice has little effect. Differentiation may possibly figure in the observed anticancer effects of pomegranate extracts in other cell lines, including breast and prostate (Salem et al., 2005a).

Olive Oil and Olive Leaf

The olive tree, *Olea europaea*, is an evergreen tree of the family *Oleaceae* and is native to the Mediterranean basin. There are thousands of ancient olive trees throughout the historic Palestine. Specifically, seven giant olive trees in the Galilee region have been determined to be over 3000 years old. All seven trees continue to produce olives.

Olive oil and olive leaf are cited in the Bible as a natural healer: "The fruit thereof shall be for meat and the leaf thereof for medicine." Prophet Muhammad (PBUH) (AD 570–632) said, "Eat olive oil and massage it over your bodies since it is a holy (mubarak) tree." He also stated that olive oil cures 70 diseases. In the Arab-Islamic world, olive oil has been commonly used in cooking, cosmetics, pharmaceuticals, and soaps and as a fuel for traditional oil lamps.

Medicinal plants and dietary factors influence carcinogenesis in a variety of tissues. A diet rich in fruits and vegetables has long been suggested to correlate with reduced risk of certain epithelial malignancies, including cancers in the lung, colon, prostate, oral cavity, and breast. In addition, there are interrelationships between diet, environmental factors, and genetics that can affect cancer risk. Potential chemopreventive agents against cancer development can be found among nutritive and/or nonnutritive compounds in inedible and edible plants. For instance, the incidence of cancer of the large bowel, breast, endometrium, and prostate is lower than in Scandinavian countries, the United Kingdom, and the United States. These forms of cancer have been linked to dietary factors, particularly low consumption of vegetables and fruits, and to a certain extent, high consumption of meat. The traditional Mediterranean diet is characterized by high consumption of foods of edible plant origin, relatively low consumption of red meat, and high consumption of olive oil, which in several studies has been reported to be more beneficial against cancer than other forms of added lipids. Although estimates can only be crude, it can be calculated that up to 25% of the incidence of colorectal cancer, 15% of the incidence of breast cancer, and 10% of the incidence of prostate, pancreas, and endometrial cancer could be prevented if the populations of highly developed Western countries could shift to the traditional healthy Mediterranean diet. Cancer prevention potential of Mediterranean diets based mainly on olive tree products is known. The major component of the leaves and unprocessed olive drupes of *Olea europaea* is oleuropein and the majority of polyphenols found in olive oil or table olives are derived from its hydrolysis. Oleuropein is a novel, naturally occurring antioxidant compound, which may possibly be used to prevent cancer and cardiotoxicity induced by doxorubicin.

Anticancer Properties

Oxidative stress has been found to increase cancer occurrence and consumption of antioxidants (found in olive oil, fruits, and vegetables) is believed to reduce the risk of carcinogenesis. Anticancer activity of olive oil is associated with its high content of antioxidants, for example, hydroxytyrosol, tyrosol, secoiridoids and lignans. In addition the anticancer effects are attributed to olive-derived compounds deemed to be anticancer agents (such as squalene and terpenoids).

In vitro investigations have found that olive oil phenols are potent antioxidants, which may provide potential chemoprotective properties. Hydroxytyrosol was found to induce apoptosis, to arrest cell cycle progression at the G1 phase, to protect cells from hydrogen peroxide-induced damage, and DNA from peroxynitrite-induced damage. In addition to antioxidant properties, oleuropein have been found to exhibit antiangiogenic effects and to inhibit cell growth, motility, and invasiveness. Furthermore, rapid tumor regression was observed when mice were given 1% oleuropein in drinking water. Saturated animal fats and polyunsaturated plant fats in the diet have been implicated in colon, breast, prostate, and ovarian cancers. The substitution of olive oil in the Mediterranean diet may explain its apparent cancer chemopreventive effects.

When olive oil was compared with other oils, it was found that fried olive oil has a protective effect against colon cancer. This agrees with data that unheated olive oil is beneficial in protecting against colon cancer. The heterocyclic amines (HCA) produced when protein-containing food is fried have been found to induce breast, colon, and pancreatic cancer in rats. When olive oil is used for frying, fewer HCAs are produced than when oils high in polyunsaturated fatty acids are used. Using specific cell lines, they investigated processes involved in cancer initiation, promotion, and metastasis—the three main stages in cancer development and concluded that olive oil phenols exert beneficial effects in all three stages. The oil extract was shown to reduce DNA damage (initiation), increase barrier function (promotion), and reduce cell invasion of surrounding tissue (metastasis) (Pasteur, 1858; Fito et al., 2007; Covas, 2008; Goulas et al., 2009).

Garlic and Onion (*Allium sativum* L. and *Allium cepa* L.)

Onion (*Allium cepa* L.) and garlic (*Allium sativum* L.), among the oldest cultivated plants, are used both as food and for medicinal applications. In fact, these common food plants are a rich source of several phytonutrients recognized as important elements of the Mediterranean diet, but are also used in the treatment and prevention of a number of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, type 2 diabetes and hypertension. Scientific research on garlic started in the nineteenth century with the work of Louis Pasteur, who in 1858 first noted antibacterial properties of garlic (Pasteur, 1858).

The association between consumption of *Allium* vegetables and risk for cancer has been assessed in several epidemiologic, mainly case–control studies, which were the first to show the protective effect of garlic and onion against cancer. For instance, death (attributed to stomach cancer) was 10-fold higher in a high-risk area where the garlic consumption is less than 1 g/day compared to the low-risk area (20 g/day) (Mei et al., 1982; Takezaki et al., 1999). Similar studies in Netherlands had also attributed the low risk for colorectal, breast, and lung cancers to onion and garlic consumption (Dorant et al., 1996). Moreover, tumor cell [including human, lung, skin and colon tumor cell lines, human neuroblastoma cells, human and murine melanoma cells, and human prostatic carcinoma cells (Welch et al., 1992; Takeyama et al., 1993; Sundaram and Milner, 1996; Sakamoto et al., 1997)] proliferation inhibition by organosulfur compounds *in vitro* has supported the *in vivo* and the case–control studies.

Onion is believed to be protective aginst cancer and to reduce the risk of cancer especially if consumed at high doses. Onion contains several active compounds, for example, flavonoids (like quercetin) and sulfur compounds. The protective effect of these compounds against cancer was tested *in vivo* (Bianchini and Vainio, 2001; Arnault and Auger, 2006). Onion and garlic organosulfur compounds when administered to mice 2–4 days prior to carcinogen challenge. Those compounds

inhibited pulmonary adenoma formation. Moreover, garlic allylic compounds induced increased glutathione-*S*-transferase (GST) activity in the mice organs especially the forestomach (Sparnins et al., 1988). Organosulfur compounds modulate the activity of several metabolizing enzymes that activate (cytochrome P450 genes) or detoxify (glutathione-*S*-transferases) carcinogens and thus inhibit the formation of DNA adducts in several target tissues.

The protective effect of *Allium* vegetables against tumor proliferation, angiogenesis, and cell cycle arrest and apoptosis is attributed mainly to its organosulfur compounds. For instance, garlic is known to be protective against cancers, especially gastric cancer. The active compounds in garlic responsible for its anticancer effects are allicin and diallyl disulfide (Arnault and Auger, 2006). For further information regarding the mechanism of these compounds in tumorgenesis and proliferation, the reader is directed to Arnault and Auger (2006), Bianchini and Vainio (2001), Colli and Amling (2009), and Gonzalez and Riboli (2006).

Bread Wheat (*Triticum aestivum* L.)

Most of the studies that had investigated the roll of *Triticum aestivum* L (bread wheat) in cancer, for example, colon cancer, were done indirectly as a cause-effect study. For instance, several fiber food sources were tested in animals and in humans, and wheat bran has been one of the most effective in protecting against colon cancer (Kritchevsky, 1999). Similarly, it was suggested that folic acid in wheat bread could prevent colon tumorigenesis (Omar et al., 2009). Lignans (found in wheat) are also thought to be involved in cancer prevention by wheat bran in mice probably by apoptotic mechanisms (Qu et al., 2005). In fact, the protective effect of the bread wheat against colon tumurgenisis could be due to its high fiber content and cell wall byproducts that contain many bioactive components such as vitamins, lignans, isoflavones, and phenolic acids. These chemicals are able to act as antioxidants or by other mechanisms related to inhibition of tumor progression (Johnson et al., 1994).

Eryngium creticum and *Ziziphus spina-christi*

Recently we screened several plant extracts for their effect on cell proliferation and induction of apoptosis using different human colon cancer (HT-29, Colo-320) cell lines. Treatment of the cells with *Eryngium creticum* and *Ziziphus spina-christi* extracts induced significant inhibition of DNA synthesis and affect cell survival. These effects were found to be dose and time dependent. We have compared the results obtained by the plant extracts to the results obtained using drugs that were used in the treatment of human cancer (topoisomerase inhibitors). The plant extracts (as crude) decreased significantly the cell proliferation in the range of 0.5–1 mg/mL (by 80–90%) as well as the topoisomerase inhibitors in the range of 1–10 μM concentration. Moreover, our results indicated that treatment of the cells with these extracts induces apoptosis which was detected by DNA fragmentation, Tunel assay, and morphological changes.

CONCLUSION

Despite the rapidly increasing understanding of the molecular and cellular processes, such as gene and protein expression, apoptosis, angiogenesis, signal transduction involved in carcinogenesis, the morbidity of this epidemiologic disease is still rising. Several drugs are used to treat and prevent the development of tumorgenesis. However, these treatments are not always effective and usually are accompanied by side effects. Hence, alternative treatment, for example, herbal plants is a potential safe candidate for use and treatment of several diseases including cancer. Several studies have been conducted *in vivo* and *in vitro* to evaluate herbal plants efficacy on carcinogenesis treatment. Care, however, is needed when extrapolating *in vitro* data to *in vivo* models because it cannot be assumed that the effects seen when cells are exposed directly to active compounds that would be candidate chemopreventive agents will be seen when they are consumed in the diet. We should

investigate whether they are capable of distribution throughout the body when they are absorbed after ingestion. Moreover, care should be taken when extrapolating *in vivo* experiments carried on animal models as Avicenna stated "The experimentation must be done with the human body, for testing a drug on a lion or a horse might not prove anything about its effect on man."

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5 Indian Vegetarian Diet and Cancer Prevention

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INTRODUCTION

Despite significant advances in the fields of genetics, molecular biology, diagnosis, and treatment, cancer is still the second leading cause of death worldwide (Mathers et al., 2001). According to the recent available information, in the year 2002, excluding the nonmelanoma skin cancers, there were more than 10 million new cases of cancer recorded globally, with nearly 7 million cancer deaths (WCRF/AICR, 2007). Projections are that by the year 2020, these figures will increase to over 16 million new cases, with 10 million deaths. In 2030 there may be more than 20 million new cases of cancer, with 70% of cancer deaths in the low-income countries, which have minimal resources to treat it (WCRF/AICR, 2007; Mackay et al., 2006). All these observations suggest that there is an urgency to work toward educating people on the causes of cancer, its detection, and prevention and also to design interventional strategies that are inexpensive and affordable to the common man (Arora, 2009).

Although cancer is of genetic origin, with several genomic changes involved and accrued during both initiation and progression stages, the incidence, the cancer types, patterns, trends, and projections vary greatly in different parts of the world (American Cancer Society, 1996; WCRF/AICR, 2007). Scientific studies performed in the recent past have helped us understand that the risk factors for carcinogenesis include sedentary lifestyle, alcohol consumption, obesity, smoking, and ingestion of heterocyclic amines and aromatic hydrocarbons (WCRF/AICR, 2007). Of these, the last three initiate and accelerate carcinogenesis, while regular exercise and judicious diet rich in fruits and vegetables prevent or retard them (American Cancer Society, 1996; WCRF/AICR, 2007).

DIET AND CANCER

Almost 25 centuries ago, Hippocrates remarked, "Let food be thy medicine and medicine thy food" (Aggarwal et al., 2003, 2008, 2009). The role of diet in cancer etiology is supported by results from preclinical studies with animals, international studies that show a large divergence in human cancer occurrence rates between countries, and studies among the immigrants who had a change in their dietary pattern and habits from when they were in their native countries (Kolonel, 1988; Donaldson, 2004; Anand et al., 2008). Reports suggest that the diet was linked with cancer risk of about 30% in the Western countries, while in developing countries it was around 20%, making diet second only to tobacco as a preventable cause of cancer (WCRF/AICR, 2007).

When compared with the Western countries, the incidences for colorectal, prostate, and lung cancers are one of the lowest in India (Sinha et al., 2003). A retrospective analysis performed on cancer mortality in Canada among the European, South Asian, and Chinese origin of the Canadian populations showed that deaths due to cancer was less in the South Asian group (Sheth et al., 1999). Both these studies suggest that this may be partly due to the diet which consists mostly of plant foods and is low in fat, while the Western diet which consists of large amounts of animal foods, is high in fat, and is lower in fiber and complex carbohydrates (Donaldson, 2004; Sengupta et al., 2004). Western meal is centered on at least one meat dish, while vegetables are the main part of South Asian dishes (Sengupta et al., 2004).

THE INDIAN DIET AND CUISINE

The Indian cuisine is a blend of both vegetarian and nonvegetarian elements. Over the centuries it has been influenced by the Arab and Chinese traders and conquerors such as the Greeks, Persians, Mongolians, Turks, British, French, and Portuguese. Today, in India, vegetarians form a nonhomogenous group consisting of semivegetarians (plant food, dairy products, eggs, and fish), lacto-ovo vegetarians (plant food, dairy products, and eggs), and vegans (plant food only). The Indian diet is very diverse. The food consists of the whole grains like wheat, rice, barley, corn, sorghum, millet, rye, and oats, which contain chemopreventive antioxidants such as vitamin E, tocotrienols, phenolic acids, lignans, and phytic acid (Miller et al., 2000).

Indian bread (made of wheat) in the form of chapati, phulka, puri, roti, and paratha is the staple diet of the northern and western India and is eaten with raw onions, chillies, or curries. Rice is the staple diet in the southern and eastern regions of India and is mostly eaten with curry. At times, special preparation like biryani, pulao, kichidi, vangibath, onion rice, jeera rice, tomato bath, coriander rice, curd rice, venpongal, sakkare pongal, mint rice, spinach rice, and so on, are prepared using specific spices and vegetables. Fermented rice dishes like idli and dosas are also an integral part of the south Indian diet and are normally prepared for breakfast. Rice congee (rice stew) is another simple dish prepared normally for a light meal for aged and sick people (Ray, 2005).

Both rice and wheat contain energy-giving complex carbohydrates, mostly in the form of starch and some dietary fiber. Whole grains contain minerals like calcium, magnesium, potassium, phosphorous, selenium, manganese, zinc, and iron and are high in vitamins B and E, phenolic compounds, phytoestrogens (lignans), antioxidants, and fibers (Marquart et al., 2002). The antioxidant content of whole grains is less than that of some berries but is greater than that of common fruits or vegetables (Miller et al., 2000).

The whole-grain intake is reported to reduce the risk of several types of cancers by 30–70%, including those of the oral cavity, pharynx, esophagus, gallbladder, larynx, bowel, colorectum, upper digestive tract, breasts, liver, endometrium, ovaries, prostate gland, bladder, kidneys, and thyroid gland, as well as lymphomas, leukemias, and myeloma (Adom and Liu, 2002; Anand et al., 2008). Several potential mechanisms have been suggested for the chemopreventive effects of grains of which the prominent is on the role of insoluble fibers, a major constituent of whole grains, in reducing the risk of bowel cancer (Eastwood and Kritchevsky, 2005). Additionally, the insoluble

fibers undergoes fermentation, thus producing short-chain fatty acids such as butyrate, which is an important suppressor of tumor formation (McIntyre et al., 1993).

The wheat bran fibers are reported to be better producers of short-chain fatty acids acetate, propionate, and butyrate. The presence of short-chain fatty acids lowers the colonic pH and inhibits the conversion of primary to secondary bile acids, which stimulate colonic cell proliferation and are thought to promote carcinogenesis. Butyrate is the most studied of all the short-chain fatty acids and is reported to mediate its chemopreventive effect by inhibiting histone deacetylase, which subsequently causes a transcription of the active P21WAF1/CIP1 gene, an inhibitor of cyclin-dependent protein kinases, and blocks the aberrant cell cycle (Jeanteur, 1999).

Recently, Scharlau et al. (2009) have reported that butyrate have differential effects on colon cells and was dependent on the different stages of cancer. In the primary colon cells, butyrate elevated the levels of drug metabolizing enzymes glutathione-*S*-transferase A2 and T2 (GSTA2, GSTT2) and catalase (CAT); the GSTM3, GSTT2, and MGST3 in the benign adenoma cells (LT97); and the GSTA4, GSTP1, GSTM2, and GSTT2 in the established colon cancer cells HT29. Butyrate augmented the protein levels of different GST isoforms and total GST enzyme activity in HT29 cells, while in the LT97 cells they were slightly reduced. The possible mechanisms of GST activation in HT29 cells may be mediated by increased histone acetylation, phosphorylation of ERK, inhibition of histone deacetylases, and modulation of MAPK signaling, which contributed to the reduction of DNA damage by hydrogen peroxide or 4-hydroxynonenal in butyrate-treated colon cells (Scharlau et al., 2009).

Fibers also increase the fecal bulk, decrease transit time, and may possibly bind carcinogens and bile acids, which overall provides chemopreventive effects by diluting and decreasing the contact and interaction between carcinogens and the intestinal epithelium (Jeanteur, 1999). Wheat and rice contain free, soluble conjugated, and insoluble phenolics. The antioxidant effect was observed to be the highest in the bound phytochemicals, which survive the gastric and intestinal digestion to reach the colon and possibly exert the chemopreventive effects (Adom and Liu, 2002).

Whole grains also mediate favorable glucose response, which is protective against breast and colon cancers (Slavin et al., 2001). Tocotrienols, a constituent of rice bran oil, palm oil, grains like barley, oats, and rye are also reported to repress NF-κB activation induced by most carcinogens, thus leading to suppression of the various genes linked with proliferation, survival, invasion, and angiogenesis of the tumors (Ahn et al., 2007; Anand et al., 2008). Tricin, a rice bran constituent is also reported to inhibit the cyclooxygenase enzymes and retard intestinal carcinogenesis in the APCmin mice (Cai et al., 2005).

Pulses are other important ingredients in the Indian vegetarian cuisine. The most important are the chickpea (*Cicer arietinum*), pigeon pea (*Cajanus cajan*), lentil (*Lens culinaris*), black gram (*Vigna mungo*), green gram or mung bean (*Vigna radiata*), lablab bean (*Lablab purpureus*), moth bean (*Vigna aconitifolia*), horse gram (*Dolichos uniflorus*), pea (*Pisum sativum* var. arvense), grass pea or khesari (*Lathyrus sativus*), and cowpea (*Vigna unguiculata*). Recently, in a hospital-based case-control study, Sumathi et al. (2009) observed that the consumption of pulses caused a 55% reduction in stomach cancer, which is the third most common cancer in southern India. This study suggests that the pulses have beneficial effects in preventing the cancer of the stomach (Sumathi et al., 2009).

Phytochemicals like daidzein, genistein, and equol present in leguminous plants have been observed to possess antiproliferative activities. Some studies have shown an inverse correlation between the cancer incidence and isoflavone-rich soy-based diet (Anand et al., 2008). Genistein and related isoflavones are reported to inhibit cell growth or the development of chemically induced cancers in the breast, stomach, bladder, lung, prostate, and blood (Anand et al., 2008).

Vegetables and Fruits in the Indian Diet

Vegetables and fruits make up a huge component of the Indian diet. Every meal includes at least one or two main vegetable dishes. Vegetables like the drum stick (*Moringa olifera*), bitter gourd

(*Momardica charantia*), bottle gourd (*Lagenaria siceraria*), snake gourd (*Trichosanthes anguina*), ash gourd (*Benincasa hispida*), cucumber (*Cucumis sativus*), pumpkin (*Cucurbita maxima*), gherkin or gooseberry gourd (*Cucumis sativus*) different varieties of spinach, yam (*Dioscorea villosa*), potato (*Solanum tuberosum*), tomato (*Lycopersicom esculantum*), beetroot (*Beta vulgaris*), carrots (*Daucus carota*), turnip (*Brassica rapa*), radish (*Raphanus sativus*), okra (*Abelmoschus esculentus*), peas (*Pisum sativum*), brinjal or eggplant or aubergine (*Solanum melongena*), and so on, are also used in numerous traditional dishes.

Fruits like mango (*Mangifera indica*), banana (*Musa balbisiana*), kagzi lime (popularly known as lemon; *Citrus latifolia* or *Citrus aurantiifolia*), ber (Chinese date; *Rhamnus zizyphus*), amla (*Phyllanthus emblica*), phalsa (*Grewia asiatica*), jackfruit (*Artocarpus heterophyllus*), bael (*Aegle marmelos*), karonda (Christ's Thorn; *Carissa carandas*), bimbli (*Averrhoa bilimbi*), star fruit (*Averrhoa carambola*), kokam (*Garcinia indica*), jamun (*Eugenia jambolana* or *Syzygium cumini*), orange (*Citrus sinensis*), litchi (*Litchi chinensis*), sapota (*Pouteria sapota*), guava (*Psidium guajava*), apple (*Malus domestica*), pineapple (*Ananas comosus*), cashew (*Anacardium occidentale*), grapes (*Vitis vinifera*), avocado (*Persea americana*) pomegranate (*Punica granatum*), papaya (*Carica papaya*), custard apple or sugar apple (*Annona squamosa*), fig (*Ficus carica*), and strawberry (*Fragaria virginiana*) are also a regular part of the Indian diet.

Results from epidemiological, experimental, and clinical studies have shown a direct correlation of consumption of vegetables and fruits to the decreased incidence of various cancers. The numerous bioactive compounds from the vegetables and fruits contribute to beneficial health effects and prevent cancers of the stomach, esophagus, lung, oral cavity and pharynx, endometrium, pancreas, and colon (Steinmetz and Potter, 1996; de Kok et al., 2008). The types of vegetables or fruits that most often appear to be protective against cancer are allium vegetables (onions and garlic), cruciferous vegetables (broccoli, cabbage, Brussel sprouts), carrots, green vegetables, and tomatoes (Steinmetz and Potter, 1996; Sengupta et al., 2004).

In India, consumption of onions is the highest. It is eaten raw (especially with Indian bread) or cooked in curries in almost all traditions and cultures (Izzo et al., 2004; Sengupta et al., 2004). Onion is also a highly valued herb in the Indian system of Medicine, Ayurveda. Long-term usage gives immense health benefits, which include antiplatelet activity, antithrombotic activity, antiasthmatic, antibiotic effects, and chemopreventive effects. Epidemiological studies have shown that in central Georgia where Vidalia onions are grown, mortality rates from stomach cancer are about one-half the average level for the United States (Craig, 2003).

Onions are rich in two classes of chemicals: the flavonoids and the alk(en)yl cysteine sulphoxides (ACSOs). Two flavonoid subgroups are found in onion: the anthocyanins, which impart a red/purple color to some varieties and flavanols such as quercetin and its derivatives responsible for the yellow and brown skins of many other varieties (Craig, 2003). The flavonoid quercetin is found in high concentration and is reported to prevent the colon against the carcinogens and also to reduce the growth of tumors in animals (Griffiths et al., 2002; Gunatillake, 2005). The other class of compounds—the ACSOs, the precursors for flavor—when cleaved by the enzyme alliinase generate the characteristic odor and taste of onion. The downstream products are a complex mixture of compounds including thiosulphinates, thiosulphonates, mono-, di-, and tri-sulphides (Griffiths et al., 2002). The thiosulfinates exhibit antimicrobial properties. Onion is effective against many bacteria including *Bacillus subtilis*, *Salmonella*, and *E. coli*. Onions are also a rich source of fructo-oligosaccharides. These oligomers stimulate the growth of healthy bifidobacteria and concomitantly suppress the growth of potentially harmful bacteria in the colon (Craig, 2003; Gunatillake, 2005).

Cruciferous vegetables like cabbage, cauliflower, broccoli, and Brussels sprouts are common. They are rich sources of glucosinolates, a biologically inert compound that on hydrolysis yields a range of bioactive compounds such as isothiocyanates, thiocyanates, nitriles, cyanoepithioalkanes, and indoles. Sulforaphane [1-isothiocyanato-4-(methylsulfinyl)-butane], is one of the well studied isothiocyanates and is reported to effect cancer prevention through stimulation of

multiple pathways. They induce cytoprotective genes mediated by Nrf2 (NF-E2 related factor 2) and AhR (arylhydrocarbon receptor) transcription factors, repress the NF-κB (nuclear factor-κB) activity, modulate AP-1, inhibit histone deacetylase, inhibit cytochrome P450, and induce apoptosis in mutated, preinitiated, and preneoplastic cells (Keum et al., 2004). They also alter the gene expression through modification of critical thiols in regulatory proteins such as Keap1 (Kelch-like ECH-associated protein 1) or IKK (IκB kinase), causing activation of Nrf2 and inactivation of NF-κB, respectively. Additional regulatory mechanisms of Nrf2 include the different signaling kinase pathways (MAPK, PI3K, PKC and PERK) as well as other nonkinase-dependent mechanisms (Keum et al., 2004; Hayes et al., 2008). Isothiocyanates and indoles are also capable of affecting cell cycle arrest and stimulating apoptosis (Keum et al., 2004; Hayes et al., 2008).

Cruciferous vegetables also contain indoles which can act as ligands for AhR. Experimental studies have shown that the indole-3-carbinol and its metabolite 3,3′-diindoylmethane modulate Akt-NF-κB signaling, caspase activation, cyclin-dependent kinase activities, endoplasmic reticulum stress, and BRCA gene expression. Indoles also induce apoptosis in the mutated, preneoplastic, and cancerous cells. It also affects estrogen metabolism in human beings, specifically the estradiol hydroxylation pathway such that a less potent form of estradiol is formed which protects against estrogen-related cancers of breast and endometrial cancers (Weng et al., 2008).

Food sources like carrots, sweet potatoes, spinach, and tomatoes are rich sources of carotenoids, a class of compounds reported to decrease the risk of chronic diseases and certain cancers. The beneficial effects of carotenoids are thought to be at least in part due to their role as antioxidants. Beta-carotene, lycopene, lutein, and zeaxanthin are some of the most studied carotenoids (Willcox et al., 2003; Krinsky and Johnson, 2005). Beta-carotene, predominantly present in orange colored vegetables like carrots, sweet potatoes, winter squash, and pumpkin and in fruits like papaya, mango, and cantaloupe are potent free radical scavengers. They can be metabolized to vitamin A, which plays a role in differentiation of normal epithelial cells. The lack of differentiation is an attribute in cancer cells and adequate intake of vitamin A, possibly has a role in inhibiting cancer initiation and development. Beta-carotene may increase cell-to-cell contact and inhibit cell proliferation. Orange colored vegetables also contain the antioxidant beta-carotene and a vitamin A precursor, which may inhibit cell proliferation (Bertram et al., 1999).

The carotenoid, lycopene, which imparts red color to vegetables like tomatoes and fruits like rosehips, watermelon, papaya, and pink grapefruit, is an effective antioxidant and a free radical scavenger. The presence of eleven conjugated and two nonconjugated double bonds make it highly reactive toward oxygen and free radicals, and this antioxidant activity probably contributes to its efficacy as a chemoprevention agent (Rao et al., 2006). Experimental studies have shown an inverse relationship between lycopene intake and prostate cancer risk and the clinical studies support this. Oral lycopene is bioavailable, accumulates in prostate tissue, and is localized to the nucleus of prostate epithelial cells. In addition to antioxidant activity, lycopene is reported to upregulate the antioxidant response element, thereby leading to the synthesis of cytoprotective enzymes and contributing toward chemoprevention, induce apoptosis in cancer cells, inhibit aberrant proliferation, and prevent metastasis (van Breemen et al., 2008).

Green leafy vegetables (also called as greens or potherbs) like spinach leaves (palak), amaranth leaves, bathua leaves, colocasia leaves, coriander leaves, cowpea leaves, curry leaves, drumstick leaves, fenugreek leaves, lettuce, mint leaves, mustard leaves, parsley, pumkin leaves, sweet potato leaves and radish leaves are a common ingredient in many Indian cuisines. They are good sources of vitamins A, C, K, and folate, minerals like iron and calcium, fibers, carotenoids (lutein), and xanthophyll pigment. The carotenoids and xanthophylls are potent antioxidants and prevent damage by free radicals. The folic acid present is important in preventing mutagenesis as its deficiency can lead to DNA damage. Folic acid is also of importance in governing the epigenetic events as its deficiency reduces methylation of DNA. This can cause an alteration in the methylation, which may cause deregulation in the expression of genes related to cancer (Johnson, 2004).

Citrus fruits like lemon, orange, tangerine, grape fruit, gooseberry, and so on, contain vitamin C, beta-carotene, flavonoids, limonoids, folic acid, and dietary fibers. Vitamin C is a powerful water soluble antioxidant and protects the cell membranes and DNA from free radical-induced damage. It also plays a role in the synthesis of the connective tissue protein; collagen and its deficiency may facilitate tumor growth. The citrus fruits also contain coumarins and *o*-limonene, especially in the rinds, which have been shown to increase the activity of glutathione transferase, a detoxification enzyme (Elegbede et al., 1993; Ju-Ichi et al., 2005).

Flavonoids are another class of pharmacologically important molecules and exhibit a number of *in vitro* and *in vivo* antioxidant, anti-inflammatory, and anticancer actions. The most abundant citrus flavonoids are flavanones such as hesperidin, naringin, or neohesperidin; flavones such as diosmin, apigenin or luteolin, and flavon-3-ols such as quercetin, kaempferol, myricetin, fisetin, and morin (Manthey et al., 2001). Experimental studies in the recent past have shown that flavonoids affect multiple cancer-related biological pathways like modulation of carcinogen bioactivation, cell signaling, cell cycle regulation, angiogenesis, oxidative stress, and inflammation. Several key studies have shown that the anti-inflammatory properties of citrus flavonoids are due to its inhibition of the synthesis and biological activities of different pro-inflammatory mediators, mainly the arachidonic acid derivatives, prostaglandins E 2, F 2, and thromboxane A 2 (Ju-Ichi et al., 2005; Benavenete-Garcia and Castillo, 2007, 2008).

The most abundant flavonoid constituents of fruits and berries are anthocyans (i.e., anthocyanins, glycosides, and their aglycons, anthocyanidins) and are widespread in fruits and vegetables of red–blue color. They are present in the Indian fruits like jamun, bedese, the exotic wild blueberry, bilberry, cranberry, elderberry, raspberry seeds, strawberry, purple sweet potato, pomegranate, red amaranth, banana flower, and red cabbages (Hou et al., 2004; Cooke et al., 2005; Zafra-Stone et al., 2007).

Experimental studies and epidemiological investigations have indicated that anthocyanins may contribute to cancer chemoprevention. They possess strong free radical scavenging, antioxidant, anti-inflammatory properties and are known to inhibit growth and proliferation of some cancer cells (Cooke et al., 2005). Animal experiments showed that oral intake of anthocyanins from purple sweet potato (*Ipomoea batatas* L.) and red cabbage (*Brassica oleracea* L.) suppressed the 1,2-dimethylhydrazine and 2-amino-1-methyl-6-phenylimidazo-[4,5-b] pyridine-induced colon carcinogenesis in rats (Hagiwara et al., 2002). The chemopreventive effects of anthocyanins are observed to be mediated through multiple pathways like targeting mitogen-activated protein kinase (MAPK) pathway and activator protein 1 (AP-1) factor, nuclear factor-κB (NF-κB) pathway and cyclooxygenase 2 (COX-2) gene, inducing apoptosis through generation of reactive oxygen species (ROS)/c-Jun NH2-terminal kinase (JNK)-mediated caspase activation (Hou et al., 2004; Cooke et al., 2005).

Spices and Condiments in the Indian Diet

Spices are defined by the U.S. Food and Drug Administration as "aromatic vegetable substances, in the whole, broken, or ground form, whose significant function in food is seasoning rather than nutrition." (Lampe et al., 2003). Spices have been grown since antiquity in India. Currently, no country in the world other than India produces as many kinds of spices and is accordingly known as the "The home of spices" (Parry, 1969). The Government of India has recognized 52 spices (Pruthi, 1987). In general, they are the dried aromatic parts of plants, generally the seeds, berries, roots, pods, and sometimes leaves (coriander leaf, curry leaf, and mint leaf) (Lampe et al., 2003). Given the wide range of botanical species and plant parts from which spices are derived, they can contribute a significant variety and complexity to the human diet (Sinha et al., 2003).

Spices are an integral part of any Indian cuisine and are added to impart tastes, flavor, and color to the curries. The use of spices in the Indian curry is more than 5000 years old and the English term "curry" is derived from the South Indian Tamil word "kaikaari" or its shortened version "kari," meaning vegetables cooked in spices. Curry is a gravy dish or a stew-like dish with spices and seasonings and is eaten with both rice and Indian bread. Spices are also acknowledged to be preservatives

and to prolong the shelf life of foods by preventing rancidity through their antioxidant or antimicrobial activity on the microbes (Lampe et al., 2003).

Most of the spices used in curries are extensively used in Ayurveda for treating various diseases and ailments (Sengupta et al., 2004). Ancient physicians, including Charaka, Sushrutha, Hippocrates, and Dioscorides, used spices extensively in their practice, and these observations have been documented in the ancient textbooks (Pruthi, 1987; Charaka, 1994). Spices possess various medicinal properties like those of soothing, healing, rejuvenating the gastrointestinal system, and helps in the digestion process (Pruthi, 1987; Platel and Srinivas, 2004). Some of the medical practices involving the spices in the alternative medicines have been still preserved and some researchers have taken an interest in understanding the potential for spices in the promotion of health (Pruthi, 1987).

Numerous studies in the past three decades have conclusively shown that most of the commonly used Indian spices possess diverse array of natural phytochemicals and that they have complementary and overlapping pharmacological and biological actions like the antioxidant effects, free radical scavenging, anti-inflammatory, antibacterial, antiviral, modulation of detoxification enzymes, stimulation of immune system, reduction of inflammation, modulation of steroid metabolism, antimutagenic, and anticarcinogenic potentials (Lampe et al., 2003; Aggrawal et al., 2009; Figure 5.1). Spices can even protect against a wide range of cancers, heart disease, and other chronic diseases (Craig, 1999). Preclinical studies in a variety of human and animal cancer cell lines of breast, cervical, colon, gastric, hepatic, leukemia, oral epithelial, ovarian, pancreatic, and prostate have shown that the extracts of spices and some of their phytochemicals possess cancer-preventive properties and are devoid of any toxicity (Aggrawal et al., 2009).

A wide variety of active phytochemicals, including the flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, and phthalides have been identified in the spices and herbs (Table 5.1). In most spices the flavors are provided by the essential oils and oleoresins present. The fragrant, aromatic, and pungent character is imparted by the essential oils including terpenes, sesquiterpenes, pinenes, alcohols, esters, ketones, and aldehydes (Lampe et al., 2003). The terpenes and terpene derivatives are probably the most important class of aromatic compounds and are associated with secretory structures such as oil cells, resin ducts, hair-like trichomes or glandular epidermis in plants. Monoterpenes contribute to the fragrance of majority of spices and occur in many different plants. The characteristic aroma of a spice results from a specific mixture of monoterpenes and not by a specific compound (Lampe et al., 2003; Bhattacharjee and

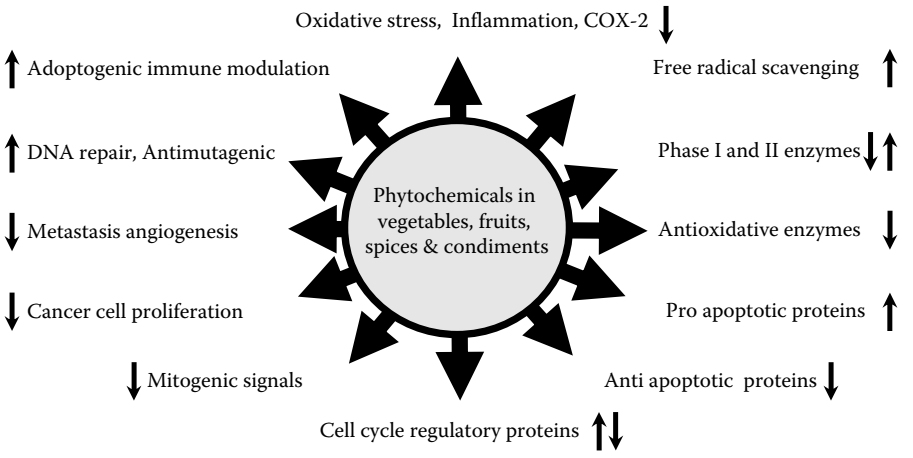

FIGURE 5.1 Molecular targets of the phytochemicals present in fruits, vegetables, and spices commonly used in Indian vegetarian cooking. (Adapted from Aggarwal et al., 2003, 2008, 2009; Anand et al., 2008; Cooke et al., 2005; Marquart et al., 2002; Arora, 2009; Miller et al., 2000; Slavin et al., 2001; Zafra-Stone et al., 2007.)

TABLE 5.1
A List of Common Spices and their Nutraceuticals

Vernacular	Scientific Name	Family	Plant Part	Active Principle/s
Aniseed	*Pimpinella anisum*	*Apiaceae*	Seed	Apigenin, luteolin, bergapten, anisaldehyde, α-himalchalene, anisaldehyde, anethole, bergapten, estragole, α-himalchalene
Asafoetida	*Ferula assafoetida*	*Apiaceae*	Gum	Auraptene, elaeochytrin A, umbelliprenin, α-pinene, phellandrenes, farnesiferoles, hendecylsulphonyl acetic acid
Bay leaf	*Laurus nobilis*	*Lauraceae*	Leaf	Kaempferol, 1,8-cineol, sabinene, α-pinene, β-pinene, β-elemene, linalool, α-terpinol, α-terpinyl acetate, thymol, caryophyllene, aromadendrene, β-selinene, farnesene, cadinene, methyl eugenol, myrcene, eugenol
Black/white Pepper	*Piper nigrum*	*Piperaceae*	Seed	Piperine, β-caryophyllene, limonene, δ-3-carene, α-pinene, β-pinene, α-phellandrene, myrcene, terpinolene
Caraway	*Carum carvi*	*Umbellifereae*	Seed	Anethofuran, carvone, limonene, L-fucitol, germacrene D, dihydrocarveol, α-pinene, β-pinene, sabinene, perillyl alcohol, carveol.
Cardamom	*Elettaria cardamomum*	*Zingiberaceae*	Seed	Eugenol, α-terpinyl acetate, 1,8-cineol, limonene, linalool, linalyl acetate, terpinolene, myrcene
Carom	*Trachyspermum copticum*	*Apiaceae*	Seed	Piperitone, alpha-pinene, limonene, 1,8-cineol, thymol, cymene, terpinene
Celery	*Apium graveolens*	*Apiaceae*	Seed	Apigenin, limonene, β-selinene, humulene, 3-butylphthalide, senkyunolide, α-pinene, β-pinene, myrcene, (Z)-β-ocimene, γ-terpinene, *cis-allo*-ocimene, (E)-β-farnesene, apiole, senkyunolide, neocnidilide
Cinnamon	*Cinnamomum zeylanicum*	*Lauraceae*	Bark	Cinnamaldehyde, cinnamyl acetate, cineol, eugenol, coumarin, ethyl cinnamate, linalool, humulene, β-caryophyllene, τ-cadinol
Clove	*Syzygium aromaticum or Eugenia caryophyllata*	*Myrtaceae*	Inflorescence	Carvacrol, cinnamaldehyde, eugenyl acetate thymol, eugenol, syzyginins, dehydrodieugenol, *trans*-coniferyl aldehyde
Coriander	*Coriandrum sativum*	*Apiaceae*	Seed leaves	Linalool, geraniol, geranyl acetate, camphor
Cumin	*Cuminum cyminum*	*Umbelliferae*	Seed	Cumin cuminaldehyde, γ-terpinene, β-pinene, *p*-cymene, *p*-mentha-1,3-diene-7-al, cuminosides, *p*-mentha-1,4-dien-7-al, sesquiterpenoid glucosides
Curry leaves	*Murraya koenigii*	*Rutaceae*	Leaf	Benzoisofuranone furocoumarins, bismurrayafoline, dimeric carbazoles, mahanine, koenine, koenigine, koenidine6, girinimbiol, girinimibine7, koenimbine, *O*-methyl murrayamine A, *O*-methyl mahanine, isomahanine, bismahanine, bispyrayafoline, scopotin, murrayanine, di-α-phellandrene, D-sabinene, D-α-pinene, dipentene, D-α-terpinol and caryophyllene
Dill	*Anethum graveolens*	*Apiaceae*	Leaves	Carvones, limonene, dillapiole, *trans*-dihydrocarvone, *cis*-dihydrocarvone, myristicin
Fennel	*Foeniculum Vulgare*	*Umbelliferae*	Seed	*trans*-Anethole, estragole fenchone, (E)-anethole, limonene, fenchone, estragole, anisaldehyde, bergapten, β-sitosterol
Fenugreek	*Trigonella Foenum-Graecum*	*Leguminosae*	Seed and leaf	Diosgenin, tricin, protodioscin 4-hydroxyisoleucine, kaempferol, methyl-protodioscin, methyl-protodeltonin, γ-schizandrin, scopoletin, trigoneosides, vitexin, tricin, naringenin, quercetin, naringenin, tricin-7-*O*-beta-D-glucopyranoside tricin-7-*O*-beta-D-glucopyranoside

Garlic	*Allium sativum*	*Alliaceae*	Bulb	Ajoene, allicin, alliin, diallyl sulfide, diallyl disulfide, diallyl trisulfide, *S*-allylcysteine, methiin, isoalliin, cycloalliin, *S*-allylmercapto cystein
Ginger	*Zingiber officinale*	*Zingiberaceae*	Rhizome	Diarylheptanoids, paradols, [6]-Gingerol, [6]-paradol, shogoal, 6-gingerdiol, gingerdione, zingiberene, citral neral, geranial, cineol, bisabolene, α-farnesene, β-phellandrene, zingerone
Basil (Holy)	*Ocimum Sanctum*	*labiateae*	Leaf	Eugenol, ursolic acid, eugenol, caffeic acid, β-sitosterol, limonene, estragole, methyl eugenol, geraniol, 1,8-cineol, linalool, citral, methyl cinnamate
Kokum	*Garcinia indica*	*Clusiaceae*	Pericarp	Garcinol, gambogic acid, hydroxycitric acid, malic acid, polyphenols, anthocyanin, ascorbic acid.
Mace	*Myristica fragrans*	*Myristicaceae*	Dried aril surrounding seed	Eugenol, myristicin, elemicin, sabinene, safrole, methyl eugenol, α-pinene, β-pinene, myristic acid, 4-terpineol
Mint	*Mentha piperita*	*Labiatae*	Leaf	Menthol, eriocitrin, luteolin, *R*-carvone, limonene, β-pinene, β-myrcene, *trans*-thujan-4-ol, dihydrocarvone, β-bourbonene, diosmin, isorhoifolin, narirutin, rosmarinic acid, caffeic acid
Mustard	*Brassica JunceaBrassica Hirta*	*Brassicaceae*	Seed andleaves	Glucosinolates, carotenoids, allyl isothiocyanate, sulforaphane
Nutmeg	*Myristica fragrans*	*Myristicaceae*	Dried kernel of seed	Eugenol, myristicin, elemicin, sabinene, safrole, methyl eugenol, α-pinene, β-pinene, myristic acid, 4-terpineol
Onion	*Allium cepa*	*Alliaceae*	Bulb	Quercetin, allicepin, allyl propyl disulphide, protocatechuic acid, quercetin dimer, quercetin
Black onion seed, black sesame Roman coriander	*Nigella sativa*	*Ranunculaceae*	Seed	Thymoquinone, nigellone, *trans*-anethole, *p*-cymene, limonene, carvone, nigellamines, α-hederin
Parsley	*Petroselinum crispum*	*Apiaceae*	Seed	Apiole, *p*-1,3,8-menthatriene, apigenin, β-phellandrene, myrcene, myristicin, rutin
Pomegranate	*Punica granatum*	*Punicaceae*	Seed	Anthocyanins, ascorbic acid, ellagic acid, gallic acid, caffeic acid, catechin, quercetin, rutin, punicic acid, anthocyanidins, tannins (punicalin and punicafolin), and flavone glycosides (luteolin and apigenin)
Poppy	*Papaver somniferum*	*poppy*	Seed	1-Pentanol, 1-hexanal, pentylfuran, caproic acid, linoleic acid, oleic acid, palmitic acid
Red Pepper	*Capsicum annuum*	*Solanaceae*	Fruits	Capsaicin, β-carotene, zeaxanthin, lutein, caffeic acid, capsanthin
Saffron	*Crocus sativus*	*Iridaceae*	Stigma	Crocin, safranal, picrocrocin, crocetin, α- and β-carotene, lycopene, zeaxanthin
Sesame seed	*Sesamum indicum*	*Pedaliaceae*	Seed	Sesamol, sesamol dimer, sesamin, sesamolin, sesaminol triglucoside, sesaminol diglucoside
Tamarind	*Tamarindus indica*	*Leguminosae*	Pulp of ripe fruit	Proanthcyanidins, epicatechin, taxifolin, apigenin, eriodictyol, luteolin naringenin, tartaric acid, limonene, geraniol, safrole, cinnamic acid, ethyl cinnamate, methyl salicylate, pyrazine, phenylacetaldehyde, 2-furfural, palmitic acid
Thyme	*Carum copticum*	*Apiaceae*	Leaf	Thymol, carvacrol, *p*-cymene, γ-terpinene, linalool, borneol, β-caryophyllene, caffeic acid, β-pinene, thymodihydroquinone
Turmeric	*Curcuma longa*	*Zingiberaceae*	Rhizome	Curcumin, zingiberene, turmerone, γ-atlantone, β-sesquiphellandrene, turmerol, bisabolone

Source: Adapted from Aggarwal et al., 2003, 2008, 2009; Anand et al., 2008.

Sengupta, 2009). Certain spices also have components other than volatile ones like the alkaloids capsaicin, piperine, and chavicene which give the pungent taste to peppers; saponins like trignollenine which impart bitter taste to fenugreek; strong coloring compounds like curcumin, carotene, saffrole, crocin, and picrocin in the turmeric, chillies, and saffron; and various acids like tartaric acid in tamarind, hydroxyl citric acid and garcinol in kokam, malic acid in mango powder, and oxalic acid in pomegranate seeds (Lampe et al., 2003).

Some of the commonly used spices in the Indian cooking include turmeric, aniseed, asafoetida, black cumin, black mustard, cardamom, cinnamon, cloves, coriander, cumin, curry leaf, fennel, fenugreek, garlic, ginger, Indian cassia, Indian dill or dill, large cardamom, kokum, lemon grass, mustard, onion, saffron, tamarind, cardamom, tulsi, yellow mustard, and xanthoxylum. Herewith we summarise the chemopreventive properties, some of the phytochemicals, and the mechanisms involved in well-studied spices like garlic, fenugreek, clove, ginger, and turmeric.

Garlic (*Allium Sativum* L.)

Garlic is a spice widely used around the world and has a long history of use as a medicinal plant that dates back to Aristotle, Hippocrates, and Aristophane (Dausch and Nixon, 1990). The antitumoral effects of garlic cloves have been recorded since very early times where in ancient Egypt (1550 BC), it was used to treat tumors. Hippocrates as well as Indian physicians reported the use of garlic as a method to reduce tumor growth (Fleischauer and Arab, 2001). Population-based case–control studies have suggested an inverse correlation between dietary intake of allium vegetables and the risk of different types of cancers, especially that of the stomach and colon (Dausch and Nixon, 1990; Dorant et al., 1993; Bianchini and Vainio, 2001).

Studies indicate that the sulfur-containing compounds are responsible for most of the health benefits of garlic, including the anticancer effect. Nonsulfur compounds in garlic including proteins, carbohydrates, flavanoids, selenium, and saponins are also reported to have beneficial effect. Two classes of organosulfur compounds are found in whole garlic cloves, the γ-glutamylcysteines and cysteine sulfoxides. Allylcysteine sulfoxide (alliin), which accumulates naturally during storage of the bulbs at cool temperature and is the odorless precursor of the organosulfur compounds accounts for approximately 80% of the cysteine sulfoxides in garlic (Herman-Antosiewicz and Singh, 2004; Nagini, 2008).

Processing of garlic bulbs by crushing, cutting, or chewing releases a vacuolar enzyme alliinase that acts on alliin to give rise to extremely unstable and odoriferous compounds, sulfenic acids, from cysteine sulfoxides. Sulfenic acids spontaneously react with each other to form unstable compounds called thiosulfinates. In the case of alliin, the resulting sulfenic acids react with each other to form a thiosulfinate, allicin. The formation of thiosulfinates is very rapid and has been found to be complete within 10–60 s of crushing the garlic. Allicin breaks down *in vitro* to form a variety of fat-soluble organosulfur compounds including diallyl trisulfide, diallyl disulfide, and diallyl sulfide, or in the presence of oil or organic solvents, ajoene and vinyl dithiins. Crushing garlic does not change its γ-glutamylcysteine content. Water-soluble organosulfur compounds, such as *S*-allylcysteine, are formed from γ-glutamylcysteines during long-term incubation of crushed garlic in aqueous solutions, as in the manufacture of aged garlic extracts (Herman-Antosiewicz and Singh, 2004; Shukla and Kalra, 2007; Nagini, 2008).

Preclinical animal studies have indicated that the analogs of organosulfur compound are highly effective in affording protection against diverse organotrophic carcinogens like DMBA-induced croton oil-promoted skin carcinogenesis in mice, benzo[*a*]pyrene (B[*a*]P)-induced forestomach, and pulmonary cancer in mice, *N*-nitrosomethylbenzylamine-induced esophageal cancer in rats, azoxymethane-induced colon carcinogenesis in rats, and 2-amino-1-methyl-6-phenyli-midazo[4,5-*b*] pyridine-induced mammary tumorigenesis in rats (Le Bon and Siess, 2000; Herman-Antosiewicz and Singh, 2004).

Several mechanisms elicited by garlic and its organosulphur compounds have been observed. These include inhibition of activation of carcinogen through modulation of cytochrome

P450-dependent monooxygenases and/or acceleration of carcinogen detoxification (via induction of phase II enzymes the glutathione transferases, quinone reductase, etc.), upregulation of antioxidant defenses and DNA repair systems, modulating the immune system, suppression of cell proliferation by blocking cell cycle by induction of G2/M phase arrest, induction of apoptosis via the intrinsic pathway by altering the ratio of the Bcl-2 family of proteins and by exerting antiangiogenic activities (Das, 2002; Herman-Antosiewicz and Singh, 2004; Shukla and Kalra, 2007; Nagini, 2008).

Fenugreek (*Trigonella Foenumgraecum* L.)

Extracts of fenugreek seeds and some of their constituents have been reported to have anticarcinogenic potency in different experimental models (Sur et al., 2001; Hibasami et al., 2003). The alcoholic extract of the seed when administered intraperitoneally, both before and after inoculation of Ehrlich ascites carcinoma in Balb-C mice was observed to produce more than 70% inhibition of tumor cell growth, enhance the peritoneal exudate cell and macrophage cell counts, and a significant anti-inflammatory effect with respect to the vehicle treated control (Sur et al., 2001). Feeding of the fenugreek seeds-incorporated diet to rats inhibited 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in rats by modulating the activities of beta-glucuronidase and mucinase (Devasena and Menon, 2003) restoring the levels of antioxidants ascorbic acid, vitamin E, reduced glutathione, glutathione peroxidase, glutathione-*S*-transferase, superoxide dismutase, and catalase with concomitant decrease in the levels of lipid peroxidation in the blood and liver (Devasena and Menon, 2002, 2007).

Fenugreek seeds are effective against the 7,12-dimethylbenz (alpha) anthracene (DMBA)-induced breast cancer in rats (Amin et al., 2005). *In vitro* studies with MCF-7 cells, an estrogen receptor positive human breast cancer cell line have shown that the ethanol extract of fenugreek seed decreased the cell viability and induced apoptotic changes, such as flipping of phosphatidylserine, decrease of mitochondrial membrane potential, degradation of cellular DNA into fragments of approximately 180–200 base pairs. Cell cycle analysis by flow cytometry showed the presence of a subG1 apoptotic population that was more prominent at higher concentrations along with cell cycle arrest at G2/M phase (Sebastian and Thampan, 2007).

Studies by Hibasami et al. (2003) have shown that protodioscin possesses strong growth inhibitory effect against HL-60 cells, but weak growth inhibitory effect on KATO III cells as evaluated by the morphological changes and flow cytometric studies (Hibasami et al., 2003). Diosgenin [(25*R*)-5-spirosten-3β-ol], a steroid sapogenin constituent of fenugreek seeds, has also been reported to inhibit cell proliferation in the human osteosarcoma 1547 cell line by induction of apoptosis and G1 phase cell cycle arrest (Moalic et al., 2001). Furthermore, in the osteosarcoma 1547 cell line, it was shown that diosgenin caused cell cycle arrest and apoptosis principally by increasing the expression of the tumor suppressor oncoprotein p53 (Corbiere et al., 2003).

Diosgenin treatment was selectively cytotoxic to the neoplastic cells and affected the growth of both MCF-7 (ER+) and MDA-231 (ER–), human breast cancer cells *in vitro.* In the normal breast epithelial cells MCF-10A at equivalent concentrations, such effect was not seen suggesting it was safe. Diosgenin caused G1 cell cycle arrest by downregulating cyclin D1, cdk-2, and cdk-4 expression in both the breast cancer cells resulting in the inhibition of cell proliferation, and induction of apoptosis. Diosgenin inhibits pAkt expression and Akt kinase activity without affecting PI3 kinase levels, resulting in the inhibition of its downstream targets, NF-κB, Bcl-2, survivin, and XIAP. The Raf/MEK/ERK pathway, another functional downstream target of Akt, was inhibited by diosgenin in ER(+) but not in ER(–) breast cancer cells. *In vivo* tumor studies indicate that diosgenin significantly inhibits tumor growth in both MCF-7 and MDA-231 xenografts in nude mice (Srinivasan et al., 2009).

Diosgenin inhibits receptor-activated NF-κB ligand-induced osteoclastogenesis, suppresses tumor necrosis factor (TNF)-induced invasion, and blocks the proliferation of tumor cells. It suppressed TNF-induced NF-κB activation through inhibition of Akt activation. NF-κB-dependent reporter gene expression was also abrogated by diosgenin, as the TNF-induced expression of

NF-κB-regulated gene products involved in cell proliferation [(cyclin D1, COX-2, c-myc), antiapoptosis (IAP1, Bcl-2, Bcl-XL), Bfl-1/A1, TRAF1 and cFLIP], and invasion (MMP-9) were also downregulated by the saponin (Shishodia and Aggarwal, 2006).

Nigella Sativa (Black Cumin, Family Ranunculaceae)

Black cumin possesses chemopreventive effect against ferric nitrilotriacetate (Fe-NTA)-induced renal oxidative stress, hyperproliferative response, and renal carcinogenesis. Feeding of rats with *Nigella sativa* caused a decrease in γ-glutamyl transpeptidase, lipid peroxidation, xanthine oxidase, H_2O_2 generation, blood urea nitrogen, serum creatinine, renal ODC activity, DNA synthesis, and incidence of tumors. It also increased the glutathione content, glutathione-metabolizing enzymes, and antioxidant enzymes in the kidneys (Khan and Sultana, 2005).

Oral feeding of the oil reduced the incidence of 1,2-dimethylhydrazine-induced aberrant crypt foci, putative preneoplastic lesions for colon cancer in Fischer 344 rats. Immunohistochemical studies showed that it had antiproliferative activity in both initiation and postinitiation stages of carcinogenesis (Salim and Fukushima, 2003). *Nigella sativa* and one of its chemical thymoquione possess inhibitory effects on AOM-induced DNA damage and inhibited the lipid peroxide levels in the rats (Al-Johar et al., 2008). The essential oil and ethyl acetate extracts of Nigella sativa are observed to be more cytotoxic against the P815 and the Vero cell line. The oil was also effective in controlling the solid tumor development, inhibited the incidence of liver metastasis development, and improved mouse survival in the DBA2/P815 (H2d) mouse (Ait Mbarek et al., 2007). Thymoquinone is also reported to possess antineoplastic effect on the SW-626 colon cancer cells. The chemotherapeutic effects were similar to that of 5-FU (Norwood et al., 2006).

Administration of *Nigella sativa* reduced the incidence and size of DMBA-induced mammary carcinogenesis in rats, decreased the levels of markers of tumorigenicity (serum levels of total sialic acid and lipid-bound sialic acid), markers of endocrine derangement (serum prolactin, estradiol, and progesterone levels), apoptotic changes [serum tumor necrosis factor (TNF)-alpha, tissue caspase-3 activity, percentage of DNA fragmentation and ultrastructural features of apoptosis], and markers of oxidative stress (tissue levels of lipid peroxides and nitric oxide) suggesting a protective role (El-Aziz et al., 2005). *In vitro* studies have shown that the aqueous and alcohol extracts of the *Nigella sativa* was also effective in influencing the survival of MCF-7 cells in the presence and absence of H_2O_2 (Farah, 2005).

Thymoquinone and α-hederin, another important constituent of *Nigella sativa*, induced both cytotoxicity and apoptosis on human cancer cell lines the A549 (lung carcinoma), HEp-2 (larynx epidermoid carcinoma), HT-29 (colon adenocarcinoma), and MIA PaCa-2 (pancreas carcinoma). Of the two, at uniform concentrations, thymoquinone was more potent in eliciting necrosis and apoptosis than α-hederin and HEp-2 cells were the most sensitive (Rooney and Ryan, 2005a). Rooney and Ryan (2005b) have also reported that in the HEp-2 cells, treatment with buthionine sulfoximine (BSO), a selective inhibitor of glutathione synthesis, significantly enhanced the α-hederin-mediated toxicity without changes in apoptosis or necrosis levels; while the opposite was observed with thymoquinone. Thymoquinone significantly decreased GSH levels, and pretreatment with BSO had a synergistic effect. Pretreatment with the caspase 3 inhibitor, Z-DEVD-fmk, decreased the thymoquinone-induced apoptosis, thereby confirming that the apoptosis is mediated through the caspase 3-activation (Rooney and Ryan, 2005b).

Sethi et al. (2008) have reported that thymoquinone suppressed tumor necrosis factor-induced NF-κB activation in a dose- and time-dependent manner and inhibited NF-κB activation induced by various carcinogens and inflammatory stimuli. It also downregulated the expression of NF-κB-regulated antiapoptotic (IAP1, IAP2, XIAP Bcl-2, Bcl-xL, and survivin), proliferative (cyclin D1, cyclooxygenase-2, and c-Myc), and angiogenic (matrix metalloproteinase-9 and vascular endothelial growth factor) gene products (Sethi et al., 2008).

Thymoquinone has also been reported to inhibit the DNA synthesis, proliferation, and viability of both androgen-sensitive as well as androgen-independent prostate cancer cells (LNCaP, C4-B,

DU145, and PC-3) but not noncancerous cells (BPH-1), clearly suggesting that the cytotoxic effects were selective only to the neoplastic cells and not to the normal cells (Kaseb et al., 2007). The effects of thymoquinone is observed to be mediated by downregulating AR and E2F-1, key regulators of cell proliferation and viability with possible role in the development of hormone-refractory prostate cancer. Studies with LNCaP cells showed that treatment with thymoquinone increased the levels of p21(Cip1), p27(Kip1), and Bax; retarded the progression of cells from G1 to S phase with a concomitant decrease in AR and E2F-1 as well as the E2F-1-regulated proteins necessary for cell cycle progression. It also inhibited the growth of C4-2B-derived tumors in nude mice and, as with C4-2B cell growth in culture, was associated with a dramatic decrease in AR, E2F-1, and cyclin. Immunohistochemical studies confirmed that thymoquinone caused a marked reduction in E2F-1 levels and induced apoptosis. In total, these findings implicate that thymoquinone suppresses the expression of AR and E2F-1 necessary for proliferation and viability of androgen-sensitive as well as androgen-independent prostate cancer cells both *in vitro* and *in vivo,* and also that it did not cause any noticeable side effects in mice (Kaseb et al., 2007).

Recently, Yi et al. (2008) have also reported that thymoquinone blocked angiogenesis in both *in vivo* and *in vivo* systems of study. It prevented the tumor angiogenesis in a xenograft human prostate cancer (PC3) model in mouse, and inhibited human prostate tumor growth at low dosage with almost no chemotoxic side effects. Thymoquinone was observed to inhibit the human umbilical vein endothelial cell migration, invasion, and tube formation and that the endothelial cells were more sensitive to thymoquinone-induced cell apoptosis, cell proliferation, and migration inhibition when compared with PC3 cancer cells. Thymoquinone inhibited vascular endothelial growth factor-induced extracellular signal-regulated kinase activation, but showed no inhibitory effects on vascular endothelial growth factor receptor 2 activation. The inhibition of cell proliferation was observed to be due to the suppressed activation of AKT and extracellular signal-regulated kinase (Yi et al., 2008). Both these above studies indicate that thymoquinone could be a potential drug candidate for both androgen-sensitive as well as androgen-independent prostate cancers and also that it could affect metastasis by affecting the angiogenesis process.

Thymoquione is reported to be both antimutagenic and chemopreventive against benzo[*a*]pyrene-induced mutagenesis and forestomach carcinogenesis in mice (Badary et al., 1997, 2007). Daily intake of thymoquione after and before or during exposure to benzo[*a*]pyrene significantly reduced the frequencies of chromosomal aberrations and damaged cells when compared with the concurrent control group (B[*a*]P alone) (Badary et al., 2007). Administration of thymoquione in drinking water resulted in significant suppression of B[*a*]P-induced tumorigenesis as observed by the reduced tumor incidence and multiplicity (Badary et al., 1999).

Curcuma Longa (Turmeric Family Zingiberaceae)

Several studies over the past few years have confirmed that turmeric and its principal constituent curcumin are of immense benefit. Compelling experimental and clinical evidence indicates that curcumin has been shown to possess chemopreventive potential in several different animal tumor bioassay systems, including colon, duodenum, stomach, prostate, and breast carcinogenesis, in both *in vivo* and animal systems of studies and also against the different groups of mutagens and carcinogens (Azuine et al., 1992; Deshpande et al., 1997, 1998; Chattopadhyay et al., 2004; Sharma et al., 2005; Jain et al., 2007; Johnson and Mukhtar, 2007; Anand et al., 2008).

Mechanistic studies have shown that curcumin is an antioxidatant; induces phase II detoxification enzymes; downregulates transcription factors (NFκB, AP-1, Egr1); enhances DNA repair; suppresses preneoplastic, mutated and neoplastic cell proliferation; induces apoptosis; inhibits angiogenesis; suppresses the expression of antiapoptotic proteins and stimulates the immune system. It downregulates enzymes such as cyclooxygenase 2, lipooxygenase, nitric oxide synthase, matrix metalloproteinase 9, telomerase, urokinase type plasminogen activator, and more. Curcumin also downregulates other factors and receptors such as tumor necrosis factor, chemokines, cell surface adhesion molecules, and growth factor receptors (e.g., EGFR, HER2) (Deshpande and Maru,

1995; Aggarwal et al., 2003; Chattopadhyay et al., 2004; Sharma et al., 2005; Johnson and Mukhtar, 2007; Anand et al., 2008; Chethankumar and Srinivas, 2008).

Phase I clinical trials have shown that curcumin is safe and well tolerated in colorectal cancer patients and the success of these trials has led to the initiation of phase II trials (Johnson and Mukhtar, 2007). The pharmacokinetic studies showed that the pharmacologically active concentration of curcumin could be achieved in colorectal tissue and that it might also be achievable in tissues such as skin and oral mucosa, where curcumin can be directly applied locally or topically (Hsu and Cheng, 2007). Studies with a small number of pancreatic cancer patients (21 evaluable) have also shown that oral administration of curcumin is well tolerated and, despite its limited absorption, has biological activity in some patients with pancreatic cancer (Dhillon et al., 2008).

Ginger

Ginger, the rhizome of the plant *Zingiber officinale* Roscoe, is one of the most important medicinal plants and has been used extensively for more than 2500 years in traditional medicine for headaches, nausea, common cold, flu-like symptoms, and in the treatment of arthritis, rheumatological conditions, atherosclerosis, migraine headaches, muscular discomfort, rheumatoid arthritis, high cholesterol, ulcers, depression, impotence, and even painful menstrual periods (Shukla and Singh, 2007). The underground stem or rhizome is an integral part of the Indian cooking and is used either as a paste or powder in the curries. Raw ginger is also used for flavoring black tea and such preparations are common during rainy season and winter (Shukla and Singh, 2007).

Some constituents of ginger are reported to possess potent antioxidant and anti-inflammatory activities, and some of them exhibit cancer-preventive activity in experimental carcinogenesis (Shukla and Singh, 2007). The nonvolatile pungent principles like gingerols, shogaols, paradols, and zingerone are supposed to account for many of the beneficial effects of ginger. Studies conducted *in vitro* with cultured mammalian cells as well as *in vivo* with experimental animals, mainly rats, and mice have shown that the anticancer and chemopreventive properties of ginger are due to the presence of the pungent vallinoids, 6-gingerol, and 6-paradol, as well as some other constituents like shogaols, zingerone, and so on. (Shukla and Singh, 2007; Kundu et al., 2009).

Studies have shown that ginger and some of its constituents possess free radical scavenging, antioxidant, and anti-inflammatory effects. The essential oil of ginger has been reported to suppress the formation of DNA adducts by aflatoxin B1 *in vivo* in a microsomal enzyme-mediated reaction *in vitro* (Hashim et al., 1994). Ginger also inhibited the Epstein–Barr virus early antigen activation in Raji cells promoted by phorbol ester, 12-*O*-tetradecanoylphorbol-13-acetate (Kapadia et al., 2002). Feeding ginger oil to mice caused increased levels of carcinogen-metabolizing enzymes, cytochrome P450, aryl hydrocarbon hydroxylase, and glutathione-*S*-transferase, and levels of acid soluble sulfhydryl in the liver (Banerjee et al., 1994). The ethanolic extract of ginger was cytotoxic to Dalton's lymphoma ascites tumor cells, human lymphocytes, Chinese Hamster ovary cells, and Vero cells in tissue culture. These extracts also inhibited the thymidine uptake into DNA (Unnikrishnan and Kuttan, 1988). Ginger was cytotoxic and induced apoptosis in the HEp-2 cells in a dose-dependent manner *in vitro*. Mechanistic studies showed that these effects were mediated by free radicals (Vijaya Padma et al., 2007). Ginger and its active constituents are reported to inhibit the growth and modulate the secretion of angiogenic factors in ovarian cancer cells (SKOV3, A2780, CaOV3, and ES2) *in vitro*. Ginger treatment resulted in inhibition of NF-kB activation as well as diminished secretion of VEGF and IL-8, important in angiogenesis (Rhode et al., 2007).

With regard to chemopreventive studies, Katiyar et al. (1996) observed for the first time observed that the ethanolic extract of ginger possesses significant antitumor-promoting effects in a mouse skin tumorigenesis model. Application of the extract to the mice skin resulted in significant inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-caused induction of epidermal ODC, cyclooxygenase, and lipoxygenase activities, ODC mRNA expression, epidermal edema, and hyperplasia in a dose-dependent manner. Ginger extract also afforded protection against

7,12-dimethylbenz[*a*]anthracene-initiated and TPA-promoted skin carcinogenesis, as both tumor incidence and multiplicity were significantly less (Katiyar et al., 1996).

Recently, ginger extract has also been reported to mediate anticancer and anti-inflammatory effects on ethionine-induced hepatoma in rats and also that this was caused by inactivating the NF-κB through the suppression of the pro-inflammatory TNF-alpha (Habib et al., 2008). Chronic treatment of the aqueous extract of ginger also inhibited the spontaneous mammary tumorigenesis in the SHN virgin mice (Nagasawa et al., 2002). However, ginger feeding is reported to have contradictory effects in preventing urothelial and colon carcinogenesis (Dias et al., 2005; Manju and Nalini, 2005; Bidinotto et al., 2006; Ihlaseh et al., 2006; Manju and Nalini, 2006).

Ginger is also reported to possess immunomodulatory effects, a property important in cancer treatment and prevention. Studies by Liu and Zhu (2002) have shown that the feeding of ginger to tumor-bearing mice increased the thymus index, spleen index, percentage of phagocytosis, rate of α-ANAE, and titer of IgM of mice with tumor, indicating that the ethanolic extract of ginger significantly improves the status immunologic function in mice with tumor (Liu and Zhu, 2002). Scientific studies also suggest that ginger has beneficial effects on nausea and vomiting associated with motion sickness, surgery, pregnancy, and also in cancer patients receiving chemotherapy suggesting its use also as an adjuvant in cancer treatment (Hickok et al., 2007).

CONCLUSIONS AND FUTURE DIRECTIONS

An impressive body of studies support the concept that dietary factors are key modulators of some cancers. Several nonnutritive phytochemicals are currently being evaluated in intervention trials for their potential as cancer chemopreventive agents. The traditional Indian vegetarian diet comprises grains, vegetables, fruits, and spices, all of which have been reported to possess chemopreventive effects in both experimental and epidemiological studies. Chemoprevention with dietary modification is a readily applicable, acceptable, inexpensive, and accessible approach to cancer control and management, and with healthcare costs being a key issue today, it would be cost-effective to promote awareness and consumption of phytochemicals as a cancer-preventive strategy for the general public.

The chemopreventive effects that most dietary phytochemicals exert are likely to be the sum of several distinct mechanisms, and accumulating evidence indicates that well-studied phytochemicals like flavanoids, anthocynanins, terpenes, curcumin, eugenol, and sulphorapane possess free radical scavenging, antioxidant, anti-inflammatory, and antimutagens effects; modulate phase I enzymes, modulate signal transduction, cell cycle progression; induce phase II enzymes and DNA repair mechanisms; inhibit angiogenesis and metastasis. These pathways either inhibit the process of carcinogenesis or retard the progression of a normal cell toward full blown malignancy and are of importance in preventing cancer.

However, the major drawbacks of these studies are that most of these phytochemicals have been evaluated in preclinical setups and at high concentrations. Detailed systematic studies are warranted on approximating the doses required to exert the chemopreventive effects in humans and also as to whether the benefits could be obtained by consuming them as supplements. Studies also need to be performed to observe whether coadministering two or more agents with different modes of action would synergize the chemopreventive effects while minimizing the toxicity, if any. Controlled clinical trials with a multidisciplinary approach or further studies with these phytochemicals must be carried out in order to elucidate and understand their efficacy in the treatment and prevention of cancer in humans. Intervention trials should also be planned, and the assay end points should revalidate the results obtained from studies mimicking human cancers in animals.

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6 Isothiocyanate-Modified Pathways in Cancer Prevention and Treatment

Jana Jakubikova and Jan Sedlak

CONTENTS

"Let your food be your medicine and your medicine your food," summed up one of the key principles espoused nearly 2500 years ago by Hippocrates, the father of medicine. This proposition was probably articulated as a resume of observation, experience, and abstraction, in a similar way of his coeval Democritus, the father of modern science and coauthor of atomic theory. Nowadays, evidence-based medicine aims to apply the best available evidence gained from the scientific method to medical decision making (Timmermans and Mauck, 2005). Properly designed experiments answer the questions and occasionally reveal unexpected mystery. Such an event occurred in Professor Drobnica's laboratory, and the obtained data about cytotoxic and cancerostatic activity of isothiocyanates (ITCs) and their effect on HeLa-cells were published in the journal *Neoplasma* (Horakova et al., 1968). The strong impulse to activate the scientific community interest in chemopreventive isothiocyanates was done at Johns Hopkins University School of Medicine, Baltimore (Zhang et al., 1992). Since then scientists discovered a lot of their properties, effects and mechanisms of possible actions such as anti-inflammatory, antiangiogenic, antineoplastic, achieved via activation of apoptosis, autophagy, modulating intracellular redox state, phoshorylation signaling pathway, cell cycle checkpoints, epigenetic regulation, epithelial-mesenchymal transition, and targeting tumor-initiating cells. Finally, the interesting property is inhered in their possible preventive or therapeutic use as combination partner with other compounds.

THE EFFECT OF LOW CONCENTRATIONS OF ITCs

The analyses of molecular and cellular changes allow defining the concentration ranges between the activation of MAPK (results in gene induction) and caspases (associates with cell death) exhibited by ITCs, which in mammalian cells may reflect their respective therapeutic windows *in vivo* (Kong et al., 2000). At low concentrations, sulforaphane (SFN) and phenethyl ITC (PEITC) activate the MAPK (ERK2, JNK1, p38) signaling pathways and defensive phase II detoxifying enzymes response (GST, QR) resulting in survival and protective mechanisms called homeostasis response. Increasing the concentrations of ITCs will additionally lead to potential cytotoxicity response that activates the caspase pathways, leading to apoptosis. Finally, further increment to suprapharmacological concentrations will lead to nonspecific necrotic cell death. Normal mammary cells treated with PEITC or SFN exhibit more changes in the expression of estrogen receptor (ER)-related genes than do breast cancer cells. Moreover, these changes occur predominantly at the low concentration (0.3 μM) achievable by dietary input of ITCs (Telang et al., 2009). Gene expression altered by treatment is associated with cell adhesion (claudin-7, fibronectin) or cancer markers (EGFR1, EGFR2). The upregulation of the pro-apoptotic gene BAD and ERβ gene belongs to novel findings and along with upregulation of tumor suppressors p21 and p27, may provide a protective effect to mammary cells against breast cancer. Concentration-dependent effect is also effective in the treatment of human colon cancer cell lines with a combination of two chemopreventive compounds SFN and 3,3′-diindolylmethane, a condensation product of indole-3-carbinol (Pappa et al., 2007). At higher doses cells are arrested in G2M phase, while at low total drug concentration all combinations are antagonistic, with increasing concentrations, the antagonistic effect gradually turns into a synergistic interaction at the highest combined cytotoxic concentration. It is clear that for better predicting beneficial health effects of bioactive food components the need for elucidating mechanistic interactions is prerequisite.

The treatment of human hepatoma HepG2 cells with SFN at nontoxic concentration (<20 μM) results in coordinate increase in the induction of metallothionein MT-I and MT-II mRNA, followed by corresponding increase in MT protein level, activation of transcription factor Nrf2 and ERK, JNK, and P38 signaling pathways (Yeh and Yen, 2005). The effective chemopreventive agents should demonstrate selectivity for transformed cells and absence of genotoxicity for healthy cells. Micronucleus test in human T-lymphocyte cultures confirmed that MTBITC (4-methylthiobutyl ITC) and SFN do not induce micronuclei, although ambivalent character of antigenotoxic potencies of ITC are suggested (Fimognari et al., 2005b; Lamy et al., 2009). At dietary achievable concentrations SFN protects cultured human lymphocytes from micronucleus induction by different mutagens such as ethyl methanesulfonate, vincristine, hydrogen peroxide, and mitomycin C (Fimognari et al., 2005a). SFN and erucin treatment induce mRNAs of NQO1, UGT1A1 and MRP2 in Caco-2 cells. Both ITCs activate ERK1/2 and Akt kinases but have no effect on JNK and p38 phosphorylation. The induction of phase II enzymes in Caco-2 cells is decreased by PI3K and MEK1 inhibitors (Jakubikova et al., 2005b). PEITC and SFN inhibit NF-κB transcriptional activity, nuclear translocation of p65, and expression of target genes VEGF, cyclin D1, and Bcl-XL. In addition, ITCs inhibit basal and UVC-induced phosphorylation of IκBα and block its degradation. These results suggest that inhibition of IKK phosphorylation, particularly IKKβ, is the main target (Xu et al., 2005). Two different concentrations of SNF (10 and 20 μM) and lyophilized broccoli sprouts reconstituted to a SFN concentration of 10 μM were used to evaluate the effect of SNF on gene expression in prostate cancer LNCaP cells (Bhamre et al., 2009). The cDNA microarray analysis confirms and expands the changes associated with the activation of phase II enzyme response as follows: NAD(P)H:quinone oxidoreductase (NQO1), leukotriene B4 dehydrogenase (LTB4DH), malic enzyme (ME1), thioredoxin reductase (TXNRD1), glutathione-*S*-transferase mu (GSTM1), microsomal glutathione-*S*-transferase (MGST1) superoxide dismutase (SOD1) and peroxiredoxin (PRDX1), γ-glutamylcysteine synthase (GCLM); thus the pathway involved Nrf2 signaling. Other modulated genes are associated with regulations of cell cycle G2M phase arrest, cell growth, and oxidative damage. A set of 575 genes was

modulated by broccoli extract and at two different concentrations of SNF. Surprisingly, the gene expression analysis did not confirm the expected SFN effect in androgen signaling pathway. SFN appears to be the primary bioactive compound present in broccoli sprouts, suggesting that broccoli sprouts can serve as a suitable source for SFN in intervention trials.

THE INTRODUCTION OF APOPTOSIS

The induction of cell arrest in G2M phase of cell cycle represents the most frequently observed cell response during ITCs treatment. At relatively higher concentration (30–100 μM, the range is cell line-dependent), SFN is a cell growth modulator. It induces apoptotic cell death characterized by DNA fragmentation, caspase-3 activation, proteolytic cleavage of poly (ADP-ribose) polymerase PARP-1, and upregulation of pro-apoptotic protein Bax and downregulation of anti-apoptotic Bcl-2 and Bcl-XL proteins (Yeh and Yen, 2005). Many studies show that SFN-induced DNA fragmentation is effectively blocked by the *N*-acetyl-L-cysteine (NAC) and catalase, suggesting that the death signaling is triggered by oxidative stress. Cells with a wild-type or mutated p53 appear to be more sensitive to the effects of SFN than cells lacking p53 (Fimognari et al., 2005c). The arrest in G1 phase seems like a departure from the common rule, but the low frequency of the event cannot be a reason to ignore it. The first demonstration of G1 cell cycle block was observed in SFN-treated prostate cancer LNCaP cell line that was accompanied with inhibition of cyclin D1 expression and DNA synthesis (Chiao et al., 2002b). The effects of SFN are maintained through the metabolic processes, because SFN and its metabolite SFN-NAC have similar activities to induce growth arrest and apoptosis. Similarly, G1 arrest of human androgen-independent DU-145 prostate cancer cells to SFN resulted downregulated expression of cyclin D1, phoshorylation of Rb protein, bcl-2 protein, and inhibited the activity of cdk4 with an up-stream induction of cdk inhibitor p21WAF-1/Cip-1. The reduced clonogenicity and induction of apoptosis may be linked to possible mechanism of dietary factor inhibition of initiation and postinitiation phases of prostate cancer carcinogenesis (Wang et al., 2004). Ovarian carcinoma cell lines OVCAR-3, SKOV-3 also respond to SFN treatment by induction of G1 arrest (Chuang et al., 2007) and HT-29 colon cancer cells in addition to upregulated p21 and decreased cyclin D1, cyclin A, and c-myc. Induced activation of ERK and p38 MAPK is involved in the upregulation of p21and cyclin D1, whereas JNK pathway activation is partially involved in the downregulation of cyclin D1 (Shen et al., 2006). PEITC-induced G1 cell cycle arrest of HT-29 resulted in downregulation of pRb protein expression and these effects are linked to activation of P38 signaling pathway, because inhibitors of P38 MAPK attenuated cell cycle arrest and PEITC's ability to decrease the level of cyclins A, D, and E (Cheung et al., 2008). The treatment of nontransformed PHA-stimulated human lymphocytes demonstrates that SFN arrests cells in G1 phase through a decrease in the protein expression of cyclin D3. Because SFN is active and even cytotoxic in normal as well as transformed T-leukemia Jurkat cells, this evidence raises questions regarding its suitability for cancer chemoprevention (Fimognari et al., 2002a). The *in vivo* daily SFN treatment of scid mice with PANC-1 s.c. tumors resulted in a decrease of mean tumor volume by 40% compared with vehicle-treated controls. The activation of caspase-8 coincides the induction of apoptosis but initial detection of caspase-3 cleavage occurs in G2M arrest, contrary to cleavage in the G1 cell cycle phase when mice were treated with higher concentration of SFN. Results show that the induction of apoptosis and the SFN-induced mitosis delay at the lower dose are independently regulated (Pham et al., 2004). The G0/G1 arrest of BITC-treated WEHI-3 cells is associated with the decreased weights of liver and spleen and inhibited differentiation of precursors of macrophage and B cells (Tsou et al., 2009).

Depending on the concentration used and the sensitivity of corresponding cells to respective compounds the G2M arrest is usually linked with apoptosis induction. This pattern is common in suspension cultures of hematopoietic cells as well as adherent cultures of carcinoma cell lines. The SFN-induced G2M cell cycle arrest in T-cell leukemia Jurkat cell line is accompanied by upregulation of p53 and proapoptotic protein bax, while Bcl-2 expression is diminished (Fimognari et al.,

2002b). Arrested pancreatic BxPC-3 cells treated with BITC downregulate Cdk1, cyclin B1, Cdc25B and increase proportion of apoptotic cells, Bax/Bcl-2 ratio, cleavage of caspase-3, PARP-1, and nucleosomal DNA fragmentation (Srivastava and Singh, 2004). Another pancreatic Capan-2 cells treated by BITC increase γ-H2AX and p21 expression, activate CHK2 (checkpoint kinase 2, Rad53) and decrease is observed for cyclin B1, Cdc2, and Cdc25C (G2M regulatory proteins). The addition of inhibitors of proteasomal activity completely block Cdc25C decline (Zhang et al., 2006). The BITC treatment of breast carcinoma MDA-MB-231 and MCF-7 cell lines suppress growth, induce G2M arrest and activate apoptosis. Cell cycle arrest is associated with a decrease of G2M regulatory proteins and apoptosis with induction of proapoptotic proteins Bax, Bak, and downregulation of antiapoptotic proteins Bcl-2, Bcl-XL. BITC induces disruption of mitochondrial membrane, leading to cytosolic release of apoptogenic molecules, cleavage of caspase-9, -8, -3, and ROS production accompanied by the formation of autophagosome-like structures. A synthetic combined superoxide dismutase and catalase mimetic EUK134 significantly attenuate apoptosis induction (Xiao et al., 2006). Analysis of prostate cancer cells treated with SFN reveals a modest increase in S phase fraction and concentration- and time-dependent increase of G2M fraction. The SFN-induced S phase arrest correlates with a reduction in protein levels of cyclin D1, cyclin E, Cdk4, and Cdk6, whereas activation of the G2M checkpoint is accompanied by induction of cyclin B1 and downregulation of Cdk1 and Cdc25C protein levels. SFN-induced G2M block is associated with Ser(10) phosphorylation of H3, and increased phosphorylation of Chk2 is accompanied by induction of p53 and p21 proteins. Knockdown of p21 increases mitotic arrest, while no apparent effect on SFN-induced apoptosis is observed (Herman-Antosiewicz et al., 2007).

Contrary to transformed neoplastic cells, the effect of ITCs is less pronounced in normal cells. For example, mammary epithelial MCF-10A cells are significantly more resistant to growth arrest and apoptosis by BITC compared with breast cancer cells (Xiao et al., 2006). Similarly, the normal prostate epithelial cells are resistant to inhibition of translation initiation (Hu et al., 2007), the cytotoxicity toward normal human T-lymphocytes is limited (Papi et al., 2008), or normal human cells of mammary and pancreatic ductal epithelium are resistant to BITC-mediated ROS generation, activation of MAPK, and induction of apoptosis (Xiao et al., 2008; Sahu et al., 2009). Normal human prostate epithelial cells are markedly more resistant to induction of autophagy (Bommareddy et al., 2009) or the cell may activate another group of genes stimulation of which is not observed in transformed counterparts (Xiao et al., 2006). There is a type of mitochondrial DNA-deficient cells known as Rho-0 cells and such variants of human prostate cancer LNCaP and PC-3 cells are more resistant to SFN-induced ROS generation, apoptosis, disruption of MMP, cytochrome *c* release, and G2M cell arrest (Xiao et al., 2009). Also SFN-induced autophagy is partially suppressed in Rho-0 variants compared with wild-type cells. Analysis of Rho-0 HeLa cells treated with BITC shows higher susceptibility to the ITC-induced necrosis-like cell death compared with the wild-type, although the ROS production is significantly inhibited. The BITC treatment more rapidly depletes intracellular ATP source in the Rho-0 cells than in wild-type cells and the decline in the intracellular ATP level plays an important role in tuning the mode of cell death by BITC (Miyoshi et al., 2008). The Rho0 MDA-MB-231 cells are resistant to ROS production and to activated mitochondrial translocation of Bax (Xiao et al., 2008). Interestingly, the stress caused by depletion of mitochondrial DNA in Rho-0 PC-3 variant also resulted in upregulation of both Bax and Bcl-2, but not Bak, in comparison with wild-type cells. It is supposed that cancer chemopreventive effect of SFN may be attenuated in the presence of antioxidants (Xiao et al., 2009).

The induction of apoptosis in SFN-treated colon carcinoma HT-29 and Caco-2 cells is associated with increased expression of the proapoptotic protein bax, the release of cytochrome *c* from the mitochondria to the cytosol, and the proteolytic cleavage of PARP-1, whereas bcl-2 was not detected (Gamet-Payrastre et al., 2000). The concentration-dependent mode of cell death is demonstrated in rat liver epithelial RL34 cells, when concentrations of ITCs below 20 μM induce apoptosis and at high concentration about 50 μM, the necrosis is the dominant mode of cell death. The mitochondrial death pathway with activated caspase-3 and caspase-9 is accompanied by generation of ROS

and superoxide, and hydroperoxides are the dominant species observed. Because glutathione depletion by diethyl maleate significantly accelerates BITC-triggered apoptosis, the apoptosis is at least partially induced through a redox-sensitive mechanism (Nakamura et al., 2002). The apoptotic effect of SFN is maintained through the metabolic processes, because both SFN and its metabolite SFN-NAC have similar activities to induce growth arrest and apoptosis (Chiao et al., 2002a). Although earlier data supposed the requirement of p53 in ITC-induced apoptosis, it seems reasonable to postulate that PEITC is effective against tumors with normal as well as mutant p53 (Xiao and Singh, 2002). The use of MAPK inhibitors shows that apoptosis is abolished in the presence of mitogen-activated protein/ERK kinase 1 (MEK-1, a kinase upstream of ERK1/2) inhibitor PD98059, while the inhibition of p38 protein kinase activation by specific inhibitor SB202190 does not prevent PEITC-induced apoptosis. The PEITC treatment creates an oxidative cellular environment that induces DNA damage and GADD153 pro-apoptotic gene activation, which in turn helps trigger apoptosis (Powolny et al., 2003). The demonstration of ROS to be the initial signal for SFN-induced apoptosis in human prostate cancer cells was published earlier (Singh et al., 2005). Mitochondrial respiratory chain complex I inhibitors, including diphenyleneiodonium chloride and rotenone, significantly attenuate the SFN-induced ROS generation. Interestingly, ectopic expression of Bcl-xL, but not Bcl-2, offers significant protection against the PC-3 cell death caused by SFN. In addition, SFN treatment increases the level of Fas, activates caspase-8, and causes cleavage of Bid protein. Thus, the results prove that ROS-initiated apoptosis in prostate cell lines uses both intrinsic and extrinsic caspase cascades. BITC and PEITC demonstrate more potent mitochondria-damaging ability than AITC and SFN. This correlates well with their stronger apoptosis-inducing potentials, induction of Bcl-2 phosphorylation, mitochondrial translocation of Bak, and disruption of Bcl-xL association with Bak and Bax in mitochondrial membrane (Tang and Zhang, 2005). The PEITC treatment of ovarian carcinoma OVCAR-3 cells induces apoptosis by caspase-3 and caspase-9 activation without the involvement of caspase-8 and anti-apoptotic Bcl-2 levels are suppressed, while Bax levels are enhanced. PEITC inhibits activation of Akt, ERK1/2 and c-Myc expression, while simultaneously activating pro-apoptotic p38 and JNK1/2 (Satyan et al., 2006). SFN-treated ovarian cancer cell line SKOV3 downregulate cyclin D1, cdk4, ckd6, total AKT, and p-AKT, and this inhibitory effect of SFN leads to a potent induction of apoptosis (Chaudhuri et al., 2007). SFN-induced apoptosis is associated with activation of Bax, downregulation of IAP (inhibitor of apoptosis protein) levels (cIAP1, cIAP2 and XIAP), which are accompanied by inhibition of nuclear translocation of p65 NF-κB. Ectopic expression of Bcl-2 fails to confer protection against SFN-induced cell death. SFN treatment markedly increases Apaf-1 protein level accompanied by an increase in transcriptional activity of E2F1 (Choi et al., 2007). BITC-induced apoptosis in MDA-MB-231 and MCF7 breast cancer cells is initiated by ROS production due to inhibition of complex III of the mitochondrial respiratory chain. BITC treatment activates JNK and P38 which function upstream of Bax activation. This MAPK activation can be abolished by overexpression of catalase and the conformational change of Bax by catalytically inactive JNK2 (Xiao et al., 2008). The exogenous expression of dominant negative caspase-8 or caspase-9 attenuates BITC-induced cell death in pancreatic cells. The phosphorylation of antiapoptotic Bcl-XL protein as well as BITC-triggered apoptosis can be decreased by inhibitors of JNK (Basu and Haldar, 2008). PEITC is a more potent inducer of apoptosis than SFN in human nonsmall lung cancer A549 cells. Similarly, the binding of PEITC to protein is higher than SNF, but the levels of oxidative damage in cells measured as ROS and protein carbonyls formation are higher in SFN- than in PEITC-treated cells. Neither PEITC nor SFN bind to DNA or RNA at detectable levels. Results suggest that direct covalent binding to cellular proteins is an important early event in the induction of apoptosis by the ITCs, and tubulin is one of the identified covalent-binding targets of ITC (Mi et al., 2007; Mi and Chung, 2008). The inhibitory effect of SFN is linked with disruption of normal tubulin polymerization and/or more subtle effects on microtubule dynamics (Jackson and Singletary, 2004). ITCs induce mitotic arrest and apoptosis with the same order of activity as disruption of microtubule polymerization (Mi et al., 2008). The use of radiolabeled ITCs reveals tubulins as major *in vivo* binding target. In contrast to

level changes in α- and β-tubulins, the levels of other cytoskeleton proteins, such as γ-tubulin, actin, and vimentin, were unchanged under the same conditions. ITC-induced tubulin depletion is initiated by aggregation and followed by proteasome-dependent degradation. Degradation is ubiquitination- and ATP-dependent, but independent of oxidative stress (Mi et al., 2009).

PEITC-induced apoptosis activates caspase 7 and 9, and cleavage of PARP-1 in MCF-7 cells (negative for caspase-3). Apoptotic cell death is characterized by downregulation of Bcl-2 and XIAP, upregulation of Bax, cytochrome *c* release, and Smad translocation. However, PEITC does not increase the expressions of p53 and p21 (Lee and Cho, 2008). BITC-induced apoptosis in some types of pancreatic cancer cells is associated with inhibition of STAT-3 signaling pathway due to reduced levels of activated and total STAT-3 protein, and, as a result, decrease of STAT-3 DNA-binding and transcriptional activities occurs (Sahu and Srivastava, 2009).

EFFECT ON IMMUNE SYSTEM SIGNALING

The chronic inflammation is associated with increased risk for tumor development. Therefore, compounds with anti-inflammatory properties have the potential to decrease the tumor incidence. The administration of SFN potentiates the NK activity in B16F-10 melanoma-induced metastasis-bearing animals. SFN enhances ADCC and ACC cytotoxicity, increases IL-2 and IFNγ production, while serum levels of proinflammatory cytokines (IL-1β, IL-6, TNF-α, and GM-CSF) are downregulated (Thejass and Kuttan, 2007). The combined treatment of RAW264.7 macrophages with SFN plus curcumin or SFN plus PEITC had synergistic effect in downregulating inflammation markers like TNFα, IL-1, NO, PGE2 probably due to induction of HO-1 and NQO-1 (Cheung et al., 2009). Ten structurally divergent synthetic ITCs were evaluated in human colorectal carcinoma cells HT-29 and RAW264.7 murine macrophages for basal transcriptional activation of NF-κB and the inflammatory response to bacterial LPS. Production of pro-inflammatory mediators and cytokines (iNOS, COX-2, IL-1β, IL-6, and TNF-α) is reduced by ITCs treatment that correlates with the downregulation of NF-κB signaling pathways. The results confirm stronger anti-NF-κB and anti-inflammatory activities of synthetic ITC than the natural ones (Prawan et al., 2009). SFN administration enhances cytotoxicity of natural killer (NK) cells and dendritic cells (DC) coculture against TRAMP-C1 target cells, which correlated with infiltration of T cells in the neoplastic lesions and increased levels of IL-12 production by the DC (Singh et al., 2009). Bactericidal effect of SFN was evaluated in mouse and 48 patients positive for *Helicobacter pylori*-infection. SFN reduces gastric bacterial colonization, attenuates mucosal TNFα and IL-1β expression, dependent on functional Nrf2 gene expression. In patients assigned to consume broccoli sprouts downregulation of biomarkers of *H. pylori* colonization and biomarkers of gastric inflammation (serum pepsinogen I and II) is confirmed. This treatment seems to enhance chemoprotection of the gastric mucosa against *H. pylori*-induced oxidative stress (Yanaka et al., 2009). Both MBTI (4-methylthiobutylisothiocyanate) and its oxidized derivative SFN inhibit growth of activated keratinocytes and arrest the activated THP-1 monocytes in the G2 stage. MTBI downregulates proinflammatory genes TNFα and IL12/23 p40, as well as ICAM-1 protein expression in activated THP-1 cells. Results show that MTBI may represent a compound possessing significant skin inflammation-preventive activities (Yehuda et al., 2009). Cell-mediated immune response of normal as well as Ehrlich ascites tumor-bearing BALB/c mice fed with SFN was followed. SFN increases NK activity, ADCC (antibody-dependent cellular cytotoxicity) and ACC (antibody-dependent complement-mediated cytotoxicity), IL-2, and IFNγ production. SFN enhances the proliferation of splenocytes, bone marrow cells, and thymocytes by stimulating the mitogenic potential of various mitogens such as concanavalin A, phytohaemagglutinin, poke weed mitogen, and lipopolysaccharide (Thejass and Kuttan, 2006). In rat glial cells SFN attenuates the LPS-induced production and release of TNFα, IL1β, IL6, and nitric oxide (NO) and increases both mRNA and activity of NQO-1 and cellular GSH content (Wierinckx et al., 2005). Overproduction of both NO and prostaglandins (PGE) is associated with numerous pathological conditions including chronic

inflammation and cancer. ITC attenuates production of PGE-2 as well as iNOS, and decreased COX-2 expression is associated with the inactivation of NF-κB and stabilization of IκBα in Raw 264.7 cells (Rose et al., 2005c).

ARE AND GENE EXPRESSION PROFILES

Antioxidant/electrophile response element (ARE)-regulated phase II enzyme and antioxidant genes are induced through activation of the Nrf2 (transcription factor nuclear factor-E2-related factor 2), which is regulated by the thiol-rich sensor protein Keap1 (Kelch-like ECH-associated protein 1). In contrast to previously studied ARE inducers, SFN treatment *in vivo* does not lead to the accumulation of ubiquitinated Keap1, because previously characterized ARE inducers target central linker domain of Keap1 and not Kelch domain, which is modified by SFN. A novel mechanism for Nrf2 stabilization by SFN-Keap1 thionoacyl adducts formation is suggested (Hong et al., 2005). The analysis of the expression profiles focused on the effect of Nrf2 acting on the antioxidant response element (ARE) located at the 5′-flanking region of regulated genes confirms modulation of detoxification phase I, II drug metabolizing enzymes and phase III transporters. Unexpected clusters of regulated genes include genes for HSP, ubiquitin/26S proteasome subunits, and lipid metabolism genes (Hu et al., 2006a). Novel xenobiotic-metabolizing genes regulated by Nrf2 and inducible by PEITC are CYP 2c55, CYP 2u1, and aldehyde oxidase (Hu et al., 2006b). Analysis of the acute effect of SFN on gene expression profile in intestinal polyps in $Apc^{-/+}$ mice using cDNA microarray reveals that genes involved in apoptosis, cell growth, and maintenance rather than the predicted phase II genes are modulated. Upregulation of proapoptotic genes MBD4, TNFR-7, and TNF (ligand)-11, downmodulation of pro-survival genes including cyclin-D2, integrin-β1 and Wnt-9A, as well as COX-2 and upregulation of 15-LOX genes involved in colorectal carcinogenesis is observed. SFN regulates different set of genes in the small intestinal polyps of $Apc^{-/+}$ mice, which could contribute to the overall chemopreventive pharmacological effects (Khor et al., 2006a). Gene expression profile of SFN and iberin effect on primary prostate epithelial and stromal cells derived from benign prostatic hyperplasia tissue are performed and real-time PCR is used to confirm changes of a subset of genes. SFN induces more changes in epithelial cells, whereas iberin is more effective in stromal cells, but similar pathways are affected such as cell cycle pathway, detoxification pathway or genes associated with cancer prevention. Both ITCs increase expression of PLAGL1, a tumor suppressor gene, in stromal cells and suppress expression of the putative tumor promoting genes IFITM1, CSPG2, and VIM in epithelial cells (Chambers et al., 2009).

INFLAMMATION

Transcription factor Nrf2 is a guardian of redox homeostasis. Nrf2 knockout mice are hypersensitive to the neuroinflammation induced by LPS. Markers of inflammation released from microglial cells—inducible NO synthase, IL-6, and TNF-alpha are attenuated by cotreatment with SNF (Innamorato et al., 2008). The physiological or biochemical factors present in the GI tract that may influence the biological properties of ITCs are rather unknown. Because endogenous concentrations of H_2S are reported to range between 0.2 and 3.4 mM in the GI tract of mice and humans, this can also influence the effect of ITC treatment. Although PEITC induces cell death in HCT116 adenocarcinoma cell line *in vitro* through classic apoptotic mechanisms, physiological concentration of H_2S prevents PEITC-mediated apoptosis (Rose et al., 2005b). Antimicrobial peptides play an important role in the innate immune system protecting the intestinal mucosa against bacterial invasion. In addition to butyrate, β-defensin-2 protein production is increased in response to SFN also. Moreover, SFN induces the expression of vitamin D receptor, peroxisome proliferator-activated receptor gamma and phosphorylated ERK1/2 but does not affect p38 MAPK activation. The data clearly demonstrate for the first time that the dietary HDAC inhibitor SFN is able to induce antimicrobial peptides in colonocytes (Schwab et al., 2008).

ANTIAGING EFFECT

SFN has a high potency as an inducer of phase II enzymes and regulates the expression and function of different cytochrome P-450 genes. Such effects may indicate a mechanism for the preventive role that SFN is believed to play against the degenerative events of aging and chronic diseases (Fimognari et al., 2008). Spontaneously hypertensive stroke-prone rats undergo premature aging of the CNS compared with the related normotensive Wistar Kyoto rats due to increased expression of inducible nitric oxide synthase and increased astrogliosis. Broccoli sprouts of cultivar containing high amounts of glucoraphanin (precursor of SFN) diet significantly decreases the aging-related degenerative changes in stroke-prone rat CNS (Noyan-Ashraf et al., 2005). Ultraviolet B irradiation induces skin damage and inflammation and severe sunburn reactions are involved in the development of skin cancer as well as in the promotion of skin aging. In immortalized human keratinocytes HaCaT SFN treatment inhibits p38, ERK and SAPK/JNK activation and reduces UVB-induced COX-2 protein expression. Moreover, UVB-induced skin thickness in hairless mice, COX-2 protein expression and hyperplasia are all suppressed by feeding SFN to the mice (Shibata et al., 2009). Results show that SFN has a potential use as a compound for protection against UVB-induced skin inflammation. Recent studies suggest that the redox equilibrium of dendritic cells is a key factor in maintaining protective cellular immunity and that a disturbance of this homeostatic mechanism could contribute to immune senescence. Aging was associated with a decreased contact hypersensitivity response that was accentuated by Nrf2 deficiency. Systemic SFN treatment reversed this decrease through Nrf2-mediated antioxidant enzyme expression and GSH synthesis. SFN and NAC upregulate Th1 immunity in aging through a restoration of redox equilibrium (Kim et al., 2008).

ENDOCRINE REGULATION

Despite the known chemopreventive activities for a variety of neoplasms including breast cancer, the molecular mechanism has not been established. The model of estrogen-stimulated cell growth provides evidence that both BITC and PEITC inhibit estrogen-stimulated cell growth, abrogate ERα transcriptional activity and hence block expression of pS2, an estrogen responsive gene. ITCs function as ERα disruptors that abrogate mitogenic estrogen signaling in ER-positive breast cancer cells, which provides a molecular explanation for the growth inhibitory function of ITCs in breast cancer development (Kang et al., 2009). Another mechanism for downmodulation of ERα protein levels may involves increased proteasome-mediated degradation, because proteasome inhibitor MG132 reverses downmodulation of ERα protein and SFN increases the expression of 20S catalytic core subunit PSMB5 in MCF7 cells (Ramirez and Singletary, 2009). Transgenic mouse model of prostate LNCaP cancer can demonstrate the effect of SFN on decrease of androgen receptor (AR) and its phosphorylation that is accompanied by decline of intracellular as well as secreted levels of PSA, an AR-regulated gene product. SFN treatment inhibits AR promoter activity as well as synthetic androgen (R1881)-stimulated nuclear translocation of AR. Naturally occurring thio analogues (iberverin, erucin, and berteroin), but not the sulfonyl analogues (cheirolin, erysolin, and alyssin sulfone) of SFN are also effective in reducing protein levels of AR in LNCaP cells (Kim and Singh, 2009). It means that dual mode of ITC action is corroborated, at transcriptional level via inhibition of the transcription factor Sp1 and at post-translational level by accelerating protein degradation (Wang et al., 2006). Novel targets are defined in Caco-2 cells, when SFN treatment downregulates serotonin receptor 5-HT, a neurotransmitter receptor and increases nicotinic acetylcholine receptor protein level (Mastrangelo et al., 2008). SFN inhibits the norepinephrine-mediated increase of cell viability in pancreatic duct epithelial cells and in addition SFN inhibits norepinephrine-mediated increase of the IL-6 levels although the VEGF levels are not affected (Chan et al., 2008). SFN is a specific antagonist of human SXR (steroid and xenobiotic receptor), binds directly to SXR and inhibits SXR-mediated induction of drug clearance, including taxol, rifampicin, clotrimazole, phenobarbital, the herbal antidepressant St John's wort, or protease inhibitor ritonavir. CYP3A4,

MDR1 and some other genes are regulated by SFN; therefore, these findings could lead to potentially important new therapeutic and dietary approaches to reduce the frequency of adverse drug reactions that are secondary to SXR-mediated induction of drug clearance (Zhou et al., 2007).

ABC TRANSPORTERS

ITCs permeate into cells rapidly and accumulate in cells primarily as glutathione (GSH) conjugates. It was demonstrated that multidrug-resistant sublines of HL-60 are sensitive to cytotoxic effect of ITCs (Jakubikova et al., 2005a), and a question remains about the interaction of ITCs with ABC transporter proteins. BCRP-overexpressing breast and lung carcinoma cell lines have lowered cellular accumulation of PEITC that is reversible by BCRP inhibitor fumitremorgin C (Ji and Morris, 2004). Thus, PEITC and/or its cellular metabolites may represent BCRP substrates, and BCRP inhibitors have the additional potential for diet-drug interactions. PEITC in its unchanged form is transported from BCRP-overexpressing MCF-7/MX100 cells and relatively high (100 μM) concentration of PEITC effectively inhibited BCRP ATPase activity (Ji and Morris, 2005). SFN is accumulated in mammalian cells by up to several hundred-fold over the extracellular concentration, primarily by conjugation with intracellular GSH, that are rapidly exported mainly from cells with overexpressed MRP-1 or Pgp-1 (Zhang and Callaway, 2002). PEITC conjugates are shown as MRP1 substrates, while PEITC is not Pgp substrate. BITC and PEITC deplete intracellular GSH, inhibit MRP1 activity, and GSH is a cosubstrate for daunomycin efflux via MRP1 (Hu and Morris, 2004). Inhibition Pgp- and MRP1-mediated efflux of daunomycin and vinblastin provide the opportunity to enhance the effectiveness of cancer chemotherapy (Tseng et al., 2002). The carcinoma cell lines HepG2, Caco-2, and A549 (liver, colon, lung) were treated with SFN and erucin to evaluate the effect of ITC on regulation of phase III detoxification system expression and activity. Neither SFN nor erucin affects P-gp expression in any of the cell lines tested. HepG2-treated cells upregulate protein levels of MRP1 and MRP2, in lung cells both mRNA and protein levels are increased more effectively by SFN than erucin, and MRP1-dependent efflux of fluorescent probes in A549 cells is more effectively increased by SFN than erucin. Dietary components modulate phase III detoxification system and this finding should be evaluated before being recommended for use during chemotherapy (Harris and Jeffery, 2008).

REDOX REGULATION

Sulforaphane can stimulate cellular adaptation to redox stressors through transcription factor Nrf2 wild-type mouse embryonic fibroblasts pretreatment for 24 hours with 3 μM SFN exhibits between 1.4-fold and 3.2-fold resistance against thiol-reactive electrophiles, including isothiocyanates, α,β-unsaturated carbonyl compounds (e.g., acrolein), aryl halides, alkene epoxides, hydroperoxides (e.g., cumene hydroperoxide, CuOOH), free radical-generating compounds (e.g., menadione), and genotoxic electrophiles (e.g., chlorambucil). Nrf2-dependent upregulation of GSH is the principal mechanism by which sulforaphane pretreatment induces resistance to acrolein, CuOOH and chlorambucil, but not menadione. By contrast, Nrf2(–/–) MEFs are typically less tolerant of these agents than wild-type fibroblasts, and SFN pretreatment does not protect the mutant cells against xenobiotics (Higgins et al., 2009). 6-HITC (6-methylsulfinylhexyl ITC) facilitates the sustained phosphorylation of ERK and the autophosphorylation of the nerve growth factor receptor TrkA. Protein tyrosine phosphatase (PTP) 1B acts as a phosphatase capable of dephosphorylating Tyr(490) TrkA and is inactivated by 6-HITC in a redox-dependent manner. This provides new clues about the chemoprotective potential of food components in the growth, survival and functional maintenance of neurons (Shibata et al., 2008). Chronic lymphocytic leukemia cells are highly sensitive to PEITC treatment (IC_{50} values about 5.5 μM), while normal lymphocytes are less sensitive (IC_{50} 27 μM). The leukemia cells exposed to PEITC generate ROS, induce GSH depletion and oxidation of mitochondrial cardiolipin and cell death. Study demonstrates the ROS-induced deglutationylation of MCL1, followed by a rapid degradation of this cell survival molecule (Trachootham et al., 2008).

Similarly, redox-based effect of PEITC is effective in killing of chronic myelogenous leukemia with T315I mutation of Bcr-Abl that is insensitive to Gleevec and other Bcr-Abl targeted drugs (Zhang et al., 2008).

COMBINED TREATMENT

The combination of drugs is a common principle to achieve better treatment efficiency, in most cases designed to hit multiple targets and reach the additive effect or to potentiate effect through the synergistic action and in some cases to sensitise the cells to the cytotoxic effect of another compound present in the mixture. ITCs are known as chemopreventive compounds and their unusually high protective potency in inhibitory or blocking phases in carcinogenesis is proved in multiple animal models. The following examples consists of ITC in combination with carcinogen, another chemopreventive compound/drug (curcumin, aspirin, TRAIL) or treatment (γ-irradiation). ITCs effectively provide significant reductions of tumor multiplicity in NNK lung tumorigenicity regardless of dose number (Morse et al., 1992), effect correlates with arylalkyl isothiocyanates with increasing alkyl chain length (Morse et al., 1989), and decreased metabolic activation of NNK (Staretz and Hecht, 1995). The inhibition of *N*-nitrosomethylbenzylamine (NMBA)-induced tumor incidence was highly correlated with the percentage inhibition of either 7-methylguanine or O6-methylguanine (Morse et al., 1993). BITC but not PEITC significantly inhibited lung tumorigenesis by BaP, whereas PEITC but not BITC significantly inhibited forestomach tumorigenesis (Lin et al., 1993). The structure of ITCs is important for the effect because tumor multiplicities is inhibited nearly up to 100% by PEITC or PPITC while BITC and PBITC have little inhibitory effect on tumor multiplicity and no effect on NMBA tumor incidence (Wilkinson et al., 1995). PEITC has tendency to lower the incidences of liver and renal tumors and is remarkably effective chemopreventive agent for the BOP-induced lung and pancreatic tumors in hamsters, (Nishikawa et al., 1996). ITCs significantly reduce the lung tumorigenesis induced by a mixture of BaP and NNK. Dietary PEITC or combination of PEITC plus BITC is more effective in these experiments than the compounds given by gavage (Hecht et al., 2000). Protective effect is usually based on inhibition of phase I enzymes (cytochrome P450) and activation of phase II enzymes (GST, NQO-1, HO-1). But there are some results that show the stimulatory effect of ITCs on chemically induced tumorigenesis.

PEITC has been found to induce urinary bladder carcinomas in F344 male rats. Dysplasia, papilloma, and carcinoma incidences and multiplicities are dramatically decreased by simultaneous *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine BBN and BITC treatment. In contrast, epithelial hyperplasia of urinary bladder is induced in rats treated with BITC alone (Okazaki et al., 2002). Bromodeoxyuridine labeling indices were increased by PEITC administration even in normal-looking epithelium. While the simple and papillary or nodular hyperplasia induced by PEITC is reversible, dysplasia is irreversible with the potential to give rise to nonpapillary carcinomas with frequent p53 mutations (Sugiura et al., 2003).

There are combinations of ITC and different compounds that potentiate the protective effect of ITCs in tumorigenic models or enhance the cytotoxic effect of ITCs alone. The combination of aspirin and PEITC has protective effects in the early stages of tumor progression initiated by tobacco-specific lung carcinogen NNK in Wistar rats (Ye et al., 2007). Treatment of PC-3 xenografts in nu/nu mice by i.p. injections of PEITC inhibits the growth of tumor and decreases activities of both PI3K/Akt and NF-κB signaling pathways. Stronger growth-inhibitory effect on xenografts, decreased incidence of tumor formation, and reduced number of high-grade PIN (prostatic intraepithelial neoplasia) is conferred by the combined curcumin and PEITC treatment (Barve et al., 2008; Khor et al., 2006b). Combination PEITC plus curcumin suppress the effect of EGF on downstream activation of EGFR. Simultaneous targeting of these signaling pathways by PEITC and curcumin could be the "complex" molecular targets by which a natural compound combination exert additive inhibitory effects on cell proliferation and ultimately lead to programmed cell death of tumor cells (Kim et al., 2006b).

TRAIL-resistant hepatoma cells treated with SFN are selectively sensitized to TRAIL-induced apoptosis but not to TNFα- or Fas-mediated one. SFN treatment induces generation of ROS and subsequent significantly upregulated mRNA and protein levels of DR5, a death receptor of TRAIL. The apoptosis induction is effective also in Bcl-2 and Bcl-xL overexpressing cells that are sensitized to apoptosis by combined treatment of SFN and TRAIL (Kim et al., 2006a). The combined treatment of Saos2 and MG63 human osteosarcoma cells with SFN plus TRAIL increases Bid cleavage, activation of caspases –8, –10, –9, –3, DR5 expression, but no such effects of combined treatment in PBMNC occurs (Matsui et al., 2006). TRAIL-resistant lung adenocarcinoma A549 cells treated with combination of SFN and TRAIL induce apoptotic cell death associated with activation of caspase-3, P38, JNK and downregulation of ERK and AKT. Inhibitors of ERK or Akt, but not p38 MAPK, resulted in significantly decreased cell viability (Jin et al., 2007). Therapeutic potential of combination TRAIL plus SFN is evaluated on orthotopically implanted PC-3 tumors in mice. SFN induces activation of caspases-3 and caspase 9; increases expression of DR4 and DR5, Bax, Bak, Bim, and Noxa and generation of ROS. Treatment decreases Bcl-2, Bcl-X(L), and Mcl-1 expression and induces inhibition of NF-κB, PI3K/AKT and MEK/ERK pathways. TRAIL in combination with SFN is more effective in inhibiting markers of angiogenesis and metastasis than single agent alone (Shankar et al., 2008).

Tumor-initiating cell (TIC)-like characteristics of pancreatic carcinoma cell lines is defined by $CD44^+/CD24^-$ phenotype, the potential to grow in immunodeficient mice, apoptosis resistance, colony- and spheroid-forming capacity, and invasion and differentiation potential. SFN reduces the DNA binding of transactivation-competent NF-κB dimer but not their nuclear localization and thereby may downregulate the expression of apoptosis inhibitors (XIAP, cIAP1 and FLIP) and induce apoptosis, together with prevention of clonogenicity. In a xenograft model, SFN strongly blocks tumor growth and angiogenesis, while combination with TRAIL has an additive effect without obvious cytotoxicity in normal cells. In addition, freshly isolated patient tumor cells expressing markers for TICs can be sensitised by SFN for TRAIL-induced cytotoxicity. Combination of SFN with TRAIL treatment represents a promising strategy for targeting of pancreatic TICs (Kallifatidis et al., 2009).

The treatment SFN plus doxorubicin restores chemosensitivity of tumor cells and induces apoptosis in doxorubicin-resistant p53(Ser220) and p53 knock-out cells, irrespective of p53 status (Fimognari et al., 2006). The treatment of human colon cancer cell lines with combination SFN and 3,3′-diindolylmethane (DIM), a condensation product of I-3C results in G2M arrest at higher doses. At low total drug concentration, all combinations are antagonistic; with increasing concentrations, the antagonistic effect gradually turns into a synergistic interaction at the highest combined cytotoxic concentration (Pappa et al., 2007). Obtained data stress the need for elucidating mechanistic interactions for better predicting beneficial health effects of bioactive food components. The combination of TNFα plus SFN induced apoptosis in TNFα-resistant leukemia cell lines. SFN inhibits TNFα-induced NF-κB activation through the suppression of IκBα degradation, leading to reduced expression of NF-κB-regulated gene products. Such inhibitory effect correlated with the suppression of NF-κB-dependent genes involved in apoptosis (IAP-1, IAP-2, XIAP, Bcl-2, and Bcl-xL), cell proliferation (c-Myc, COX-2, and cyclin D1), and metastasis (VEGF and MMP-9). Production of ROS and subsequent activation of caspase-3 is required for apoptosis (Moon et al., 2009). The combined treatment of pancreatic cell line BxPC3 with γ-irradiation plus BITC potentiated apoptosis-inducing effect of γ-irradiation, enhances arrest of cells in G2M associated with DNA damage leading to phosphorylation of ATR, Chk2, Cdc25, Cdk1, and induction of p21. The apoptosis induction is associated with NF-κB inhibition and p38 activation (Prakash et al., 2009).

ANGIOGENESIS AND INVASIVENESS

SFN interferes with all essential steps of neovascularization from proangiogenic signaling and basement membrane integrity to endothelial cell proliferation, migration, and tube formation. SFN

treatment of HMEC-1 (immortalized microvascular endothelial cells) in a human *in vitro* anti-angiogenesis model induces inhibition of hypoxia-induced VEGF and two angiogenesis-associated transcription factors HIF-1α and c-Myc expression. Other downregulated genes are vascular endothelial growth factor receptor KDR-flk-1, MMP-2, the predominant endothelial collagenase and its tissue inhibitor TIMP2 (Bertl et al., 2006). SFN-induced HIF-1α downmodulation was through activated JNK and ERK signaling pathways in human tongue squamous cancer and prostate cancer DU154 cells, without involvement of AKT pathway (Yao et al., 2008).

PEITC inhibits hypoxia-induced activation of HIF1α—a transcription factor that plays an important role in expression of pro-angiogenic factors, including the treatment with hypoxia mimetic effect of cobalt chloride, and the inhibition of angiogenesis may be an important mechanism in cancer chemoprevention by PEITC. PEITC inhibits induction of HIF-1α target genes CAIX, GLUT1, BNIP3, and VEGF-A independently of the activity of prolyl hydroxylases, the Von-Hippel-Lindau protein and the proteasome, all of which are required for the normal rapid turnover of HIF-1α in normoxia. PEITC decreased phosphorylation of translational inhibitor 4E-BP, that stimulated binding to eIF4E leading to repression of cap-dependent translation initiation. Thus, the attenuated cap-dependent translation of HIF-1α RNA is responsible for inhibitory effect of PEITC on HIF-1α activity (Wang et al., 2009). PEITC treatment caused a decrease in survival of HUVEC cells. The capillary-like tube structure formation (*in vitro* neovascularization) and migration (invasion potential) by HUVEC cells are inhibited significantly in the presence of PEITC. Inhibition of angiogenic features of HUVEC is mediated by suppression of VEGF secretion, downregulation of VEGFR2 expression, and inhibition of AKT. PEITC-mediated inhibition of PC-3 cell migration is significantly attenuated by ectopic expression of constitutively active Akt (Xiao and Singh, 2007). The SFN treatment of MDA-MB-231 cells downregulates MMP7 and MMP14 mRNA as well as Twist1 and POU5F1, transcription factors that mediate epithelial-mesenchymal transition and the self-renewal of undifferentiated embryonic stem cells. SFN reduces also the production of pro-inflammatory cytokines IL-1β, IL-6, TNF-α, IFN-γ, immunomodulating cytokine IL-4 and growth factors involved in angiogenesis PDGF and VEGF (Hunakova et al., 2009). The invasiveness of MDA-MB-231 cells and TPA-induced MMP-9 activity are suppressed by extracts of broccoli and Rorripa in a concentration-dependent manner and inhibitory effects of each vegetable correlate with the presence of SFN and 7-methylsulphinylheptyl ITC in extracts (Rose et al., 2005a). PEITC treatment of human hepatoma SK-Hep1 cells inhibits proliferation, invasion, and migration of cells. Theses effects on cellular level are accompanied by downgulation of MMP-2, MMP-9, MT1-MMP mRNA levels, while expression of TIMP-1 and TIMP-2 inhibitors is upregulated (Hwang and Lee, 2006). Using the HUVECs model (human umbilical vein endothelial cells) SFN inhibits tube formation on matrigel, but does not affect MMP production. Results demonstrate the anti-angiogenic activity of SFN (Asakage et al., 2006). Human brain microvascular endothelial cells treated with SFN selectively decrease the PMA-induced secretion of MMP-9 but not that of MMP-2 which is proportional to a decrease in the expression of the mRNA stabilizing factor HuR protein. SFN selectively inhibit MMP-9-activated, but not basal, HBMEC migration, and fails to inhibit PMA-induced tubulogenesis (Annabi et al., 2008).

EFFECT ON EPIGENETIC REGULATION

The novel mechanism of chemoprevention by SNF is described in Myzak et al., (2004). SFN inhibits HDAC activity and subsequently increases the level of acetylated histones. Enhanced interaction of acetylated H4 with promoter region of P21 and BAX genes leads to increased protein levels of p21 and Bax (Myzak et al., 2006b). The p21 expression due to inhibition of HDAC activity is increased in ileum, colon, prostate, and PBMNC (Myzak et al., 2006a). PEITC treatment downmodulates the activity and protein level of HDAC in prostate LNCaP cancer cells and concurrently demethylates the promoter and restores the unmethylated GSTP1 to the level found in normal prostatic cells. PEITC mediates the cross-talk between the DNA and chromatin in demethylating and

reactivating GSTP1 genes (Wang et al., 2007). Downregulated HDAC activity and increased global histone acetylation in PBMNC of human subjects on SFN-rich diet provide evidence that one mechanism through which SFN acts as a cancer chemopreventive agent *in vivo* is through the inhibition of HDAC activity, that in addition may be used as a biomarker for assessing exposure to novel dietary HDAC inhibitors in human subjects (Myzak et al., 2007).

- Dietary isothiocyanates can inhibit many types of tumor formation in animal models and their consumption is inversely correlated with the risk of human cancers including lung, breast, and colon.
- The complex pattern of the effects of ITCs may depend on both the genetic background of cells and/or the concentration ranges used.
- Chemopreventive activities of ITCs are based on the modulation of enzymes required for the activation or detoxification of carcinogens, inhibition of pro-inflammatory and pro-angiogenic pathways.
- ITCs potentiate the cytotoxic effect of chemotherapeutic drugs, sensitise cells to TRAIL treatment and influence the epigenetic regulation of genes expression, modulate epithelial-mesenchymal transition, and effectively target the tumor-initiating cells.

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7 Is Tulsi a Panacea for Cancer Prevention and/or Therapy? *An Evidence-Based Revisit*

Manjeshwar Shrinath Baliga, Farhan Fazal, Rajesh Wilson, and Rajesh Arora

CONTENTS

INTRODUCTION

Ocimum sanctum L. syn *Ocimum tenuiflorum* L. (Mint family: *Lamiaceae*), commonly known as "Holy Basil" in English, "Tulsi" in Hindi and Sanskrit is a bushy fragrant plant found in the semi-tropical and tropical regions of the world (Chopra et al., 1982). The plant is grown all over India for

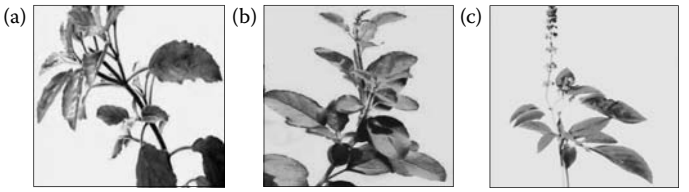

FIGURE 7.1 Different Tulsi varieties commonly used in India. (a) Shyama or Krishna Tulsi. (b) Rama Tulsi or Sri Tulsi. (c) Vana Tulsi (or forest Tulsi).

its medicinal and religious purposes in houses, temples, and gardens. It is also grown on commercial basis in vast stretches of farm lands to cater to the herbal, cosmetic, and pharmaceutical industries (Sathyavathi et al., 1987; Gupta et al., 2005). There are two main morphotypes of Tulsi, the purple leafed or dark variety, commonly known as the *Shyama* or *Krishna Tulsi* (Figure 7.1a) and the green or light-colored leafed variety known as *Rama Tulsi* or *Sri Tulsi* (Figure 7.1b). Rama Tulsi is regularly used for worshiping and is the more common of the two (Chopra et al., 1982; Sathyavathi et al., 1987; Rastogi and Mehrotra, 1993; Singh and Hoette, 2002; Gupta et al., 2005). A third type, commonly known as *Vana Tulsi* (or forest Tulsi), is *O. gratissimum* (Sathyavathi et al., 1987) (Figure 7.1c).

TULSI IN INDIAN TRADITION

In ancient Hindu scriptures, Tulsi occupies the supreme position among the herbs, so much so that it is referred to as "Mother." The ancient works of *Padmapurana* and *Tulsi Kavacham* describe Tulsi as a protector of life, accompanying the human being from birth up till death (Dymock et al., 1893). The ancient sages or rishis of yore ensured its integration into daily life by incorporating it in religious rituals.

The medicinal use of Tulsi is well documented and is extensively used in the Indian traditional systems of medicine, the Ayurveda, Siddha, Unani, and the Asian folk medicine in India, Srilanka, Nepal, Indonesia, Malaysia, and Burma for treating various diseases either alone or in combination with other plants (Satyavathi et al., 1987). Tulsi has been used for thousands of years for its diverse healing properties and is regarded in Ayurveda as "*elixir of life*" that promotes longevity. In the ancient ayurvedic text, the *Charaka Samhita*, Tulsi has been documented to be of immense use in the treatment of common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning, and malaria (Gupta et al., 2002).

All parts of Tulsi are known to possess therapeutic potentials and used for various ailments either alone or in combination with other medicinal plants, and its modern medical applications are receiving widespread attention (Gupta et al., 2002; Prakash and Gupta, 2005; Singh et al., 2007). The leaf juice is used in ayurvedic eye drop preparations and is recommended for glaucoma, cataract, chronic conjunctivitis, and other painful eye diseases. The juice of fresh leaves are given to patients to treat chronic fever, dysentery, hemorrhage, and dyspepsia (Uma Devi, 2001; Gupta et al., 2002; Prakash and Gupta, 2005; Singh et al., 2007).

In home remedies, Tulsi is traditionally taken in many forms: as herbal tea, dried powder, fresh leaves, or mixed with honey or ghee. The aqueous decoction of Tulsi leaves is given to patients suffering from gastric and hepatic disorders (Gupta et al., 2002; Prakash and Gupta, 2005). For centuries, the dried leaves of Tulsi have been mixed with stored grains to repel insects. A decoction of Tulsi leaves is a popular remedy for cold (Gupta et al., 2002; Prakash and Gupta, 2005; Singh et al., 2007). Tulsi leaves also check vomiting and has been used as an antihelminthic. Fresh Tulsi leaves are taken with black pepper in the morning as a prophylactic against malaria (Gupta et al., 2002; Prakash and Gupta, 2005; Singh et al., 2007).

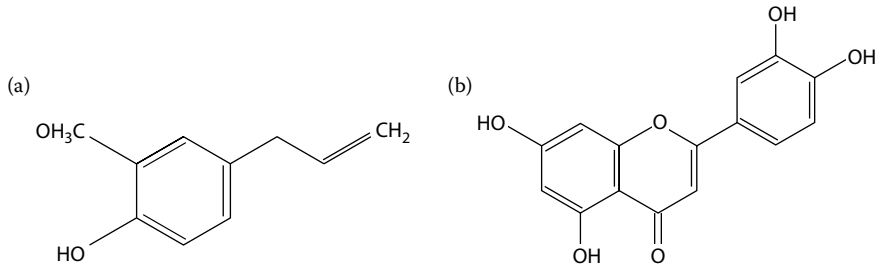

FIGURE 7.2 Chemical structure of eugenol (a) and luteolin (b).

The pharmacological properties of *O. sanctum* have been investigated in detail (Dadkar et al., 1987; Singh et al., 1991). Experimental studies in the recent past have shown that the aqueous and alcoholic extract from the leaves have various pharmacological activities like anti-inflammatory, analgesic, antipyretic, antiasthmatic, antiemetic, diaphoretic, antidiabetic, hepatoprotective, hypotensive, hypolipidemic, and antistress agents (Sarkar and Pant, 1989; Sarkar et al., 1990; Singh et al., 1996; Prakash and Gupta, 2005). The seeds are reported to possess hypoglycemic, hypouricemic and uricosuric, anti-inflammatory and analgesic properties (Sarkar and Pant, 1989; Sarkar et al., 1990; Singh et al., 1996; Uma Devi, 2001; Gupta et al., 2002; Prakash and Gupta, 2005; Singh et al., 2007). On distillation, the Tulsi plant yields pale yellow-colored fixed oil, and studies have shown that the leaves contain the highest percentage of oil (Singh et al., 2007). The oil of Tulsi has antibacterial, antioxidant and anti-inflammatory properties and is used extensively in the pharmaceutical industry mainly for skin cream preparations (Singh et al., 2007).

Eugenol (Figure 7.2a) is identified as one of the major active constituent and is reported to possess myriad benefits (Prakash and Gupta, 2005). Tulsi is also reported to possess eugenol methyl ester, caryophyllene, terpinene-4-ol, (+)-δ-cadinene, 3-carene, alpha-humulene, citral, (–)-trans-caryophyllene, eugenal, 6-allyl-3′,8-dimethoxy-flavan-3,4′-diol, 6-allyl-3-(4-allyl-2-methoxy phenoxy)-3′,8-dimethoxyflavan-4′-ol, 5-allyl-3-(4-allyl-2-methoxyphenoxymethyl)-2-(4-hydroxy -3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran,1,2-bis(4-allyl-2-methoxyphenoxy)-3-(4-hydroxy-3-methoxyphenyl)-3-methoxypropane, 1-(4-hydroxy-3-methoxyphenyl)-1,2,3-tris (4-allyl-2-methoxyphenoxy) propane, 1-allyl-4-(5-allyl-2-hydroxy-3-ethoxyphenoxy)-3-(4-allyl-2-methoxyphenoxy)-5-methoxybenzene, 3-(5-allyl-2-hydroxy-3-methoxyphenyl)-1-(4-hydroxy-3-methoxyphenoxy)-prop-1-ene, α-pinene, β-pinene, α-camphor, carvacrol, luteolin (Figure 7.2b), methylchavicol, limatrol, caryophylline, decyladehyde, cirsilineol, cirsimaritin, isothymusin, isothymonin, apigenin, rosmarinic acid, and cervacrol (Kelm et al., 2000; Prakash and Gupta, 2005; Anandjiwala et al., 2006; Singh et al., 2007; Zheljazkov et al., 2008; Suzuki et al., 2009).

CANCER PREVENTIVE AND THERAPEUTIC PROPERTIES

Cancer is the second-leading cause of death worldwide, only preceded by cardiovascular diseases. According to IARC-WHO estimates, cancer rates are set to increase at an alarming rate, from 10 million new cases globally in 2000 to 15 million in 2020 (Mignogna et al., 2004). Recently there has been an exponential growth in the use of complementary and alternative medicine as natural therapies to prevent cancer and also to reduce the treatment-induced adverse side effects (WCRF/AICR, 2007). Compelling scientific studies with both *in vitro* and *in vivo* systems have shown that Tulsi possesses anticancer, chemopreventive, and radioprotective effects and at nontoxic concentrations (Uma Devi, 2001; Gupta et al., 2002; Arora et al., 2005; Prakash and Gupta, 2005; Singh et al., 2007). Here an attempt is made to summarize the use of Tulsi as an anticancer agent, chemopreventive agent and as a radioprotective agent.

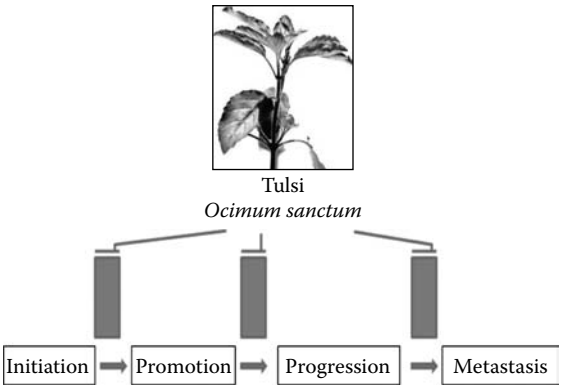

FIGURE 7.3 Putative steps where Tulsi can be used to stop/retard carcinogenesis.

Chemopreventive Effects

Cancer chemoprevention has traditionally been defined as a dietary or therapeutic approach for the prevention, delay, or reversal of carcinogenesis with nontoxic agents (Sporn and Suh, 2000). Epidemiological studies have provided convincing evidence that natural dietary compounds can modify the process of carcinogenesis which includes the three decisive steps: initiation, promotion, and progression in several types of human cancer. Experimental studies have also validated the efficacy of a number of bioactive dietary components, supporting the acceptance of natural dietary compounds as chemopreventive agents in the near future. Tulsi is able to stop initiation, promotion, and progression of cancer (Figure 7.3) and the ability of Tulsi to render chemopreventive effect are discussed in the ensuing sections.

Prevention of Oral Carcinogenesis

The chemopreventive effects of fresh paste, aqueous extract, and ethanolic extract of Tulsi leaves were studied against the 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced hamster buccal pouch carcinogenesis. Topical application of the leaf paste and oral administration of the Tulsi extracts reduced the incidence of papillomas and squamous cell carcinomas with an increase in the survival rate. Application of the leaf paste (1 g/kg body weight), aqueous extract (30 mg/kg body weight) and ethanolic extract (30 mg/kg body weight) reduced papillomas at 10 weeks and in carcinomas at the end of 16 weeks (Karthikeyan et al., 1999a).

Oral feeding of the ethanolic extract decreased the papillomas by 23.06% and 38.52%, while the carcinomas decreased by 71.16% and 73.12%, respectively, for 500 mg and 800 mg/kg body weight (BW). The aqueous extract was observed to be better than the ethanolic extract as it decreased the papillomas by 30.79% and 44.29%, respectively, while the carcinomas were less by 80.73% and 86.62%, respectively for 500 mg and 800 mg/kg BW. Histopathological observations made on the mucosa of DMBA and the topical as well as oral administration groups confirmed these findings (Karthikeyan et al., 1999a).

The survival rates of the animals were inversely proportional to the number of papillomas and carcinoma during the 24-week observation. In the experimental group, the DMBA treated animals showed lowest survival rate when compared with any other experimental groups. The survival rate of topically applied leaf paste, orally administered ethanolic and aqueous extract groups were higher when compared with that of the topically applied ethanolic and aqueous extract groups (Karthikeyan et al., 1999a).

Prevention of Gastric Carcinogenesis

Aruna and Sivaramakrishnan (1992) in their exploratory studies with some commonly consumed spices (cumin seeds, poppy seeds, asafoetida, turmeric, and pepper) and leafy vegetables (drumstick

leaves, Solanum leaves, basil leaves, and Alternanthera leaves) showed for the first time that the feeding of Tulsi leaves in the diet prevented benzo[*a*]pyrene-induced forestomach carcinogenesis in mice. In this study, the authors observed that the administration of benzo[*a*]pyrene alone caused 77% tumor incidence while administering Tulsi two weeks prior to and during the period of benzo[*a*]pyrene administration for eight weeks reduced the incidence to 29% (Aruna and Sivaramakrishnan, 1992).

Administration of 70% ethanolic Tulsi leaf extract is also observed to reduce the incidence of cancer caused by *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG), a nitroso compound widely used as an experimental gastric carcinogen. MNNG is a potent mutagen and induces erosions of the gastric mucosa, an initial precancerous change integral for initiation of forestomach carcinogenesis. Intragastric administration of MNNG induces well-differentiated squamous cell carcinomas with increased cell proliferation, and angiogenesis with evasion of apoptosis (Manikandan et al., 2007).

Administration of Tulsi decreased these changes. It selectively induced apoptosis in MNNG-treated gastric carcinomas but not on the normal stomach tissue in rats. Tulsi extract influenced the critical molecules involved in cell proliferation, invasion, angiogenesis and apoptosis. A significant decrease in the levels of cytokeratin, CK (infiltration), vascular endothelial growth factor, VEGF (angiogenesis), proliferating cell nuclear antigen (PCNA), glutathione-*S*-transferase pi, GST-pi (key proteins involved in proliferation), and antiapoptotic protein Bcl-2 with simultaneous increase in the proapoptotic proteins Bax, cytochrome *c*, and caspase 3 were reported (Manikandan et al., 2007) (Figure 7.4).

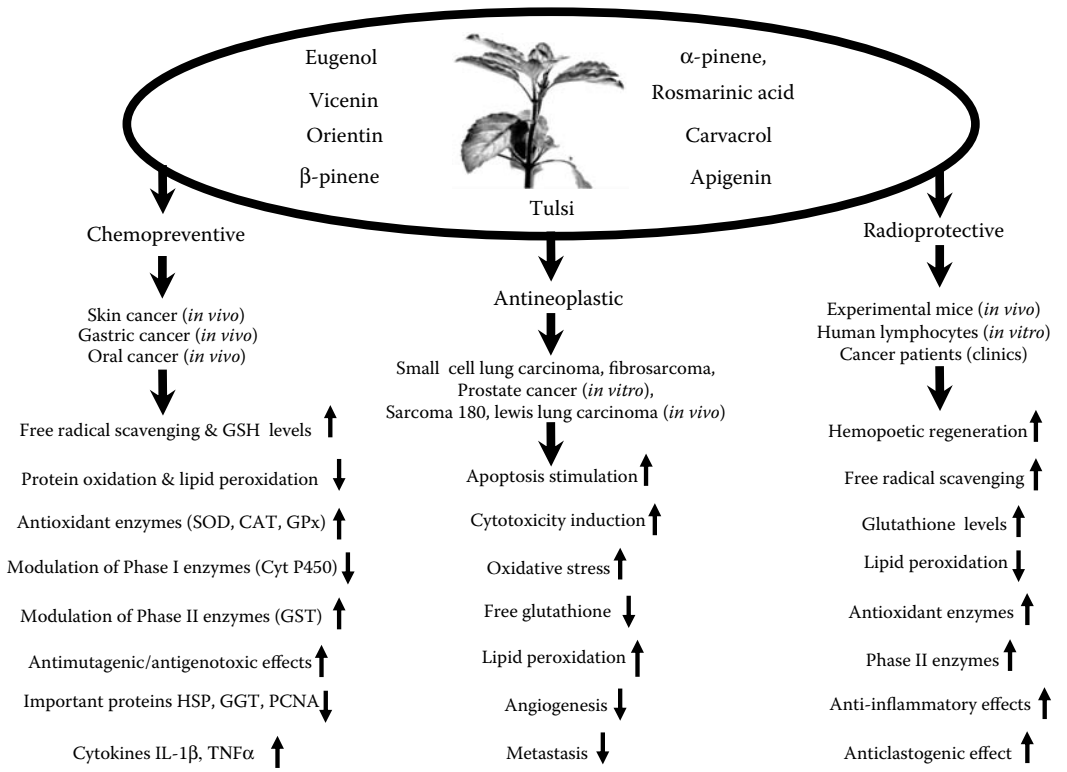

FIGURE 7.4 Mechanisms responsible for the anticancer, chemoprevention, and radioprotective effects of Tulsi.

Prevention of Liver Carcinogenesis

In vitro studies with the primary hepatocytes from the Fischer F-344 rat have shown that the pretreatment with ethanolic extract of Tulsi leaves (20, 50, 100, or 500 μg/mL) prior to 7,12-dimethylbenz[*a*]anthracene (DMBA) exposure (10 μg or 50 μg), caused a significant reduction in the levels of DMBA–DNA adducts. This effect was more pronounced at the lower dose of 10 μg than at 50 μg of DMBA (Prashar et al., 1998). This result suggests that the leaf extract blocks or suppresses the biochemical events associated with chemical carcinogenesis by inhibiting metabolic activation of the procarcinogen to carcinogen. From the data it can also be inferred that the protective effects were mediated at nontoxic concentrations as experiments have also shown that Tulsi extract alone did not affect the cell viability even at a high concentration of 100 μg/mL (Prashar et al., 1998).

In vivo studies with Wistar rats also showed that administration of Tulsi significantly prevented the 3′-methyl-4-dimethyl aminoazo benzene-induced hepatomas (Aruna and Sivaramakrishnan, 1992). Feeding the carcinogen-incorporated diet (0.05%) for 12 weeks resulted in the development of hepatomas (adenocarcinoma) in 82% of the control animals (without administering Tulsi). Feeding of the Tulsi leaves (200 mg/g diet) two weeks before and during carcinogen administration rendered significant protection as it reduced the tumor incidence to 25% (Aruna and Sivaramakrishnan, 1992).

Further recent support to these observations by Manikandan et al. (2007) have shown that pretreatment with alcoholic extract of Tulsi leaf before administering 7,12-dimethylbenz[*a*]anthracene caused (1) Decreased phase I enzymes; (2) reduction in the levels of lipid and protein oxidation, and (3) a concomitant enhancement of the antioxidant and phase II enzyme activities in the liver. Tulsi also caused a decrease in the 7,12-dimethylbenz[*a*]anthracene-induced genotoxicity, as evaluated by the micronuclei formation in bone marrow cells in mice (Manikandan et al., 2007). These results suggest that in association to the modulation of the phase I and II detoxification enzymes Tulsi possesses antigenotoxic effects, and all these might have contributed to the reduction of chemical carcinogenesis.

Prevention of Skin Carcinogenesis

Studies have shown that the Tulsi extract, as well as the seed oil, possess significant chemopreventive effects against the carcinogenic chemicals such as 3-methylcholanthrene (MCA), 7,12-dimethylbenz[*a*]anthracene (DMBA), and aflatoxin B1 (AFB1)-induced skin tumorigenesis in mice (Prashar et al., 1994; Prakash and Gupta, 2000; Rastogi et al., 2007).

Singh et al. (1999) investigated the modulatory effect of Clocimum [a eugenol-rich variety of *Ocimum gratissimum* developed by Indian Institute of Integrative Medicine, Jammu formerly Regional Research Laboratory (RRL), Jammu] oil on murine skin papillomagenesis and the xenobiotic detoxification system. Topical application of clocimum oil (50 μl/animal/day) during peri-initiation stage (1 week before and 2 weeks after initiation) of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced papillomagenesis and/or during the tumor promotion stage reduced the tumor burden significantly. Significant elevation in the hepatic levels of glutathione-*S*-transferase (GST), sulfhydryl (-SH) and cytochrome b5 was observed by the respective topical treatment of clocimum oil. The authors concluded that the chemopreventive potential of clocimum oil against murine skin papillomagenesis may be due to the modulated xenobiotic detoxification system enzymes.

The ethanolic extract of Tulsi leaves prevented 7,12-dimethylbenz[*a*]anthracene-induced skin papillomagenesis in Swiss albino mice. Topical application of the extract caused a decrease in tumor incidence, average number of tumors per tumor bearing mice and the cumulative number of papillomas at peri-initiational, postinitiational stages or continuously at peri- and postinitiational stages of papillomagenesis when compared with the corresponding control group. Tulsi extract increased the levels of reduced glutathione content and activity of glutathione-*S*-transferase activity in the skin (Prashar et al., 1994).

The seed oil of Tulsi was evaluated for chemopreventive activity against another skin carcinogen, 20-methylcholanthrene (MCA), which induces fibrosarcoma in the Swiss albino mice. Administration

of the oil at 100 mL/kg body weight once daily by oral route reduced the MCA-induced tumor incidence, tumor volume and was comparable to that of 80 mg/kg of vitamin E (Prakash and Gupta, 2000). The decrease in the tumor incidence as well as tumor volume had an inverse correlation with the survival of the mice. Biochemical assays in the liver showed that when compared with the MCA-treated control group of mice, the levels of the antioxidant enzymes superoxide dismutase, catalase, glutathione-*S*-transferase and the nonenzymatic antioxidant, the reduced glutathione (GSH), increased, while the levels of the lipid peroxidation end product, malondialdehyde, decreased. These results of this study suggest that the potential chemopreventive activity of the oil is partly attributable to its antioxidant properties (Prakash and Gupta, 2000).

Topical application of the alcoholic extract of the Tulsi leaves prior to the carcinogenic treatments [(1) MCA application, (2) DMBA-initiated tetradecanoyl phorbol acetate (TPA) promoted two-stage carcinogenesis, and (3) AFB1-initiated TPA promoted two-stage carcinogenesis], decreased the number of tumors, when compared with the respective carcinogen alone groups (Rastogi et al., 2007). Application of Tulsi decreased the expression of cutaneous γ-glutamyl transpeptidase (GGT), a late marker of tumor progression (Aldaz et al., 1988), and glutathione-*S*-transferase-P (GST-P), which is increased in chemically induced hepatic tumors (Satoh et al., 1985). The heat shock protein, which is altered during carcinogenesis, is also decreased in amount (Ferrarini et al., 1992).

Application of Tulsi extract decreased the activity of ornithine decarboxylase, an enzyme involved in the regulation of cell proliferation and development of cancer. Tulsi increased the phase II enzymes, levels of free glutathione suggesting greater conjugation of carcinogenic metabolites to glutathione and their elimination as mercapturic acid. There was also a concomitant decrease in the phase I enzymes and lipid peroxidation suggesting that Tulsi inhibits the activity of carcinogen induced cytochrome P-450-dependent enzymes and that this leads to a decrease in formation of ultimate carcinogenic moiety (Prashar et al., 1998).

The histopathological studies showed that in the tumors treated with Tulsi, an increase in the infiltration of polymorphonuclear, mononuclear and lymphocytic cells were observed. An increase in the levels of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) were also seen in the serum of the MCA-induced tumorigenic animals (Rastogi et al., 2007). These results when considered along with the histological observations suggest that the Tulsi extract induced cytokines and increased infiltration of polymorphonuclear, mononuclear and lymphocytic cells into the tumors and that this might have imparted cytostatic property that checked the rapid proliferation (Rastogi et al., 2007).

All these studies suggest that Tulsi provides chemopreventive effects by acting as an antioxidant, by modulating phase I and II enzymes, restoring the free glutathione levels, by exhibiting antiproliferative activity, by immunomodulatory function, decreasing the levels of lipid peroxides, and heat shock proteins (Prashar et al., 1994; Prakash and Gupta, 2000; Rastogi et al., 2007) (Figure 7.4).

Antineoplastic Activities

The ethanolic extract of Tulsi has been studied for its cytotoxic and apoptosis-inducing effects in human fibrosarcoma cells and human nonsmall cell lung carcinoma (NSCLC) A549 cells *in vitro* (Karthikeyan et al., 1999b; Magesh et al., 2009). In human fibrosarcoma cells, the cytotoxicity was not significant at lower concentrations (<50 μg/mL). However, with increase in concentration, the cytotoxic effects increased exponentially (Karthikeyan et al., 1999b). Tulsi caused oxidative stress as a decrease in the levels of free glutathione with concomitant increase in the levels of products of lipid peroxidation was seen (Karthikeyan et al., 1999b).

Tulsi extract caused apoptosis as evaluated by the DNA fragmentation assay. Microscopic studies showed that morphologically the cells had condensed nuclei with shrunken cytoplasm. Administration of both aqueous and ethanolic extracts of Tulsi caused a reduction in tumor volume and an increase in the lifespan of the Sarcoma-180 bearing animals, suggesting that the observed *in vitro* occurrence extends even into the *in vivo* systems as well (Karthikeyan et al., 1999b).

The hydroalcoholic (70% ethanolic) extract was also evaluated for its antineoplastic activity on three human prostate cancer cell lines differing in the metastatic potential, that is, poorly (LNCaP), moderately (DU-145) and highly metastatic (PC-3M) prostate cancer cell lines *in vitro*. At 20 and 40 μg/mL the extract did not possess noteworthy inhibition of cell proliferation indicating that at the concentration used Tulsi was not effective in preventing the proliferation and growth of human prostate cancer cell lines (Rao et al., 2004).

In the A549 cells, when compared with the untreated control, Tulsi extract significantly exerted cytotoxicity in a concentration dependent manner. The cytotoxicity observed at 200 μg/mL was almost comparable to 1 μM paclitaxel that was used as a positive control (Magesh et al., 2009). The treatment of A549 cells with Tulsi extract for 24 hours increased the TUNEL positive apoptotic cells and the apoptotic sub-G1 peak apoptotic cells in a concentration dependent manner. Mechanistic studies have shown that Tulsi extract decreased the expression of antiapoptotic protein Bcl-2 in a concentration-dependent manner without affecting the levels of the proapoptotic protein Bax in the A549 cells (Magesh et al., 2009).

The levels of cytochrome *c* release were also increased into the cytosol (Magesh et al., 2009), suggesting that Tulsi induces apoptosis through the intrinsic mitochondrial-dependent pathway (Susin et al., 1999; Hengartner, 2000). Cytochrome *c* binds to Apaf-1, leading to activation of caspase-3, the key executioner of apoptosis with the end result being the PARP cleavage, DNA fragmentation, and apoptosis (Thornberry and Lazebnik, 1998; Wolf and Green, 1999). Tulsi also decreased the phosphorylation of the survival gene Akt and ERK in A549 cells. Together all these results suggest that Tulsi induces apoptosis via activation of caspases and inhibition of Akt and ERK in A549 cells (Magesh et al., 2009) (Figure 7.5).

To further validate the *in vitro* observations, the authors performed *in vivo* studies in the Lewis lung carcinoma bearing $C_{57}BL_6$ mice. The study showed that Tulsi extract decreased the tumor weight, and the final tumor size at necropsy, when administered at 50 and 100 mg/kg through the intraperitoneal route every other day, untill 21 days post-tumor inoculation caused a dose-dependent inhibition of tumor growth (Magesh et al., 2009).

Ethanolic extract being nonpolar will invariably contain eugenol, luteolin, ursolic acid, and oleanolic acid in differing ratios (Singh et al., 2007). Of these, experimental studies have shown that eugenol (Figure 7.2a) and luteolin (Figure 7.2b) possess anticancer effects *in vitro* (Kim et al., 2005; Yoo et al., 2005). Luteolin, depending on the concentration can biochemically function as either an antioxidant or a pro-oxidant. Multiple studies have shown that luteolin's anticancer property is coupled to stimulation of apoptosis, inhibition of cell proliferation, angiogenesis, and metastasis. Luteolin suppresses the cell survival pathways phosphatidylinositol 3′-kinase (PI3K)/Akt, nuclear factor kappa B (NF-κB), and X-linked inhibitor of apoptosis protein (XIAP) with concomitant stimulation of the apoptosis pathways including those that induce the tumor suppressor p53 (Lin et al., 2008).

In human hepatoma HepG2 cells, luteolin induced apoptosis via mechanisms involving the cytosolic release of cytochrome *c*, activation of CPP32, and mitochondrial translocation of Bax/Bak. Pretreatment with SP600125, a specific inhibitor of c-Jun NH2-terminal kinase suppressed luteolin-induced apoptosis, thereby confirming it was important in the biochemical mechanism (Lee et al., 2005).

Treatment of HL-60 cells with graded doses of eugenol containing media also caused apoptosis specific DNA fragmentation and formation of DNA ladders in the agarose gel electrophoresis. Eugenol-induced apoptosis was mediated through the generation of reactive oxygen species, mitochondrial permeability, release of cytochrome *c*, and decrease in the levels antiapoptotic protein bcl-2 (Yoo et al., 2005).

These studies when considered in total suggest that the combination of these antitumor phytochemicals in the ethanolic extract of *O. sanctum* might have played an important role in inducing apoptotic activity, and also that this may have affected the tumor proliferation and growth (Figure 7.4).

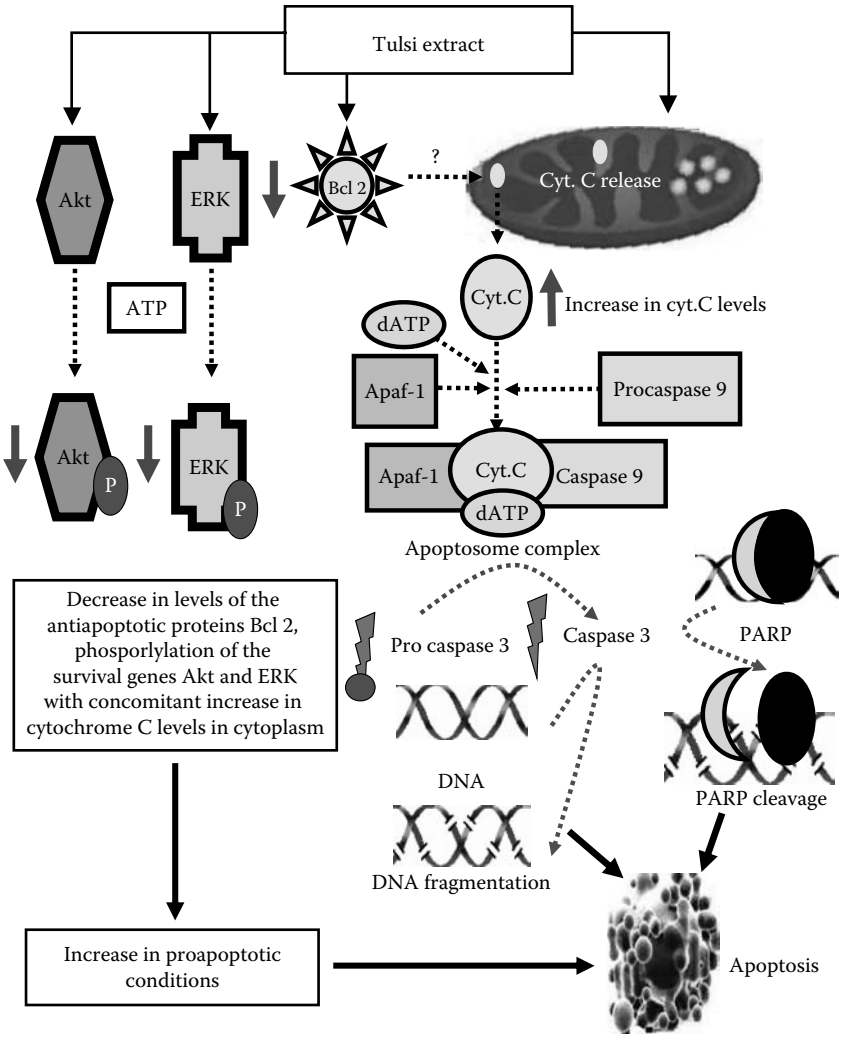

FIGURE 7.5 Mechanism of apoptosis induced by Tulsi extract in human nonsmall cell lung carcinoma A549 *in vitro*. (Adapted from Magesh et al. 2009. *Phytother Res*. 23:1385–91 [Epub ahead of print].)

Antitumorigenic and Antiangiogenic Activities

Tulsi extract has been observed to prevent the migration of breast cancer cell (MDA-MB-435) toward matrigel, and the human umbilical vein endothelial cells (HUVEC) toward galectin-3. The extract inhibited the induction of cycloxygenase COX-2 induced by the treatment of tetradecanoyl phorbol acetate (TPA) to the MDA-MB-231 breast cancer cells.

Tulsi repressed the *in vitro* capillary tube formation in the three-dimensional cultures of HUVEC on Matrigel. In the presence of Tulsi extract a significant reduction in the number of blood vessels and COX-2 expression in the matrigel plug assay was observed with MDA-MB-231 cells. All these results suggest that the leaf extract possess an antitumorigenic and antiangiogenic agent in breast cancer models, and that this may play a role in the anticancer and chemopreventive effects of tulsi (Nangia-Makker et al., 2004) (Figures 7.3 and 7.4).

Radioprotective Effects

The use of ionizing radiation in medicine has led to major improvements in cancer treatment. Recent estimates suggest that each year, nearly 60% of all cancer patients receive radiation therapy, either alone or in conjunction with surgery, chemotherapy, or other forms of cancer therapy (Hall, 2000). While being beneficial in tumor remission, radiotherapy also causes severe untoward effects due to the normal tissue toxicity. Especially of importance is the hematopoietic and gastrointestinal syndrome. These two organs are highly radiosensitive life supporting organs and damage to these organs can force the physicians to discontinue or reduce the dose of treatment, which can consequently affect the treatment outcome (Hall, 2000). In such situations, an agent that can provide a therapeutic differential between the cancer and normal cell will be highly beneficial (Hall, 2000).

Therapeutic differential may be achieved with chemical compounds that may selectively enhance the radiation cell kill effects in tumor cells (radiation sensitizers) or selectively protect the normal cells from the deleterious effects of radiation (radioprotectors) (Hall, 2000). Seminal studies carried out by Uma Devi et al. (2000) have shown that Tulsi and its flavonoids, orientin and vicenen possess selective normal tissue radioprotective effects at nontoxic concentrations. Eugenol, a nonpolar compound rich in the Tulsi oil, is also reported to possess radioprotective effects (Tiku et al., 2004).

Protection against Radiation-Induced Sickness and Mortality

In their first report Uma Devi and Ganasoundari (1995) evaluated the radioprotective effects of Tulsi by survival studies. The study showed that the dried leaves of Krishna Tulsi (dark-leafed variety of *O. sanctum*) were effective in protecting against the radiation-induced sickness and mortality. The study showed that the aqueous extract was more effective than the 95% ethanolic extract in increasing the 30-day mouse survival; the optimum dose of the aqueous extract by intraperitoneal administration was 50 mg/kg; the acute LD_{50} for the mice was more than 6 g/kg body weight suggesting the optimal dose to be devoid of any inherent toxicity. The extract was also effective when given orally in increasing mouse survival, although to a lesser extent than intraperitoneal route of administration. Administration of the optimum dose in fractions of 10 mg/kg/day for five consecutive days before irradiation gave better protection when compared with a single administration of 50 mg/kg of the extract.

The optimum dose of the extract, given intraperitonealy before radiation gave a dose modification factor (DMF, the ratio of the radiation dose needed to produce the same effect in the presence or absence of the protector) of 1.28 for 30-day mouse survival (Uma Devi and Ganasoundari, 1995). Animal survival at 30 days after lethal whole body irradiation indicates recovery and regeneration of both gastrointestinal and hemopoetic progenitor cells in the bone marrow, the two most life supporting radiosensitive organs affected by radiation treatment in clinics (Hall, 2000).

Protection against Radiation-Induced Chromosome Damage and Stem Cell Death

Aqueous extract of Tulsi protected against the sublethal doses (1–6 Gy) of radiation-induced chromosomal aberrations in the mice bone marrow cells, while the extract alone was devoid of any significant effect at all time points studied (on days 1, 2, 7, and 14 postirradiation). The extract significantly reduced the percent aberrant metaphases as well as the different aberrations, including the dicentric and rings, induced by radiation. Tulsi pretreatment reduced the exchange and multiple aberrations in comparison with radiation-alone groups, demonstrating protection against DNA DSBs and multiple lesions. Tulsi extract administration also resulted in a faster recovery compared with radiation-alone group (Ganasoundari et al., 1997a).

Administering the optimum dose of Tulsi leaf extract before whole body exposure to a clinically used sublethal gamma irradiation dose of 2 Gy, produced a significantly higher bone marrow stem cell survival (CFU-S) and the effect was better than that by the clinically approved radioprotector WR-2721 (300 mg/kg at 40% of its LD_{50}). This result suggests that in terms of protective dose and

toxicity, the extract of Tulsi may be a better protector than the synthetic compound (Ganasoundari et al., 1997b).

Enhancement of Bone Marrow Radioprotection and Reduction in Late Toxicity of WR-2721

The radioprotective (antigenotoxic) effect of the leaf extract of Tulsi (10 mg/kg on five consecutive days) in combination with a single dose of WR-2721 (100–400 mg/kg) was investigated on the mouse bone marrow on days 1, 2, 7, and 14 post-treatment after whole-body exposure to sublethal dose of gamma irradiation (4.5 Gy). Individually, both Tulsi and WR-2721 caused a significant decrease in aberrant cells as well as different types of aberrations. However, the increase in the protection when both were combined was almost twofold more when compared with 400 mg/kg WR-2721 alone, the highest dose used in the study (Ganasoundari et al., 1998).

The percent aberrant cells decreased in all groups with time, though the values remained higher than normal even on day 14 in the radiation alone group as well as those treated with Tulsi or WR-2721 before radiation. Combination of Tulsi with WR-2721 enhanced the protection and reduced the delayed toxicity observed at higher doses of WR-2721. These results suggest that Tulsi may also act as a detoxifier of WR-2721. The protective effect of the combination of Tulsi and WR-2721 is reflected by the enhancement of bone marrow colony forming unit survival (CFU-S). Either agent individually checked the radiation-induced stem cell death. However, the combination of the two agents [Tulsi + WR-2721 (300 mg/kg BW)], further enhanced this protection (Ganasoundari et al., 1998).

In vitro hydroxyl radical scavenging activities showed that both Tulsi and WR-2721 possessed significant scavenging activities and also that the combination of the two synergistically increased the effect. These results indicate that the enhanced protection by the combination of WR-2721 and Tulsi against radiation-induced chromosome damage could be due to the more efficient removal of the highly reactive free radicals. In total, these studies suggest that the combination of Tulsi and WR-2721 increased chromosome protection and CFU-S, with concomitant reduction in the toxicity of WR-2721 (at higher doses 400 mg/kg BW). Together this study suggests that the combination of Tulsi with WR-2721 may promise better radioprotection and reduce the inherent delayed toxicity of the synthetic compound WR-2721 (Ganasoundari et al., 1998).

Prevention of Radiation-Induced Lipid Peroxidation and Restoration of Glutathione and Activity of the Antioxidant Enzymes

Administering Tulsi leaf extract alone either as a single dose of 50 mg/kg or as five fractions of 10 mg/kg each did not produce any significant change in liver lipid peroxide values. Exposure to 4.5 Gy of gamma radiation alone caused a significant increase in the levels of lipid peroxides at all time points studied from 15 minutes to 2 hours. Treatment with a single dose of 50 mg/kg of the aqueous extract produced a higher reduction in the lipid peroxides when compared with the fractionated dose (10 mg/kg × 5) at all study points. These observations clearly indicate that Tulsi extract in both conditions (when given as a single dose or as fractions) protects the lipid membranes against radiation-induced oxidative damage (Uma Devi and Ganasoundari, 1999).

Administering only the Tulsi extract increased the levels of free GSH and the antioxidant enzymes glutathione transferase (GST), reductase (GSRx), peroxidase (GSPx) and superoxide dismutase (SOD) above the baseline normal levels, while exposure to only irradiation significantly reduced them. Pretreatment with the extract prevented the radiation induced depletion of GSH and all the antioxidant enzymes and maintained their levels within or above the control range. Increase in the GSH levels offers protection against oxygen-derived free radicals and cellular lethality and Tulsi might have mediated the protective effects in part through the GSH. It also caused a significant increase in the levels of SOD in the Tulsi treated group, and this might have facilitated the removal of radiation-induced superoxide anions, and the H_2O_2 formed in the process may have been efficiently removed by the GSPx and GST, which are also increased by the Tulsi treatment (Uma Devi and Ganasoundari, 1999) (Figure 7.4).

Protective Effect against High-Dose ^{131}I Exposure

Aqueous extract of Tulsi when orally fed at 40 mg/kg BW for 15 days also protected mice against the oxidative stress induced by high doses (3.7 MBq) of oral ^{131}I. Pretreatment with Tulsi caused a reduction in lipid peroxidation in both kidneys and salivary glands, prevented depletion in GSH level, thereby indicating its utility in ameliorating ^{131}I exposure-induced damage to the salivary glands (Bhartiya et al., 2006).

Nonprotection against Radiation, Cisplatin or Withaferin-Induced Tumor Cell Kill

For optimal use and application in clinics in the treatment of cancer, a radioprotective agent should be specific in protecting only the normal cells/tissues. Uma Devi et al. (2004) studied the effect of aqueous Tulsi extract (50 mg/kg) on the response of fibrosarcoma to radiation (10 Gy), cisplatin (5 mg/kg), and withaferin (40 mg/kg) in the lung colony assay. It was observed that the pretreatment with Tulsi extract before radiation or cisplatin or withaferin or their combinations did not bring about any notable change in their tumor killing effect (Uma Devi et al., 2004).

Ocimum Flavonoids, Orientin and Vicenin Protect against Radiation-Induced Ill Effects without Interfering with Tumor Cell Kill

Detailed studies lead to the understanding that the flavonoids, orientin (Figure 7.6a) and vicenin (Figure 7.6b) present in the aqueous extract provided significant protection against radiation-induced sickness and death from both gastrointestinal syndrome and bone marrow syndrome. Both are readily soluble in water and did not show any systemic toxicity in mice even at a dose of 100 mg/kg BW. The optimum drug doses for protection for both the compounds were 50 μg/kg BW, when administered intraperitoneally 30 minutes before irradiation. However, changing the interval between drug injection and irradiation, or the route (oral, intramuscular, and intravenous), significantly increased survival, but to a lesser extent. Postirradiation drug treatment was not very effective suggesting their efficacy to be only when administered before irradiation. At the optimum

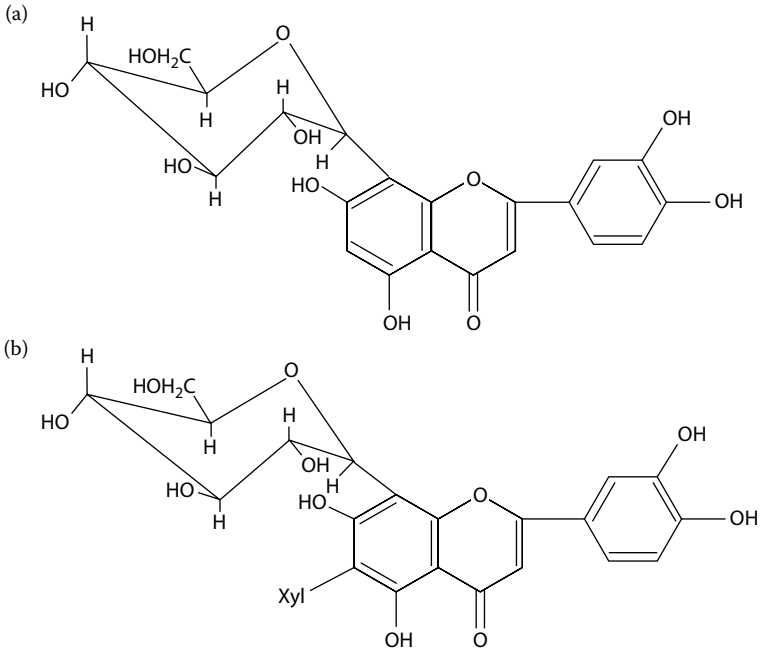

FIGURE 7.6 Chemical structures of Ocimum flavanoids. (a) Orientin (8-*C*-b-D-glucopyranosyl-luteolin). (b) Vicenin 1 (6-*C*-b-D-xylopyranosyl-8-*C*-*C*-D-glucopyranosyl apigenin).

dose, vicenin gave a slightly higher protection (DMF = 1.37) than orientin (DMF = 1.30) (Uma Devi et al., 1999).

Mechanistic studies showed that both the flavonoids prevented radiation-induced lipid peroxidation in the liver of mice whole body exposed to 3 Gy of gamma radiation. Both compounds showed better free radical-inhibiting activity than DMSO *in vitro*, did not possess any pro-oxidant activities at the concentrations tested, and inhibited free radical formation in the absence of EDTA (Uma Devi et al., 2000). Both compounds at 50 μg/kg BW were observed to protect mice against 2 Gy of whole body gamma radiation-induced chromosomal aberrations in bone marrow cells. Vicenin also caused a maximum reduction in percent aberrant cells, while 2-mercaptopropionyl glycine (MPG, 20/kg BW) was the least effective; orientin and WR-2721 (150 mg/kg BW) showed an almost similar effect. However, WR-2721 was the most effective against reduction of complex aberrations, followed by vicenin. Neither flavonoid had any systemic toxicity, even at 200 mg/kg BW (Uma Devi et al., 1998).

The flavonoids also rendered bone marrow protection against the deleterious effects of sublethal doses of gamma radiation (0–6 Gy ^{60}Co). Stem cell survival was studied using the exogenous spleen colony (CFU-S) assay and the DNA damage by chromosomal aberrations. Pretreatment with either flavonoid significantly increased the number of CFU-S and reduced the aberrant cells and different aberrations (breaks, fragments, rings, and dicentrics), when compared with the respective radiation-alone groups. The DMFs for 50% reductions in the number of CFU-S were 1.6 for orientin and 1.7 for vicenin (Nayak and Uma Devi, 2005). Orientin and vicenin protected against foetal irradiation-induced genomic damage and instability, thereby reducing the delayed chromosomal abnormalities and tumorigenesis in adult (Uma Devi and Satyamitra, 2004).

Pretreatment with orientin or vicenin also reduced the DNA damage as evaluated by enumerating the micronuclei in the cytochalasin B-induced cytokinesis block method with human peripheral lymphocytes. The optimum protective dose for orientin or vicenin was selected by treating HPBLs at 6.25, 12.5, 15.0, 17.5, or 20 μM, 30 minutes before exposure to 4 Gy gamma radiations. The optimum concentration of orientin and vicenin 17.5 μM is very low compared to the other protectors. In the radiation dose-dependent studies, pretreatment with 17.5 μM of orientin and vicenin before exposure to 0.5–4 Gy gamma irradiation gave a DMF of 2.62 for orientin and 2.48 for vicenin. Both compounds showed significant antioxidant activity *in vitro* at the above concentrations, which was significantly higher than that of DMSO at equimolar concentrations (Vrinda and Uma Devi, 2001).

The effect of orientin and vicenin on the tumor response to radiation (30 Gy) and cyclophosphamide was studied on fibrosarcoma and melanoma bearing mice. In B16F1 melanoma, none of the radiation treatments produced any complete response. Pretreatment with orientin or vicenin produced a slight increase in volume doubling time and growth delay even though the difference was not significant (Uma Devi et al., 2004). Cyclophosphamide alone produced no complete response, while treatment with either orientin or vicenin before cyclophosphamide resulted in 20% complete response. Pretreatment with orientin or vicenin produced a marginal increase in volume doubling time and almost equal growth delay as cyclophosphamide alone (Uma Devi et al., 2004).

In fibrosarcoma, all the radiation groups had almost similar complete response, volume doubling time, and survival up to 120 days post-tumor inoculation. Cyclophosphamide alone as well as in combination with orientin or vicenin produced complete response. In the cyclophosphamide-alone group, 90% of the animals survived without tumor recurrence while with the combination, all animals survived without tumor recurrence (Uma Devi et al., 2004).

Eugenol in Radioprotection

Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), a hydrophobic compound present in many of the plants like bay leaves, allspice, oil of cloves, and Tulsi possesses myriad therapeutic uses in medicinal, cosmetic, and pharmaceutical preparations (Prakash and Gupta, 2005). Studies by Tiku et al. (2004) have shown that the administration of different doses of eugenol (75, 150, and 300 mg/kg)

before exposure to 1.5 Gy of gamma radiation caused a significant reduction in the frequencies of micronucleated polychromatic erythrocytes with all three eugenol doses.

A detailed radiation dose-dependent studies (0.5, 1, 1.5, and 2 Gy) with 150 mg/kg of Eugenol showed it was effective in preventing radiation-induced DNA damage at all doses when compared with the concurrent radiation-alone groups. Eugenol decreased the incidence of radiation-induced micronuclei (with 1.5 Gy) at all time points up to 72 hours postirradiation, suggesting efficient recovery. Biochemical studies showed that eugenol protected against the peroxidative damage and decreased the specific activities of lactate dehydrogenase (LDH) and increased the levels of methylglyoxalase I (Gly I) in the liver of mice when compared with the untreated radiation-alone group of mice (Tiku et al., 2004).

Clinical Trials with Tulsi as a Radioprotector

A number of clinical trials of *O. sanctum* have been conducted for various diseases in three phases (Ghosh, 1984). Phase I and phase II trials were conducted on the basis of determination of most appropriate dose and evaluation of pharmacokinetics data on a small number of patients (Uma Devi, 2006). Further, on the basis of results of phase III trials data were obtained on a large number of patients to establish those results and safety and efficacy of the drug, and compare with therapeutic effects with placebo and/or established drug through double blind studies (Uma Devi, personal communication).

Clinical trials have been on-going at Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai, India utilizing Tulsi. To evaluate the radioprotective efficacy of drugs, a human clinical system for testing novel radioprotectors has been developed by Dr. Rajiv Sarin. Since it is not possible to evaluate radioprotectors in humans, testing is restricted only to radiotherapy patients who are subjected to radiation (either whole body or large parts of the body receive a significant dose of radiation). Total body irradiation (TBI) is given as a part of the conditioning regiment prior to hematopoietic stem cell transplant for various benign and malignant lesions.

The limitations of TBI as a clinical model for testing radioprotectors, however, include the following: (i) alkylating agents used with TBI in most conditioning regimens, (ii) masking effect of planned marrow rescue by an immediate BMT, (iii) fractionation of radiation doses during TBI, (iv) extremely low dose rate radiotherapy in single fraction regimens, (v) already immunocompromised state of patients further creating a complex situation. Sarin and his group have developed the use of hemibody irradiation (HBI) for bone metastasis as a clinical sytem for evaluation of *O. sanctum* in a phase III randomized trial.

In this system, eligible patients were randomized to receive either placebo or Ocimum 48 hours prior to HBI (6–8 Gy; single dose upper or lower body and uniform irradiation of entire salivary tissue in upper HBI). Just before starting placebo/Ocimum treatment, and 48 hours before HBI, biochemical analysis, salivary amylase activity, and various enzymes like glutathione reductase, glutathione peroxidase, and superoxide dismutase were estimated. Similar analysis was carried out 48 hours after the placebo/Ocimum treatment and just before HBI and also 30 minutes, 2 hours and 6 hours after HBI, and 24 and 72 hours post-HBI-salivary amylase was tested only for upper HBI. Post-HBI, data was recorded 1 week later and every week up to 5 weeks, including data on TLC, DLC, and platelets. The results have been quite promising though it is a long way before the drug can see the light of the day in the form of an over-the-counter drug for management of radiation injuries.

CONCLUSION

Considerable information from preclinical studies in the recent past, suggest the usefulness of *O. sanctum* in preventing cancer and in ameliorating the toxic effects of ionizing radiation at nontoxic concentrations. While most research has been with experimental animals and validated clinical applicability of Tulsi to humans, *in vitro* studies with relevant propagatory cell lines and primary cultures will help in understanding the mode of action responsible for the chemoprevention and radioprotection.

Further studies on determining the chemopreventive and radioprotective activity of Tulsi and its active components should ideally include human intervention trials as its effectiveness against human cancers and as a selective radioprotective agent in treating cancer in the clinics can be investigated. Study should also focus on how genetic variability in the humans will influence the chemopreventive and radioprotective benefits attributed to Tulsi against different environmental carcinogens and mutagens.

As there is considerable variation in the chemical composition among various samples of Tulsi, it is imperative that a quality control be established for the authenticity of the plant and the presence of active phytochemicals in the required levels. In this regard, the availability of authentic metabolite standards for quantification of the secondary metabolite will make the scientific observations more reliable and reproducible.

Due to its abundance, low cost, and safety in consumption, Tulsi remains a species with tremendous potential and countless possibilities for further investigation. Tulsi has the potential to develop as a nontoxic chemopreventive and radioprotective agent when the gaps existing in the knowledge are bridged.

Apart from applications in the clinic for preventing/treating cancer patients, *O. sanctum* can be used as a radiation countermeasure in the management of chemical, biological, radiological/nuclear (CBRN) incidents; for the protection of defense personnel from nuclear weapon radiations; for protecting reactor workers and rescue crew; protection of astronauts from exposure to space radiation; protection of embryos against maternal exposure during pregnancy; protection against radiation-induced genomic instability and radiation carcinogenesis.

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8 Culinary Spices in Cancer Chemoprevention

John R. Endres, Irfan Qureshi, Amy Clewell, and Alexander G. Schauss

CONTENTS

INTRODUCTION

Recently published epidemiological investigations have revealed significant variation in international cancer incidence rates. The most remarkable differences in these rates are seen between Indians living in India and around the world compared with the U.S. white population. The data reveal a lower rate of incidence for most cancers for Indians living in India and increasing rates for Indians living in the United Kingdom or in the United States. The U.S. white population was found to have the highest incidence of cancer overall. Rastogi et al. (2008a,b) found the total cancer incidence rates to be the lowest in India with 111 per 100,000 for men and 116 for women. In contrast, the rates for the U.S. white population are 362 and 296 per 100,000 for men and women, respectively. For Indians living outside of India the rates increase but do not reach the rates observed in the U.S. white population. In the United Kingdom the rate of incidence for the Indian population increases to 173 and 179 and in the United States those rates range between 152 and 176 and 142 and 164 per 100,000 for men and women, respectively.

While there are many potential hypotheses for why the rates differ so greatly between these populations, clearly differences in lifestyle seem the most plausible. Genetic defects or mutations were once thought to play the major role in cancer development. It is now thought that only 5–10% of all cases of cancer are due to genetic causes whereas 90–95% are thought to be due to lifestyle and environmental factors—and therefore may be preventable (Anand et al., 2008; Aggarwal et al., 2009). The fact that the cancer incidence rate increases for Indians living in Western countries—but does not reach that of the U.S. white population—supports this idea because there is most likely some degree of adaptation to the lifestyle and culture of the country of residence.

A comparison of the lifestyles between the U.S. white population and Indian populations reveals great contrast. One particular aspect of lifestyle that is clearly different is the diet. More specifically, a wide variety of culinary spices not commonly used by the U.S. white population are consumed more often and in much greater quantity by Asian Indians. Spice consumption in the United States is, however, on the rise. From 1970 to 2005, U.S. spice consumption per capita increased from 1.6 to 3.3 pounds per year with the majority (a sixfold increase) attributed to garlic (Kaefer and Milner, 2008). It has been reported that some Americans may consume as much as 4 g of spices per day. To illustrate the difference, an individual in India is likely to consume to 4 g daily of turmeric alone and as much as 7 g of garlic per day (Tapsell et al., 2006). Since a huge body of evidence is building for the health benefits from culinary spice consumption, a deeper look was required to see if there is support for the hypothesis that greater spice consumption may be the key to understanding why Indians residing in India have the lowest overall rate of cancer incidence in the world.

Interestingly, the relationship of spice production by country to cancer incidence was recently investigated and an inverse relationship between incidence of cancer and spice production was found. Spice production in India is ~2.25×10^6 tons compared with ~6.0×10^5 tons for China, and 2.5×10^5 tons for the United States (Aggarwal et al., 2009). Since exported and otherwise lost spices were subtracted from the results of this comparison, the spice production should approximate actual consumption. Therefore, when compared with the United States, Indians produce approximately nine times more spices and have roughly one-third the rate of cancer incidence.

This logically leads to the question as to what it is about the spices consumed in India in such relatively large quantities compared with the rest of the world that support this unfolding hypothesis. This introductory chapter focuses on the chemopreventive properties of three of the main culinary spices used in India as presented in peer-reviewed published works available in the public domain. Namely, turmeric (*Curcuma longa*), garlic (*Allium sativum*), and ginger (*Zingiber officinale*). Of these three culinary spices, turmeric and garlic have the greatest quantity of research regarding their chemopreventive properties. This is interesting because of all the land area in India devoted to spice cultivation, turmeric alone comprises 60% (Krishnaswamy, 2008). Interestingly, turmeric also comprises roughly 60% of typical curry blends that are so often used in meal preparation. Research published on ginger and its constituents in no way pales in comparison.

Many culinary spices share common properties that published research has demonstrated may function to prevent cancer. These properties include: antioxidant properties, cytokine modulation, upregulation of phase I and phase II detoxification pathways, antiangiogenesis, cell cycle modulation, apoptosis, as well as antimetastasis properties. The mechanisms responsible for these properties specific to the culinary spices reviewed are outlined in the following sections along with evidence for chemoprevention in animal models. These mechanisms include: inhibition of and decreased gene expression of NF-κB (nuclear factor kappa B), decreased TNFα (tumor necrosis factor alpha) activation of NF-κB, decreased expression and activity of COX (cyclooxygenase) enzymes, as well as LOX (lipoxygenase) enzymes. Therefore the end result from consumption of these spices is a reduction in chronic inflammation.

A strong link has been established between cancer formation and chronic inflammation (Rakoff-Nahoum, 2006; Meteoglu et al., 2008). While inflammation is a normal and natural process necessary for health and healing, when excessive or sustained over many years it is thought to be a causative factor for many degenerative conditions such as Alzheimer's disease, cardiovascular disease, diabetes, arthritis, and cancer. NF-κB activation may be at the heart of the matter. NF-κB is a complex protein that controls the transcription of a host of pro-inflammatory substances. Its excessive activation has been closely associated with cancer (Aggarwal et al., 2008, 2009). NF-κB creates an environment favorable for the survival of malignant cells (Karin, 2008) by supporting cell proliferation, invasion, and angiogenesis (Brown et al., 2008; Naugler and Karin, 2008).

Below is a detailed description of research suggesting how these particular culinary spices are able to positively affect the physiological and cellular environment (e.g., by inhibiting NF-κB, reducing inflammation, and oxidative stress) making it less favorable for carcinogenesis and tumorigenesis.

FIGURE 8.1 Turmeric rhizome.

TURMERIC (*CURCUMA LONGA* AND OTHER *CURCUMA* SPP.)

Turmeric (*Curcuma longa* and other *Curcuma* spp.) has a long history of culinary and medicinal use dating back to at least the seventh century BC. These plants are members of the *Zingiberaceae* family, which also includes its relatives, ginger (*Zingiber officinale)* and galanga (*Kaempferia galanga*). Texts from the traditional Indian system of health known as Ayurveda list turmeric as an ingredient used for both external and internal healing applications. Assyrian inscribed clay tablets dating back to 650 BC describe the use of turmeric as a spice, digestive aid, and coloring dye, while ancient Chinese writings from the seventh and eight centuries BC mention the use of turmeric root and related species for the creation of yellow dyes. Marco Polo described turmeric in writings from his travels to China in the thirteenth century, whereafter it was introduced into Europe and began to be used as a food colorant and dye. Today, turmeric is the most prevalent spice in Indian and South Asian curries, where it can make up 60% of the seasoning mixture added to a wide range of foods.

The rhizome, or root, of the plant is most prized for both culinary and medicinal uses. It is an extremely rich source of the phenolic compounds known as the curcuminoids, which have a distinct bright orange or yellow coloration. Three major curcuminoids have been isolated from turmeric, including curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin was first identified in the turmeric rhizome and isolated by Vogel in 1815. Currently available preparations of curcumin for commercial use generally contain the three curcuminoids in the following percentages: curcumin 77%, demethoxycurcumin 17%, and bisdemethoxycurcumin 3%. Purified extracts containing simply curcumin are also available. In addition to these important phenolic compounds, turmeric contains protein, fat, minerals, carbohydrates and essential oils (Pari et al., 2008).

Turmeric is widely renowned for its anti-inflammatory properties and is used in the Ayurvedic tradition to treat liver disorders, coughs, rheumatic conditions, anorexia, and sinusitis, among other conditions. Topically, turmeric is known for its beneficial wound-healing properties and is used for the treatment of diabetic wounds. In modern times, turmeric is widely used for treating digestive disorders because of its purported protective action on mucosal tissue and its ability to induce bile flow. Turmeric and its curcuminoids also have utility in supporting cardiovascular health, likely due to antioxidant effects and ability to prevent lipid peroxidation. Its anti-inflammatory activities lend credibility to turmeric's use for arthritis and joint pains (Pari et al., 2008). Significantly, these and other beneficial aspects of turmeric also have earned it a great reputation as a useful compound for cancer chemoprevention and treatment. Given its wide dietary use and relative cost-effectiveness, along with its broad spectrum of activity, turmeric is a prime candidate for health maintenance.

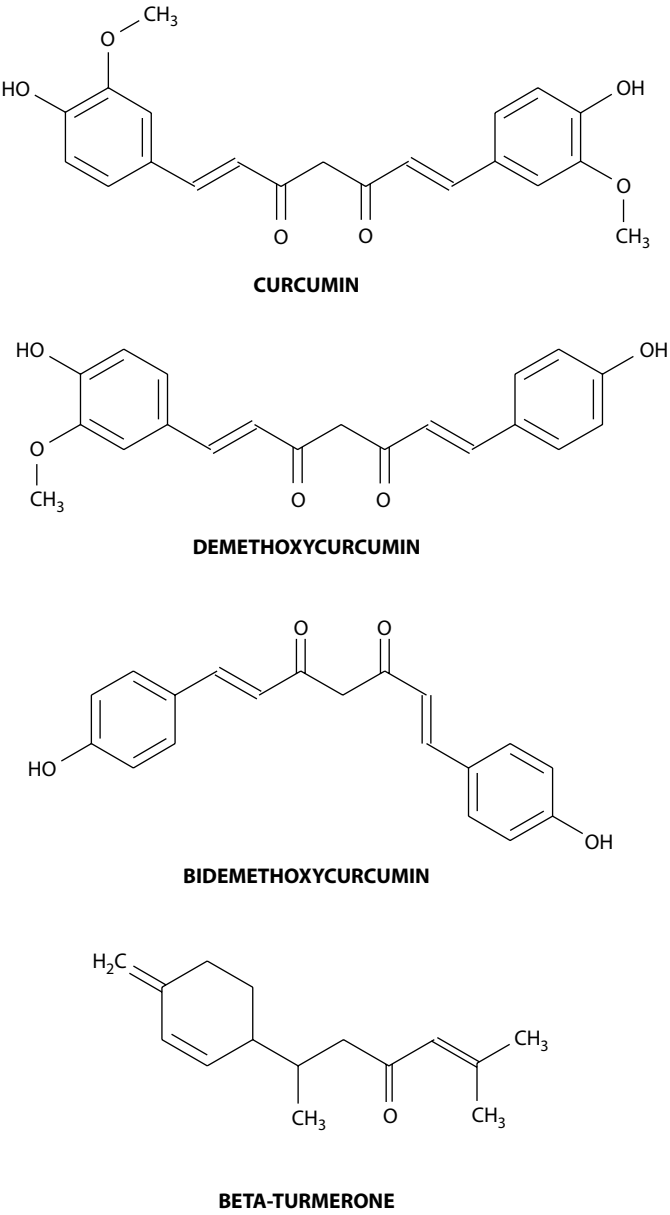


FIGURE 8.2 Major phytochemical constituents in Turmeric.

Turmeric, Curcumin, and Chemoprevention

Evidence for the chemopreventive effects of turmeric and curcumin comes from the results of epidemiological studies showing that the incidence of several cancers, including those of the lungs, breasts, colon, and prostate, are lower in countries such as India and Pakistan, where significant amounts of turmeric and curcumin are consumed, in comparison to Western countries such as the United States. Furthermore, evidence from animal studies shows significant cancer preventive effects of curcumin in various models of carcinogenesis. Curcumin has shown preventive effects on cancers of the colon, lung, breast, stomach, esophagus, liver, skin, lymphoma, leukemia, and others (Lopez-Lazaro, 2008).

Research on curcumin in animal models of skin cancer where tumors were initiated with the chemical DMBA (7,12-dimethylbenz[*a*]anthracene) and promoted with the chemical TPA (12-*O*-tetradecanoylphorbol-13-acetate) found that oral supplementation of the diet with curcumin led to significant reductions in number of tumors and tumor volume. Furthermore, studies conducted with mice in which tumors were promoted with topical applications of TPA and curcumin was topically coadministered showed marked inhibition of tumorigenesis. Similar studies in which DMBA was applied topically once weekly for 10 weeks, followed by application of TPA twice weekly for 15 weeks as a tumor promoter, found that topical application of curcumin decreased the incidence of skin tumors (Khan et al., 2008).

Curcumin also has been shown to greatly inhibit lung metastasis associated with breast cancer. Human breast cancer cells of the cell line designated MDA-MB-231 were injected into the hearts of nude mice to induce lung metastases of hematogenous origin. Control mice were fed a control diet while the treatment group was fed a diet supplemented with 1% curcumin. After five weeks of treatment, the animals were assessed for the number of metastatic sites in their lungs. The results of the study showed a dramatic reduction in the number of metastases in curcumin-fed mice. More than 21% of the treated animals were free of metastases whereas none of the control animals were. Moreover, 68% of the curcumin-treated animals had fewer than three metastatic sites in the lung whereas only 17% of the controls showed less than three metastases (Bachmeier et al., 2008).

Several studies have also shown the chemopreventive effects of curcumin on colon cancer carcinogenesis. In one such study, rats five weeks of age were fed either a control diet or one fortified with 2000 ppm curcumin. Weekly subcutaneous injections of azoxymethane were used to induce colon carcinogenesis beginning at seven weeks of age. The animals were followed for 52 weeks and the results showed that curcumin-treated animals had a significantly lower incidence of colon adenocarcinomas. Curcumin also suppressed colon tumor volume by more than 57% compared with the control diet (Rao and Hashim, 1995). A number of other studies have found similar results with respect to curcumin's ability to suppress colon carcinogenesis (Lopez-Lazaro, 2008).

Curcumin has shown several benefits in models of liver cancer as well. For example, curcumin administered orally to rats as a component of the diet suppressed the development of intrahepatic foci after induction by aflatoxin B1, an occurrence that is considered a precursor to the development of hepatocellular neoplasms. Further studies have found that curcumin administration to rats inhibited diethylnitrosamine-induced liver inflammation and hyperplasia (Khan et al., 2008), which is suggestive of curcumin's ability to support healthy liver function.

Mechanisms of Chemoprevention

As previously mentioned, evidence from epidemiological studies suggests lower rates of several cancers in areas of the world where large amounts of curcumin are consumed in the diet. The evidence from animal studies presented above is intriguing in that it clearly demonstrates curcumin's chemopreventive potential *in vivo*. A look into the mechanism of curcumin's chemopreventive activity reveals that curcumin acts on multiple levels and in multiple pathways that influence carcinogenesis and the proliferation of cancers as well as their ability to metastasize.

A significant aspect of curcumin's biological activity is its ability to inhibit tumor growth at the initiation and promotion phase. Cancer cells activate the NF-κB pathway as a means of protecting themselves against various cancer therapeutics. This family of transcription factors helps cancerous cells survive via incompletely understood antiapoptotic mechanisms. Curcumin may be effective in this scenario due to its ability to inhibit the activation of NF-κB induced by TNFα as well as other cytokine-mediated activation. This has been shown in human lung epithelial cells, myeloma cells, Hodgkin's lymphoma, and prostate cancer cells. Beyond these effects, curcumin has the potential to induce apoptosis in tumor cells by various mechanisms, many of which have been reviewed in other papers (Singh and Khar, 2006).

One of the most important activities of curcumin for health maintenance and chemoprevention is its ability to scavenge various free radical species. Among other antioxidant mechanisms, curcumin has been found to modulate the activity of the enzyme glutathione-*S*-transferase, the enzyme involved in recycling glutathione, the major innate antioxidant defense system in cells. By modulating this activity, curcumin may also be able to sensitize tumor cells to apoptotic mechanisms, which serve to prevent tumor cell proliferation. On the other hand, curcumin's modulatory activity on antioxidant enzymes also allows it to enhance antioxidant defenses, as curcumin has been shown to increase glutathione concentrations in various cell types (Singh and Khar, 2006).

Curcumin has significant anti-inflammatory effects, which can lead to an inhibition of cancer cell proliferation and tumor growth. The COX and LOX enzyme systems play a large role in tumor cell growth, cellular adhesion, proliferation, and metastatic potential. Curcumin has the ability to inhibit both the COX and LOX systems, and inhibit arachidonic acid metabolism, which serves to decrease overall inflammation and the production of reactive oxygen species in cells. Furthermore, its inhibitory effect on NF-κB activation also significantly reduces inflammation by decreasing the production of several pro-inflammatory cytokines.

Additional data suggests a role for curcumin in regulating the cell cycle of precancerous and cancerous cells as a further means of chemoprevention. Several studies have shown that curcumin, through various mechanisms, influences cell cycle progression, generally in a dose-dependent fashion. In several cell lines, turmeric has been found to enhance the expression of tumor suppressor proteins and kinase inhibitors (Meeran and Katiyar, 2008). Curcumin also has direct antiangiogenic effects. This activity has been shown both *in vitro* and *in vivo*, and provides a further explanation for its tumor growth and metastasis inhibitory effects. Possible mechanisms that have been postulated include downregulation of the transcription of pro-angiogenic genes, a decrease in the invasion of endothelial cells, as well as the suppression of angiogenic growth factor production (Villegas et al., 2008).

Turmeric Essential Oil Compounds and Chemoprevention

Major compounds in the essential oils of turmeric are sesquiterpenoids: α-turmerone, β-turmerone, and *ar*-turmerone. The proportion of these terpenoids varies according to the rhizome's origin and the method of extraction (Ji et al., 2004; Jain et al., 2007).

Studied individually, each compound has been shown to have bioactivity *in vitro* and activity that is suggestive of several chemopreventive benefits. *ar*-turmerone, isolated from *C. longa*, has been shown to induce apoptosis in a dose and time-dependent manner in various cancer cell lines including K562 (an erythroleukemia cell line), L1210 (a lymphoid leukemia cell line), and U937 (a diffuse histiocytic lymphoma) (Ji et al., 2004). Beta-turmerone and *ar*-turmerone from *Curcuma zedoaria* has been demonstrated to inhibit lipopolysaccharide (LPS)-induced prostaglandin E2 production in mouse macrophage cell RAW 264.7 in a dose-dependent manner, as well as inhibit LPS-induced nitric oxide production (Hong et al., 2002; Lee et al., 2002).

As it is now known that type 2 diabetics are at increased risk of many cancers, the finding that these compounds *in vivo* can bind to peroxisome proliferator-activated receptor gamma (PPAR-gamma) in type 2 diabetic KK-A(y) mice is of interest. The study, carried by Nishiyama and colleagues, indicates that both these major sesquiterpenoids and curcuminoids work together either via an additive or synergistic mechanism to induce ligand-binding activity, shown in both human PPAR-gamma and the GAL4-PPAR-gamma chimera assay (Nishiyama et al., 2005). PPARs may play critical roles in cellular development and differentiation as well as tumorigenesis.

As cancer is associated with oxidative stress, recent evidence that sesquiterpenoid-rich curcuma oils from *C. longa* provide neuroprotection during oxidative stress is noteworthy. In one study, 500 mg/kg given intraperitoneally 15 minutes before a two-hour middle cerebral artery occlusion followed by a 24-hour reflow in rats significantly diminished infarct volume, mitigated neurological deficits, and counteracted the damaging effects of oxidative stress (Rathore et al., 2008). The percentage of ischemic lesion volume was lessened as shown by diffusion-weighted imaging. Two other

studies have shown similar effects using a rat embolic stroke model and in response to induced cerebral ischemia-reperfusion injury (Dohare et al., 2008a,b). In the former study, curcuma essential oil, whose major turmerone was *ar*-turmerone, downregulated iNOS-derived NO production during ischemic injury. This downregulation is crucial for inhibiting the upregulation of NO in tissues subject to ischemic injury.

What remains to be shown is whether the terpenoids derived from *C. longa* are safe when consumed individually or in various combinations. However, exposure to terpenoids as a result of turmeric consumption appears to be safe.

Curcumin has been shown to be safe, even at relatively high doses when taken orally. Doses of curcumin as high as 3600 mg to 8000 mg given daily for four months in one study of colorectal cancer were found to cause mild nausea and diarrhea in a few patients. One concern regarding curcumin administration is its seemingly low oral bioavailability. Only a fraction of an oral dose is systemically absorbed. Thus, some researchers feel that curcumin may have its greatest chemopreventive effects on cancers of the gastrointestinal tract and skin. However, studies using high doses of curcumin have shown detectible levels in the blood and urine, while metabolites of curcumin were also detectible in both (Pari et al., 2008). Importantly, studies in animals, many of which were reviewed above, also show clear chemopreventive effects of curcumin with oral administration, suggesting that either curcumin or its metabolites are absorbed to the extent necessary to have the desired effect.

GARLIC (*ALLIUM SATIVUM*)

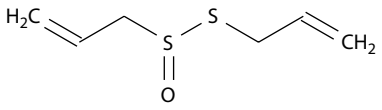
Allium sativum L., is among the oldest of cultivated plants, and has been used both to enhance the taste and flavor of foods and as a folk medicine for thousands of years. Appropriately, its genus, which comprises more than 500 species is named after the Celtic word for "pungent" (Block, 1985; Sengupta et al., 2004). As long ago as 1550 BC, the Egyptians documented the use of garlic in at least 22 different formulations for medicinal use, including the treatment of tumors (Shukla and Kalra, 2007). A poem written in 1609 by Sir John Harrington reads:

> "Garlic then have power to save from death, Bear with it though it maketh unsavory breath, And scorn not garlic like some that think, It only maketh men wink and drink and stink." (Block, 1985, p. 119)

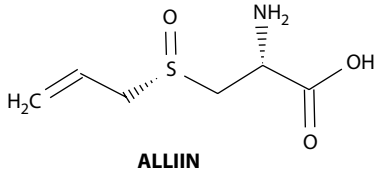
The unique components of garlic that appear to have the most activity with regard to health benefits are the organosulfides. These compounds appear to be produced by the plant to protect them from insult by bacteria, fungi and animals, as they have both antimicrobial properties, and function as a deterrent for animals that taste them (Ariga and Seki, 2006).

The chemistry behind the formation and metabolism of these compounds has been intensely studied and much is known about them. The major sulfur component in garlic is γ-glutamyl-*S*-

FIGURE 8.3 Garlic bulbs.



ALLICIN



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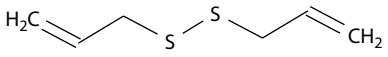
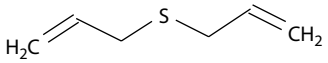
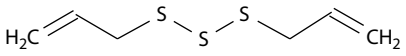


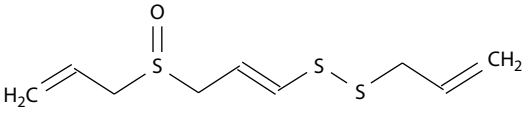
DIALLYL DISULFIDE



DIALLYL SULFIDE



DIALLYL TRISULFIDE



AJOENE

FIGURE 8.4 Organosulfur compounds in garlic.

alk(en)yl-l-cysteine, which becomes hydrolyzed and oxidized to form alliin (*S*-allyl cysteine sulfoxide). Alliin is an odorless compound that accumulates in the garlic bulb when it is stored in cool temperatures. When a clove of garlic is crushed or chopped, the enzyme alliinase (which makes up roughly 10% of the total protein weight of garlic) is released from its respective compartment and acts upon alliin, turning it into a number of highly odorous compounds, one of which is allicin. These compounds are quite unstable and quickly degrade to form a number of oil soluble organosulfur compounds including diallyl sulfide, diallyl disulfide, diallyl trisulfide, dithiins, and ajoene (Ariga and Seki, 2006; Herman-Antosiewicz et al., 2007). The properties of the latter compounds have been studied quite extensively.

Garlic and Chemoprevention

Scientific evidence associating garlic and *Allium* species in general with chemoprevention has been developing for many years and continues to build. The National Cancer Institute of the U.S. National Institutes of Health placed garlic at the top of its list of potential foods that may play a role in the prevention of cancer during its Designer Food Program in the 1990s (Tanaka et al., 2006).

Many published epidemiological studies show an association between garlic consumption and a decreased risk for a wide variety of cancers. One of the more recent epidemiological publications on the subject was a collection of case–control studies performed in southern Europe (Galeone et al., 2006). The paper reported on over 9000 subjects with one of nine different histologically confirmed cancers (along with over 17,000 controls—subjects admitted to the same hospital networks, but for reasons other than cancer treatment or long-term dietary modifications). Individuals were interviewed using a structured food frequency questionnaire. The authors concluded that increased garlic intake was inversely related to the incidence of oral, esophageal, colon, larynx, ovarian, and renal cancers (but not to prostate and breast cancer) in a significant manner. This was true even after adjusting for total vegetable intake.

Chan et al. (2005) published a case–control study specifically on pancreatic cancer and food intake. They directly interviewed 532 cases and 1701 controls, and found that the subjects in the highest quartile of onion and garlic consumption compared with the lowest resulted in an odds ratio of 0.46 (95% CI 0.33–0.63). Of all the vegetables investigated, garlic and onions showed the strongest inverse relationship to cancer (Chan et al., 2005). Similarly, Hsing and colleagues found a statistically significant decrease in the risk for prostate cancer in men who consumed the most garlic (odds ratio = 0.47, 95% CI 0.31–0.71). The results were independent of body size, consumption of other foods, and total calorie intake (Hsing et al., 2002).

A number of reviews have also been published on this subject, including one published in 2009 that utilized the U.S. Food and Drug Administration's evidence-based review system for the scientific evaluation of health claims to determine the relationship between garlic and cancer prevention (Kim and Kwon, 2009). The paper reviewed 19 independent studies and concluded that there is credible, while limited, evidence to suggest an association between garlic intake and lowered risk of colon, prostate, esophageal, larynx, oral, ovarian, and renal cancers. A meta-analysis published in 2000 took data from 22 studies that reported relative risks for garlic and cancer and calculated that greater consumption of garlic may be associated with decreased risk of stomach and colorectal cancers (Fleischauer et al., 2000).

Mechanisms of Chemoprevention

Numerous studies in animals are in overall agreement with epidemiological evidence for chemopreventive properties of garlic and its constituents (Sengupta et al., 2004; Ngo et al., 2007). Studies of the mechanisms responsible for the chemopreventive actions have been progressing for more than 20 years. A number of distinctly different mechanisms may independently play a role. For example, garlic's organosulfur compounds have the ability to modulate enzymes in both the phase I and phase II detoxification pathways. Diallyl trisulfide strongly induces hepatic phase II enzymes in rats; these particular enzymes are critical as they complete the detoxification of otherwise innocuous compounds that can become toxic intermediates formed during phase I detoxification (Fukao et al., 2004). Diallyl trisulfide can protect the liver from injury from carbon tetrachloride, which causes lipid peroxidation of hepatocytes via the trichloromethyl radical formed by the phase I enzyme, cytochrome p450 2E1. The protective effect was congruent with the degree of induction of phase II enzymes by diallyl trisulfide. Additionally, garlic powder is protective against aflatoxin B1-induced carcinogenicity in rats, which is due in part to upregulating enzymes involved in aflatoxin B1 detoxification (Berges et al., 2004).

While garlic-derived sulfur compounds do not tend to have strong antioxidant activity *in vitro*, they appear to affect antioxidant status indirectly by enhancing the activity of endogenous antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase. This may function as a second chemopreventive mechanism (Ariga and Seki, 2006). Diallyl sulfides also have shown inhibitory effects on COX-2 gene expression. This is important because certain malignant and premalignant lesions have been found to overexpress the COX-2 enzyme, which is induced by NF-κB, and there is some evidence for use of COX-2 inhibitors in chemoprevention (Elango et al., 2004).

In vitro studies have shown antiproliferative and or differentiation-inducing effects of garlic's organosulfur compounds, especially diallyl trisulfide, in a number of different cancer cell lines such as colon, lung, skin, prostate, neuroblastoma, and leukemic (Sengupta et al., 2004; Ariga and Seki, 2006; Shukla and Kalra, 2007). Induction of cell cycle changes such as increased cell cycle arrest in the G_2/M phase and apoptosis through mechanisms such as induction of caspase-3 and or phosphorylation of Bcl-2 have been observed *in vitro*. Garlic extract and/or garlic compounds have also shown inhibitory activity on adenosine deaminase and cyclic AMP phosphodiesterase enzymes, disassembling activity on microtubule networks, protection from the formation of DNA adducts and nitrosamine, and even dose-dependent anti-angiogenic activity in tumor bearing mice (Agarwal, 1996; Sengupta et al., 2004; Shukla and Kalra, 2007). While there is currently evidence to support each of these mechanisms of chemoprevention of cancer, how they fit together into a succinct model is still a matter of speculation.

In conclusion, there is good evidence from epidemiological, animal, and *in vitro* experiments that garlic and its sulfur compounds exert cancer preventative qualities. In future studies, it may be prudent to flush out what role cultivation and preparation techniques play in the chemopreventive activity of garlic. For example, as one might suspect, garlic grown in an environment with greater concentrations of sulfur has dramatically higher levels of sulfur-containing compounds found in the bulb and demonstrates a more protective effect against hepatocellular cancer in rats (Berges et al., 2004). Additionally, the manner in which garlic is prepared for consumption (e.g., exposure to heat with cooking) will likely have an effect on the stability of the sulfur-containing compounds, and may result in diminished efficacy (Ariga and Seki, 2006). In fact, decreased efficacy after cooking was shown in an *in vitro* study looking at the antiplatelet activity of the sulfur compounds (Cavagnaro et al., 2007). Thus while eating garlic appears to provide beneficial effects in relation to cancer prevention, determining the differences between various cultivation conditions and how cooked versus raw garlic affects cancer prevention deserves further investigation.

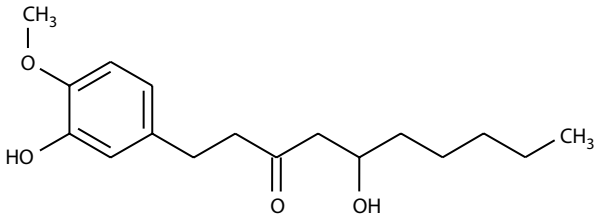
GINGER (*ZINGIBER OFFICINALE*)

Ginger (*Zingiber officinale*) has a long history of use as a spice and traditional medicine dating back several thousands of years. The root or rhizome of the plant has been used in traditional Chinese medicine, the Indian Ayurvedic system, and by Unani practitioners for therapeutic purposes since ancient times. Chinese medical practitioners have used the plant to treat headaches, nausea, and colds. Mediterranean and Western herbal medicine practitioners have used ginger root for the treatment of inflammatory conditions including arthritis, rheumatological disorders,and muscle pain. Modern uses of ginger root based on research and practical application include the treatment of atherosclerosis, migraine headaches, high cholesterol levels, gastric ulcers, depression, and rheumatoid arthritis. Many of these therapeutic uses are based on the reputation of ginger as a potent anti-inflammatory botanical.

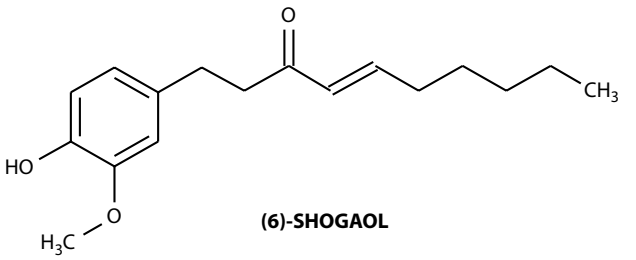
Ginger is widely consumed as a culinary spice throughout the world. It is highly regarded in China, India, and other Asian countries, where it is used as a component of vegetable and meat

FIGURE 8.5 Ginger rhizome.

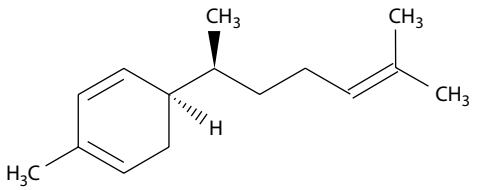
dishes, and as a part of the mixture of spices that constitute curry dishes. Ginger root contains lipids, waxes, carbohydrates, several vitamins and minerals, along with proteolytic enzymes that have demonstrated anti-inflammatory properties. The root is also a rich source of volatile oils, including zingiberene, curcumene and farnosene. More than 40 distinct monoterpenoid hydrocarbons also make up the volatile fraction and contribute to the unmistakable taste and smell of ginger root. Additionally, several nonvolatile pungent compounds are present in the root, including the gingerols and shogaols (Shukla and Singh, 2007). These compounds have garnered the most attention for their anti-inflammatory and cancer chemopreventive potential. Ginger's broad use, coupled with the fact that countries such as India and others in South Asia have relatively low rates of several types of cancer, make it an interesting spice to investigate for its cancer preventive properties.



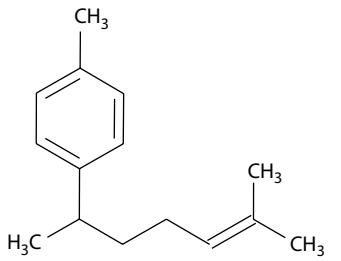
(6)-GINGEROL



(6)-SHOGAOL



ZINGIBERENE



CURCUMENE

FIGURE 8.6 Volatile and pungent compounds in ginger.

Ginger and Chemoprevention

Several animal models of carcinogenesis have been used to highlight the chemopreventive properties of ginger extracts and individual components such as the gingerols and shogaols. The most active anti-cancer compounds present in ginger root based on the results of several lines of research include (6)-shogaol and (6)-gingerol.

In an animal model of colon carcinogenesis, male rats were given a weekly injection of the potent carcinogen dimethylhydrazine (DMH) for 15 weeks to induce colon cancer. In the group of rats that received only DMH injections and no ginger (the positive control), 100% developed colonic tumors. A second group of rats received ginger (50 mg/kg body weight per day) orally beginning one week prior to the initiation of DMH injections (15 weeks) and continuing for one week after cessation of the injections. A third group of rats received weekly DMH injections for 15 weeks and then received the same ginger powder (50 mg/kg bw/day) beginning at week 16 and continuing to week 30. Interestingly, none of the rats in the group pretreated with ginger developed tumors, while only one rat (10%) in the third group developed colonic tumors. The dried, powdered ginger potently inhibited the development of colon cancer (Manju and Nalini, 2005).

Similarly, a study in nude mice found that the administration of (6)-gingerol significantly suppressed the development of colonic tumors. Mice were fed (6)-gingerol three times weekly for two weeks prior to injection with the HCT116 colon cancer cell line. Of the 20 mice in the vehicle-treated group, 13 measurable tumors were identified whereas of the 21 mice in the (6)-gingerol treated group, only four measurable tumors were identified. The effects of (6)-gingerol were first assessed *in vitro* in this set of experiments. The researchers found that (6)-gingerol significantly inhibited the proliferation of both HCT116 and HT-29 colon cancer cell lines (Jeong et al., 2009), suggesting the potent effect of this ginger compound against colon carcinogenesis both *in vitro* and *in vivo.*

In a rat model of chemically induced bladder cancer, a ginger extract given at a level of 1% of the diet for 26 weeks, and containing a concentration of 2.54% gingerols, was found to confer complete protection against the development of neoplasias and offer significant protection against the development of hyperplastic lesions in the bladder. The control group, as well as a group consuming the ginger extract at a level of 0.5% of the diet, developed neoplastic lesions and had a significantly higher incidence of hyperplasia (Ihlaseh et al., 2006).

Further evidence suggests that (6)-gingerol can delay the onset of skin tumorigenesis. Tumors were induced in mice with the topical application of the known carcinogen benzo[*a*]pyrene. Two groups of animals were also given (6)-gingerol via topical application. (6)-gingerol was applied either 30 minutes before or 30 minutes after the application of benzo[*a*]pyrene in these groups. The results from both (6)-gingerol groups revealed a delayed onset of tumor formation as compared to the group treated with benzo[*a*]pyrene alone. Furthermore, the benefits seen in the groups treated with (6)-gingerol included increased tumor-free survival of the animals and reductions in tumor volume as well as significant reductions in the average number of tumors per mouse (Nigam et al., 2009).

Research into the various compounds present in ginger, as well as the whole rhizome itself, has provided insight into potential chemopreventive benefits associated with the plant. Given ginger's wide use as a medicinal herb and culinary spice, its cancer preventive benefits add to the utility of this wonderful food. Evidence highlighted here is highly suggestive of numerous chemopreventive benefits associated with the gingerols and shogaols present in ginger, as ginger may have potential impacts on slowing the development of a wide variety of different cancers. Ginger also clearly acts on multiple mechanisms that affect the process of carcinogenesis. *In vitro* studies have highlighted the effects of ginger and ginger compounds on various cell lines, while evidence from animal studies shows that oral administration of ginger compounds affects several processes associated with cancer development, adding to the positive effects associated with its culinary use.

Mechanisms of Chemoprevention

Data from cancer registries in India suggest relatively low rates of skin cancer, colon cancer, and others. The consumption of phytochemicals from several spices on a daily basis may provide an explanation for the differences seen in cancer prevalence in India versus the Western world. Ginger is a spice that is widely consumed in India and other Asian countries. Evidence suggests that ginger as a whole and its individual compounds act on multiple pathways constituting the carcinogenic process that render it a useful chemopreventive agent. Ginger has displayed antioxidant and anti-inflammatory effects, while also exhibiting anti-angiogenic effects and cell cycle modulating activity. Several compounds from ginger have also been shown to induce apoptosis, the normal process of programmed cell death (Shukla and Singh, 2007).

Recent research has found that (6)-shogaol has the ability to induce apoptosis in a human hepatoma cell line that is commonly resistant to treatment by a number of cancer chemotherapeutics. Mahlavu cells are poorly differentiated mutant cells of a human hepatoma cell line that highly express multidrug resistance genes and anti-apoptotic proteins. (6)-shogaol induced apoptosis in these mutant cells by promoting the production of reactive oxygen species and depleting intracellular glutathione concentrations, making the cells highly susceptible to oxidative damage (Chen et al., 2007).

Further studies have found that compounds from ginger exhibit antimetastatic effects in various cancer cell lines. For example, one of the attributes breast cancer cells must exhibit to manifest their metastatic potential is the ability to degrade the extracellular matrix and escape through the basement membrane. This is accomplished through the activation of enzymes, an important class of which includes the matrix metalloproteinases (MMPs). Treatment of MDA-MB-231 breast cancer cells with (6)-gingerol resulted in decreased expression of MMP-2 and MMP-9, two important metalloproteinases involved in extracellular matrix degradation, indicating (6)-gingerol's anti-metastatic potential. In the same experiment, (6)-gingerol was also found to reduce cell motility in this breast cancer cell line. By decreasing cellular motility and impacting the MMPs to inhibit cell adhesion, invasion and migration, (6)-gingerol acts on multiple mechanisms to inhibit metastasis (Lee et al., 2008).

Proliferation of several tumor cell types is regulated by NF-κB. Constituents in ginger have been shown to inhibit NF-κB activation. Specifically, in an experiment conducted in two chemoresistant ovarian cancer cell lines, a ginger extract was found to produce a significant inhibitory effect on NF-κB in both cell lines. NF-κB-mediated cancer cell proliferation often involves the secretion of IL-8. Thus, IL-8 inhibition is a potential therapeutic target for limiting proliferation and growth of several cancers. Expression of IL-8 is characteristic of the ES-2 and SKOV3 ovarian cancer cell lines. Treating these cell lines with dried whole ginger root powder extract (1:1 extraction solvent: ethanol 50%/water 50%) standardized to 5% gingerols *in vitro* was found to significantly inhibit IL-8 production. Furthermore, several angiogenic proteins are under transcriptional control of NF-κB. A particular angiogenic factor known as VEGF (vascular endothelial growth factor) is produced in significant amounts by several ovarian cancer cell lines. Treating these cell lines with ginger extract was shown to inhibit the secretion of VEGF (Rhode et al., 2007). Thus, ginger extracts prevent cancer cell proliferation *in vitro* by inhibiting numerous pathways related to NF-κB activation, exhibiting significant chemopreventive activity.

Further evidence of this comes from studies in rats with liver cancer. As chronic inflammation mediates a number of steps in the carcinogenic process, including cellular transformation, proliferation, invasion, angiogenesis, and metastatic potential, inhibition of pro-inflammatory cytokines may significantly decrease carcinogenesis. TNF-α is one of the main pro-inflammatory cytokines and a strong inducer of NF-κB. In rats with liver cancer who were fed an ethanolic ginger extract by gavage for eight weeks at a concentration of 100 mg/kg body weight, a significant inhibitory effect on the expression of NF-κB and TNF-α was seen (Habib et al., 2008), indicating the anti-inflammatory effect of ginger. Additional evidence for the involvement of inflammation in the pathogenesis of

cancer, and of ginger's anti-inflammatory role, is provided by studies that show ginger's inhibitory effect on leukotriene synthesis. Leukotriene B_4 is a potent chemoattractant that has been implicated in cancer development, including colorectal cancer. (6)-Gingerol has been shown to suppress colon cancer growth by binding to leukotriene A_4 hydroxylase, the enzyme that catalyzes the final step in leukotriene B_4 synthesis. The inhibition of leukotriene A_4 hydroxylase has been found to lead to a reduced cancer incidence in several animal models (Jeong et al., 2009).

DISCUSSION

Carcinogenesis is a process in which abnormal cell growth and proliferation takes place due to a disruption of the physiological function of normal cells. It starts with transformation of normal cells. Certain normal genes called proto-oncogenes are thought to be susceptible to carcinogens. When these genes are altered, a transformation in a cell can result, which affects the normal regulation of cellular division and differentiation. Following alteration, these proto-oncogenes become oncogenes. In stark contrast to and in opposition to oncogenes are the tumor suppressor genes, which are also referred to as anti-oncogenes and play the opposite role as negative regulators of cell growth. When mutations in these genes occur the tight regulation of cell growth is lost and a tumor can form (tumorigenesis). Cancer cells are able to grow uncontrollably when they escape one or more processes that function to eliminate them or they cause physiologic changes that function to promote their growth. Apoptosis is a normal process of programmed cell death that functions to remove abnormal or dysfunctional cells. As tumors proliferate, they may manipulate the environment in a manner that causes the formation of new blood vessels (angiogenesis), which supply the tumor with nutrients and thus allow for continued unregulated growth. Metastasis, the most lethal stage of carcinogenesis, takes place when the cells are able to break through the basement membrane—often by interfering with matrix metalloproteinase (MMP) inhibition. Once the basement membrane is compromised, the tumor cells are able to enter into circulation where they can be deposited in distal locations forming new tumors (IUCC, 1999).

Since the rate of cancer incidence is the lowest in India and the highest in the U.S. white population, a closer look for evidence to explain this difference is warranted. The authors propose a hypothesis that greater consumption of culinary spices offers a plausible explanation. The hypothesis is supported by a great deal of published research demonstrating the anti-inflammatory and anti-oxidant properties of culinary spices such as turmeric, garlic, and ginger. Since these culinary spices are consumed in large quantities in India and because the link between chronic inflammation and cancer is well established, while not proven, recommending the regular use of these culinary spices is a reasonable conclusion. Further research is needed to prove this conclusion for the spices presented in this chapter as well as for other commonly consumed spices in India and elsewhere.

The fact that the rate of diseases like cancer that are associated with inflammation are on the rise, with cancer expected to become the number one killer in the United States in 2010 (Aggarwal et al., 2009), necessitates continued public education on the importance of lifestyle modifications such as diet and exercise to reduce the risk to individuals, while also reducing the burden to overly taxed healthcare systems.

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Section II

Bioactive Foods and Supplements in Cancer Prevention

9 Bioactive Foods in Cancer Prevention

Rakesh Sharma and Jose Katz

CONTENTS

INTRODUCTION

The term "bioactive food" was first defined "as foods, food ingredients, or dietary supplements that demonstrate specific health or medical benefits including the cancer prevention and treatment of disease beyond basic nutritional functions" (Baichwal, 1999, pp. 19–20). Now bioactive foods

have emerged as potential supplements in cardiovascular and cancer preventive natural sources from food (Baichwal, 1999; Amin et al., 2009).

Bioactive foods are fortified usable for daily use diets with vitamins, minerals, and nutraceuticals or any food or part of a food that provides health or disease prevention benefits with nutrition. The bioactive foods are served as cuisine line of frozen foods on the shelf such as Camden (balanced meal program) for hypertension, high cholesterol, or adult-onset diabetes; Tropicana Products, orange juice; Procter & Gamble's FruitCal®, calcium citrate malate, and so on, Growth in the bioactive food market has also rocked new combinatorial chemistry and profoundly accelerated the pace of discovery of new bioactives such as new high-oleic soybean contains no *trans* fatty acids reduces the cancer disease (Bougnoux et al., 2009).

Both food industries and pharmaceutical industries have roped up to use bioactive food, pharmaceutical and nutrition products—from drinkable yogurt to mainstream designer bone, heart, and digestive health foods to calcium chews, from sports nutrition bar makers to soy burger manufacturers—bioactive foods are poised to undergo very rapid growth in the coming years. Bioactive foods are designed basically to meet four consumer demands: taste, convenience, simple proposition, and price. A successful bioactive food product's bioactive role needs its perceptible health benefit. If a health benefit is clearly understandable, or if the health benefit is clearly perceptible—such as weight loss or stress reduction, or can be easily measured—such as a product that reduces cholesterol, then the product is much more likely to succeed. Now interest is growing for use of drugs mixed with bioactive foods in cancer prevention. Recently JIVA™, a bioactive food made of resveratrol combined with garlic, has been advocated as potential anticancer formula (Condori et al., 2009).

Similar bioactive foods are in market by various companies including Kellogg Company, Ensemble products, Johnson & Johnson, Benecol®, Balance Bar Company, Nestlé and Vevey (Switzerland) and several Asian companies such as Reddy's lab, Himalaya Products, Dabur, Baidhyanath, Zhundu Pharmacy to manufacture dietary products with potentials in cancer prevention. These bioactive foods work on the principle that cancer disease is a concern of environmental toxicants, fatigue/energy, and stress. Tropicana Ultimate Smoothie combined with Galaxy's soy, rice, and oats has preventive characteristics Veggie Milk® base with Tropicana's fruit juices. It was estimated that major benefactors were subjects with heart disease (75%), cancer (81%) including breast cancer (48%), colon cancer (37%), and prostate cancer (25%) using bioactive foods (Sloan Trends, 1999).

TIME SERIES OF AWARENESS IN BIOACTIVE FOODS

Untill 1990, the concept of bioactive foods was initially considered as natural foods in diet to provide energy as recommended daily requirement in the body for health. Later the importance of bioactive foods was realized as beneficial in different nutritional and internal organ disorders with growing use of the traditional practices, such as Ayurvedic principles in India. In the last decade, use of bioactive foods as self-prescription has grown in cardiovascular, cancer, developmental conditions. In last nine years, national and federal bodies accepted bioactive foods as possible neutraceutical therapy in the main stream of medical education and health with the name of "Complementary and Alternative Medicine." The healthcare industry demonstrated the shift of growing population from medical treatment of cancer toward nonprescription supplements and self-medication in cancer management and prevention. The growing awareness of benefits and shift of healthcare economics in favor of bioactive foods has brought bioactive foods in the spotlight of government health policy on systematic use of bioactive food active principles (so-called nutraceuticals) in prevention and control of various chronic diseases. Since 2005, the efforts of the National Cancer Institute (NCI) and other global efforts have documented fact sheets and several health documents on bioactive foods, mainly herbs and their different plant parts, in cancer management. The major efforts were devoted to investigation of inhibitory effects of active chemical component(s) on cell proliferation, cancer oncogenesis resulting in reduced metastasis, delayed apoptosis, reduced necrosis, and rate of malignancy growth in initial stages. In last two years, the use of herbs in cancer prevention

and disease control has been extended further as protective dietary supplementation policy of Centers for Disease Control and Prevention (CDC) under its independent supervision. However, mechanisms of herb action still remain unproven and unvalidated but practice of bioactive foods or food supplement formulations in cancer prevention is acceptable.

What are Bioactive Foods?

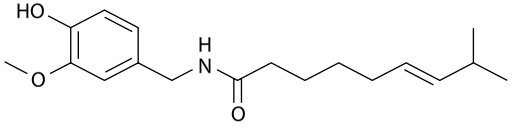
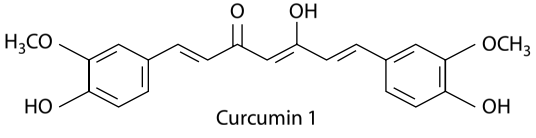
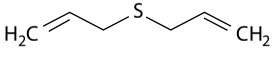
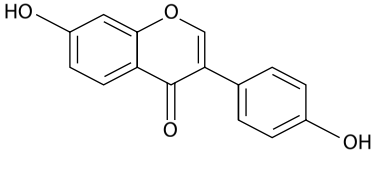
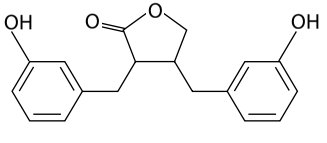
Bioactive foods are natural forms of herbs, whole plants and their parts such as flowers, roots, oils, stems are rich in bioactive chemical compounds, the so-called "nutraceuticals." The main difference between pharmaceutical drugs and nutraceuticals is their isolation methods and purification levels. The pharmaceutical drugs are available with the highest purity as mono component of artificial chemical(s) while nutraceuticals are partially purified extracts containing mixtures of natural chemicals from bioactive foods. Bioactive foods may be used as "nutraceutical formulations" as combinations of different parts of plants or the parts that have value in health promoting, disease preventing or semi-medicinal properties. Bioactive foods may be fortified with vitamins, proteins, amino acids, minerals, and carbohydrates. Different food companies have advocated their bioactive foods as natural products from (a) food industries, including herbals and dietary supplements, and (b) products from pharmaceutical industries, including newly emerged bioengineered microorganisms, agroproducts, or active biomolecules. It may range from isolated nutrients, herbal products, and dietary supplements and diets to genetically engineered "custom" foods and processed products such as cereals, soups and beverages. Chemically, the active components in bioactive foods may be classified as isoprenoid derivatives (terpenoids, carotenoids, saponins, tocotrienols, tocopherols, terpenes), phenolic compounds (curamines, tannins, ligrins, anthrocynins, isoflavones, flavonones, flavanoids), carbohydrate derivatives (ascorbic acid, oligosaccharides, nonstarch PS), fatty acids and structural lipids (n-3 PUFA, CLA, MUFA, sphingolipids, lecithins), amino acid derivatives (amino acids, allyl-S compounds, capsaicnoids, isothiocyanates, indols, folate, choline), microbes (probiotics, prebiotics) and minerals (Ca, Zn, Cu, K, Se).

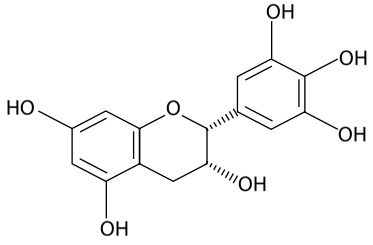
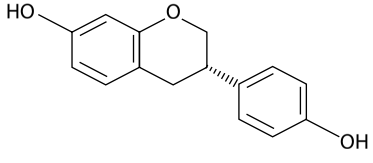
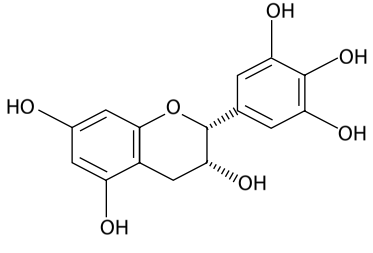
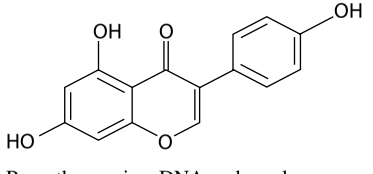
Broadly, the bioactive foods were reported as whole medicinal plants with active natural chemical compounds. Recently, Wildman (2005) reported the therapeutic value of bioactive foods in cancer and other chronic diseases mainly cardiovascular, diabetes, kidney, and lung diseases. The majority of cancer prevention evidence comes from animal experiments on phytochemicals, fats, flavones, phytoesterogens, isoflavonones, genestein, curcumin, capsaicin, epigallocatechin-3-gallate, gingerol, lycopene, antaoxidants, vitamins, minerals (Wildman, 2005). Self-described testimonies of herbal medicine and its success accrued over years in favor of liquorice (for peptic ulcer), isoflavones (for cholecarotenoids, saponins, tocotristerol lowering, osteoporosis), phosphatidylcholine (for hepatitis), ginger (for emetic disorder, dizziness, carminative), kambocha tea (for arthritis), glucosamine (for chondroitin), vitamins C, D, and E, minerals Zn, Se, and Cu (for lycopene), lutein (for antipain), leupeptin, urokinase inhibitor (for prostate cancer), fenugreek (osteoarthritis), lycopene, glucans (for cardiovascular disease), green tea (for cancer), carotenoids, *Trigonella foenum-graceum* (as antidiabetic, anticancer), noni *Morinda citrifolia* (for relief blood pressure, muscle pain), *Thymus vulgaris*, *rhus coriaria* (for Antibacterial activity), sorrel (for immune system), *Geranum sanuineum* (as antiviral) over years. Lycopene, silbinin, shark cartilage, vitamin D (to decrease osteoporosis and bone pain), green tea, selenium and vitamin E, grape seed extract, modified citrus, pectin, soy, PC-SPES are cited as prostate cancer protective food supplements (Cherukuri, 2008; Gromadzińska, 2008; Sharma, 2009a,b). Still, a lot of herbs remains to be tested as anticancer in nature. A comprehensive list of potential herbs is presented in Table 9.1.

How Bioactive Foods Act?

Bioactive foods may act as a whole plant or part of a plant rich in essential nutrients showing improvement of diseased condition or as single component drugs affecting specific biochemical

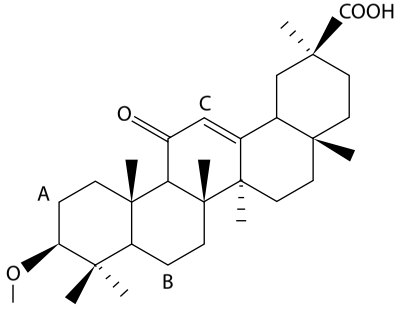
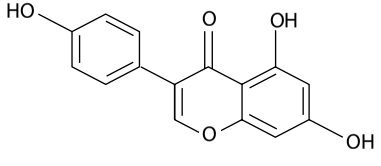
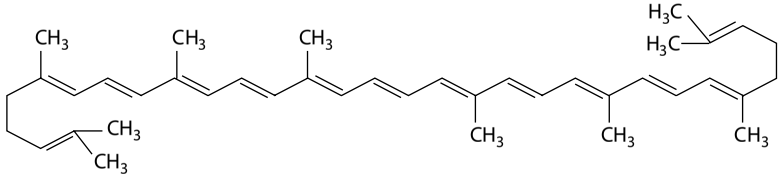
TABLE 9.1
Examples of Nutraceuticals and Active Components of Bioactive Foods Shown with their Benefits in Different Cancers and Mechanism of Chemoprotective Action in the Body Along with their Mode of Action and Formula in Chemical Nomenclature

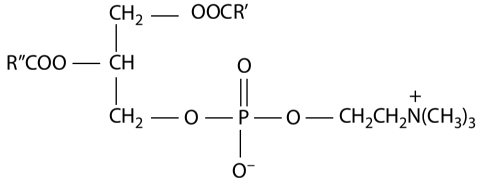
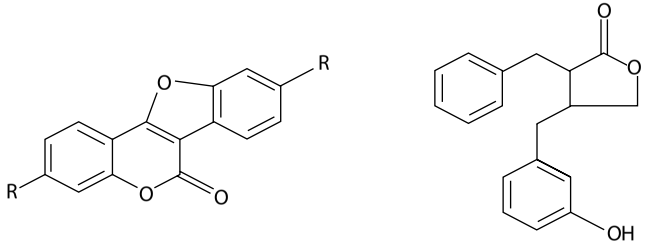
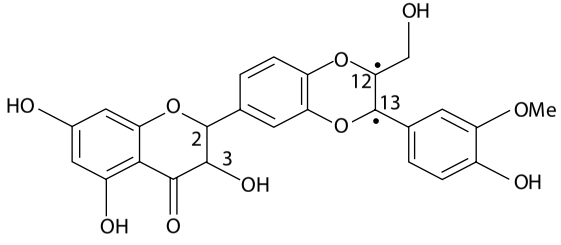
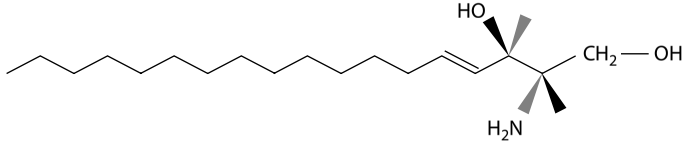
Active Component	Cancer	Mechanism	Structure
Ajoene (garlic)	PtK2	Antineoplasia cytotoxicity	Trithiadodeca-1,6,11-triene-9-oxide
Antioxidants	B, Br, C, O, G, P	Free radical scavenger	HO^-
Citrus	G	Delayed apoptosis	Limonoids
CLA	Br	?	Conjugated linoleic acid
Capsaicin	Br, P	VR1 receptor/ion channel	
Carnosol	C	O•H scanenger	Diterpenes
Curcumin	C, P	NKX 3.1gene, cytokines	 Curcumin 1
Dallyl sulphide (garlic)	B, C, P	Cyt oxidase, LDH, Glu reductase	
Daidzein	C, I	Antioxidation regulation	
Enterolactone	G, I, P	Antioxidative	

Epigallocatechin-3-gallate	C, L	DNA metTrans, LDH inhibitor	
Ellagic acid	Br, C, P	Antioxidant protection	3,4,5-Hydroxybenzoic acid
Equol	C, P	??	
Fenugreek	Br, C, CO, G, I, P, PN	Cytokines, redox reactions	??
Gingerol	Br, C, G, I, CO, P	VR1 receptor, caspase	Methoxy phenyl decanone
Green tea	Br, C, CO, P	Reduced MMP 2,9; cell proliferation	
Genestein	Br, P		
Grape seed extract	CO, G, L, P, PN	Cytotoxicity, antioxidant, antiesterogenic, antiangiogenic	Proanthocyanins, DNA endonuclease

continued

TABLE 9.1 (continued)
Examples of Nutraceuticals and Active Components of Bioactive Foods Shown with their Benefits in Different Cancers and Mechanism of Chemoprotective Action in the Body Along with their Mode of Action and Formula in Chemical Nomenclature

Active Component	Cancer	Mechanism	Structure
Glycyrrhizin	CO, G, I	Peroximase proliferation	
Isoflavones	Br, P, PN	HMG CoA-LDH inhibitor, caspase	
Kambocha tea	Br, CO, C, G, I	Free radical scavenger	??
Liquorice	G	Estrogen receptor agonism	Methyl glabridins
Limonene	Br, CO	Farnesyltransferase inhibitor	1-met-4-Propenyl-cyclohexene
Lutein	B, P, S	Antiooxidant	Xiazanthin
Lycopene	Br, CO, G, I, P	Antioxidant	
Meditarranian diet	CO, G, I, P	Hemocyanins, low fat	??
Pectin	G, P	Leminin, fibronectin conjugation	Galectins 2, 3 in apoptosis

Phosphatidylcholine	CO, G, I	Choline transport, phosphorylation	
Phytoesterogen (soy)	Br, C, G, I, CO, P	Antioxidant (lunasin, coumestan lignan)	
Silbinin	CO, L	Apoptosis, reduced cell growth	
Selenium and vitamin E	Br, C, CO, G, I, P, PN	cdk2, PKC, G1/S DNA breaks	Selenoproteins, S-Se-S, S-Se
Sphingolipid	Br, G, I, P	Cell cycle arrest, apoptosis senescence and differentiation	
Soy, PC-SPES	Br, C, CO, G, I, O, P, PN	G_2m cell cycle kinase, cytokines	Chalcones
α-Tocopherol	Br, CO, P	Antioxidant, antiproliferation	α-Tocopherol

continued

TABLE 9.1 (continued)
Examples of Nutraceuticals and Active Components of Bioactive Foods Shown with their Benefits in Different Cancers and Mechanism of Chemoprotective Action in the Body Along with their Mode of Action and Formula in Chemical Nomenclature

Active Component	Cancer	Mechanism	Structure
		Cyclooxygenase inhibition, apoptosis	γ-Tocopherol
Vitamins and minerals	All cancers	Oxidative phosphorylation	Active vitamin forms
A		Proton pumps	Retinal
B		Phosphorylation, redox reactions	Pyrophosphates
D		1,25-Dihydroxycholecalciferol	Calciferol
Folic acid		5-Methyltetrahydrofolate	Folate bound form
Calcium		Calmodulin, Ca^{2+} channels	Hydroxyapatite
Copper		Catalase inhibitor, angiogenesis	Ceruloplasmin
Potassium		Na/K channels	K^+ or bound protein
Zinc		Zn-endopeptidases (mmp) inhibition	Cofactor in enzymes

Note: PtK2, protein tyrosine kinase 2; B, blood; Br, breast; C, colon; O, oral; G, gastine; P, prostate; I, intestinal; CO, colorectal; PN, pancreas; S, spleen; L, liver.

pathway(s) or as regulatory biochemical metabolites to inhibit cancer pathway(s) or as phytohormones showing global effects in the human body. These foods have active component(s) so called "nutraceutical" with anticancer properties. Cancer development depends on several factors such as presence of environmental and dietary oxidants, industrial organometallic contaminants, and toxicants in the air. In our view, cancer progresses in the body slowly and damages the cells at the cellular level in three stages: (1) intracellular metabolic integrity loss due to energy depletion; (2) metabolic integrity loss causes molecular imbalance (cancer pathways with impaired protein, lipid, carbohydrate, and other synthesis/catabolic regulatory processes at the gene level); and (3) reduced or accumulated metabolic products interact with regulatory cascade to disrupt the normal cells to transform them into cells in "cancerous state" (active proliferation, multiplication, elevated endonucleases). The intervention of nutraceuticals induces or inhibits these said imbalances and several intracellular processes. These processes may be the inhibition of cell proliferation, apoptosis, metabolic enzyme reactions, cytokines, cytochromes and endothelial functions, or may be biotransformation, senescence, and cancer pathways. For details see Table 9.1, Figures 9.1 through 9.3, and a descriptive citation (Sharma, 2009).

What remains as bottleneck is the bioactive food extract potency calibration as a drug, documentation of side effects if bioactive foods have any, as reported time to time in literature. In order to make it easy to understand the intricacies at this point, this chapter throws light on evidences, emerging concepts, some established anticancer mechanisms, tradeoffs in drug vs. nutraceuticals as prescription, verbal testimonies, and clinical trials with government policy to make public aware of the reality of bioactive foods and nutraceiuticals in general health. Broadly, today it is believed that bioactive foods get digested by natural enzymes and their digested metabolite products target many cancer related intracellular metabolic abnormalities of both focal (targeted cure) and whole body in origin (whole individual or global cure) while its counterpart artificially synthesized pharmaceutical

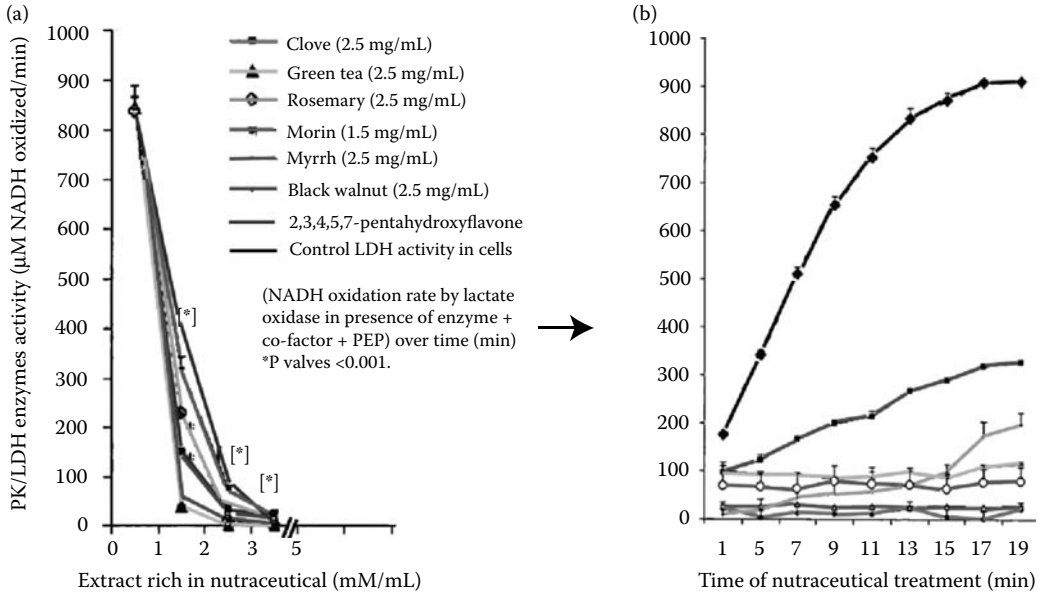


FIGURE 9.1 Evidence of pyruvate kinase and lactate dehydrogenase enzyme inhibitory action of different bioactive foods in cancer cells are shown in (a). The rate of inhibition was concentration dependent. Initially the inhibition was rapid and later the inhibitory effect was delayed showing the evidence of additional role of enzyme regulatory proteins in the action (b). The time series of enzyme changes are shown at different intervals. (Modified from US patent. Mazzio, EA, Suleman, K. Inhibition of anaerobic glucose metabolism and corresponding composition as a natural non-toxic approach to cancer treatment. U.S. patent 2006/0035981.)

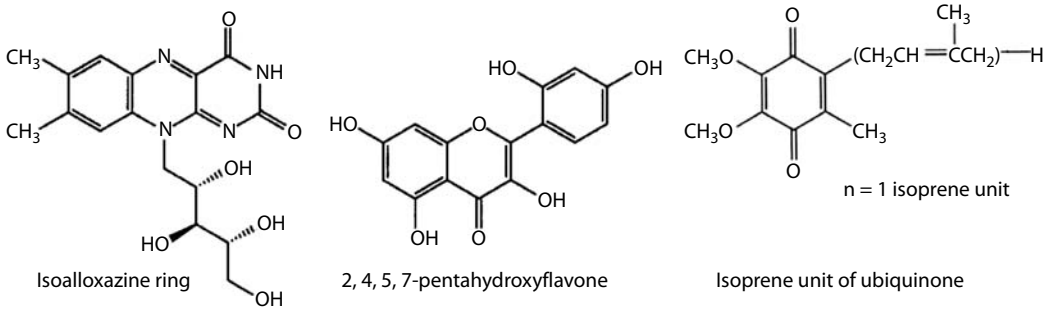


FIGURE 9.2 Chemical structure of bioactive components abundant in herbs, roots, and medicinal plants. They are used in in designing nutraceutical therapeutic formula due to possible presence of anticancer components. (Adapted from Sharma R. *Open Nutraceut. J* 2009a; 2:92–106.)

drug either inhibits or elevates only one biochemical reaction with assumption of complete cure. In this single-step-cure approach, several naturally active enzymes, cofactors, and assembly proteins loose their conformation and functionality (bioactive behavior) leading to several side effects. These side effects of bioactive foods or nutraceuticals are less common becuase of their wider acceptance in the body, but side effects caused by pharmaceutical drugs are very frequent and still it remains a challenge as to how to minimize their side effects.

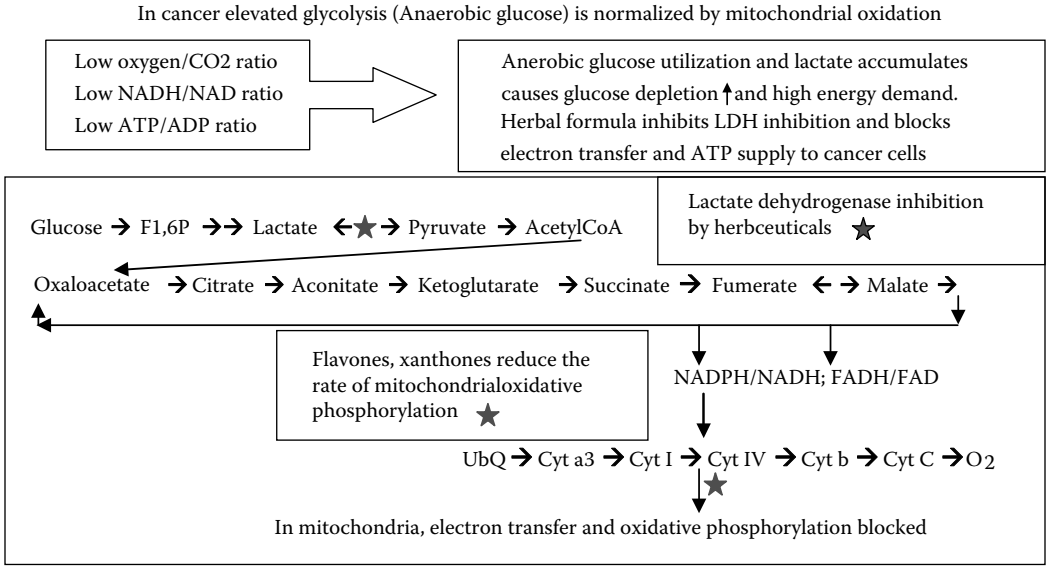


FIGURE 9.3 A diagrammatic sketch of blocked anaerobic glucose utility sites in glucose oxidation and TCA cycle. The metabolic steps are shown for enzymes: fructose 1,6-bisphosphatase, acetate-CoA ligase, malate synthase, isocitrate lyase, aconitase, phosphoenol carboxylase,/carboxykinase, glycolate oxidase, phosphoglycolate phosphatase, glycolaldehyde dehydrogenase, pyruvate carboxylase, citrate lyase, ferridoxin oxidoreductase, 2,3-diphosphoglycerate mutase, propionyl CoA carboxylase, malic enzyme and acetyl CoA carboxylase. The mitochondrial respiration shows metabolic steps of oxidative phosphorylation and electron transport chain. (Adapted from Kwon KB, Park BH, Ryu DG. *J Bioenerg Biomembr.* 2007; 39(1):31–4 and Sharma R. *Open Nutraceut. J* 2009a; 2:92–106.)

Evidences of Bioactive Foods as being Anticancer

Some important evidences are reported in favor of cancer inhibitory metabolic activity of bioactive foods in the human body:

1. The plants rich in essential amino acids act both as drug and as essential nutrients. For example, tryptophan is needed for protein synthesis at low dose in humans. At high dose, it increases brain 5-hydroxytryptamine levels and thus acts as a drug to treat the insomnia (Rishi, 2006).
2. The bioactive foods as blended preparations containing phytosterols are effective in lowering LDL cholesterol and osteoporosis.
3. Bovine milk fat globule acts as anticancer nutrient formula (Spitberg, 2005).
4. The phytonutrients prevent cell proliferation and play significant role in the prevention of chronic degenerative diseases. Notable examples are ginseng, spirulina, gingko biloba, amino acids, glucosamine, chondroitin, and Aegle marmelos. Herbal and medicinal plants have shown significant inhibition of cell proliferation (Baichwal, 1999). Phytoesterogens play a role in reducing necrosis (German and Dillard, 2000).
5. Vitamin C, vitamin E, β-carotene, lycopene (carotenoids), lipoic acid, glutathione (thiols) play a role in cancer prevention and inhibition of necrosis; Coenzyme Q-10, superoxide dismastase (enzyme), selenium, copper, manganese, and zinc (minerals) act as anticancer nutraceuticals in cancer management by delayed apoptosis observed in isolated cancer cells (Hennekens, 1994; Kim and Milner, 2001).
6. Bioactive foods rich in oligosaccharides were tested in animals. Fructo oligosaccharides, inulins, lactilol, lactulose, galacto-oligosaccharides, soybean oligosaccharides, lactosucrose, isomalto-oligosaccharides, gluco-oligosaccharides, xylo-oligosaccharides, and oligonols showed reducing cancer cell divisions (Aruoma et al., 2006; Zhao et al., 2007).
7. Polyunsaturated fatty acids (PUFA)-containing substances such as safflower oil, corn oil, soybean oil, mustard oil, evening primrose oil, flax oil, hemp seeds, and borage seeds showed protective effects in heart disease and stroke, rheumatoid arthritis, inflammatory arthritis, inflammatory bowel disease, asthma, cancer, chronic lung failure, kidney transplant, and bone formation (Conkin, 2009).
8. Foods such as oats, dried beans, legumes, and chicory contain water soluble fibers; apple, orange, apricot, plum, pine apple have 18–30% fiber content. The vegetable sources such as cabbage, carrot, lettuce, onion, and tomato containing 9–12% fiber content showed antioxidant and cell proliferation inhibitory properties (Nair, 2004).
9. Wild foods are other major source of nutraceuticals and phytoesterogens. Most of the wild plants, wild mushrooms, wild fungi, wild vegetables, wild nuts, wild fruits and wild flowers as a whole are considered as being potential natural therapy alternatives (German and Dillard, 2000; Jung and Haywood, 2005).
10. Soy isoflavones, genistien, curcumin, capsaicin, epigallocatechin-3-gallate (EGCG), gingerol, and lycopene have emerged as established cancer protective nutraceuticals (Lambert et al., 2003).
11. The glutathione is the liver's most abundant protective constituent of antioxidant glutathione reductase enzyme. Glutathione functions as a substrate for the two key detoxification processes in the liver: (1) transforming toxins into water soluble forms and (2) neutralizing and "conjugating" with toxins for elimination through the gut or the kidneys. If either of these processes is impaired for any reason, toxins will accumulate in the body and lead to disease. The best treatment for subjects with liver cancer focuses on improving the body's glutathione reserves (Tripathi et al., 2005).
12. Some nutraceuticals rich in opiads are tumor-inhibiting, and these nutraceuticals showed the ability to get rid of toxins such as heavy metals, chemicals, digestive byproducts, and

so on, Tobacco plants may also help people to fight against lymphoma (Stagnaro et al., 2004; Lin et al., 2006b).

13. The soy isoflavone Haelan951 (genistein and genistin) was reported to have some role as a chemopreventive agent against cancer in humans (Béliveau and Gingras, 2004, 2007). Beta-glycoside conjugate, genistin is abundant in fermented soybeans, soybean products such as soymilk and tofu. The beta-glycosyl bond of genistin is cleaved to produce genistein by microbes during fermentation to yield miso and natto. Soy sauce has high isoflavone but low miso and natto contents. How much soy isoflavones are needed? 1.5–4.1 mg/person miso isoflavone and 6.3–8.3 mg/person natto, respectively (Béliveau, 2004, 2007; Cooper et al., 2005).
14. Green tea has always been considered by the Chinese and Japanese people as a potent medicine for the maintenance of health, endowed with the power to prolong life. Recently, investigators looked at the effects of the main active green tea constituent, epigallocatechin-3-gallate (EGCG) on chronic lymphocytic leukaemia B cells isolated from leukaemic patients (take away Yean Lee). These cells were characterised by their resistance to apoptosis because they secrete and bind vascular endothelial growth factor (Lambert et al., 2003; Rana et al., 2008).
15. Some herbal plants act as medicines. The herbal extracts are known to reduce the cell proliferation. Cumin (*Cuminnum cyminum* L.) seeds stimulate lipid peroxidase (LPO), detoxifying enzyme (GST), and antioxidant defense enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Sharma, 2009a,b,c).

The following section is devoted to a glimpse of potential bioactive foods, and of herbs as a whole or their parts with promise in cancer prevention. Most of the information was accrued based on personal and verbal testimonies of people or documents from nonmedical and traditional therapy institutions around the world. The information serves as a platform to scientists, alternative physicians, academicians, nutraceutical industries, pharmacists, and government regulatory bodies to become aware of the existing or growing knowledge of bioactive foods and their anticancer claims with promise of future potentials.

ANTICANCER PROPERTIES OF BIOACTIVE FOODS AND HERBS

Most of the herbs are part of whole plant or some preserved part(s). Based on available data, potential bioactive food with herbal bioactivity are reported for interested readers. A tentative list of herbs is presented with experimental values of anticancer screening (lethal value LC_{50} in mg/mL).

Anticancer herbs proved as killers: Alkanet root (0.138); babul bark (0.492); bakuchi seed (0.102); berch leaf (0.365); bhumy amalaki (0.497); black papper (0.495); blood root (0.04); blue cohosh root (0.218); buckthrone bark (0.107); butternut bark (0.506); chapparral (0.124); cinnamon (0.479); cubeb berry (0.263); dragon blood (0.242); dragon blood (0.242); elecampane root (0.447); eucalyptus leaf (0.305); feverfew (0.307); green tea (0.507).; kava kava (0.491); kochea seed (0.147); male fern rhizome (0.232); osho root (0.509); psoralea fruit (0.243); red sandalwood (0.326); rosemary (0.299); sage (0.519); senna leaf (0.275); sweet myrrh (0.158); Turkey rhubarb (0.466); wild cherry bark (0.360); yellow dock root (0.348).

Less known herbs or those with one-time evidence of their anticancer properties are listed below as tentative list for readers to become aware of these herbs either tested one time in preclinical Chinese or Ayurvedic use or likely to be prospective anticancer herbal candidates.

Potential anticancer herbs: Yam root, abalone shell, tertraphylla root, ailanthus bark, mimosa bark, alfafa leaf and seeds, alum, angelica, snise seed, ash bark, ashwanda root, astralgus root, bamboo leaf, barley grass, bee pollen, bilberry fruit, black haw, blue green algae, blue verian, borage, buddleia flower bud, bugleweed, burdock root, cardamom, carob powder, carpesium fruit, cassia seed, catnip, chamomile, chervil, chickory root, chickweed, chinese holly leaf, chlorella,

cilantro, cleavers, clemetis root, club moss, codonopois root, coix seed, coltsfoot, comfry leaf, corn silk, cortyceps, couch grass, cranberry powder, dandelion root, dill seed, dittany root bark, dog grass root, don quai root, dulse, echinechea, eleuthro root, erend herb, eucomnia, eyebright, false unicorn root, fennel seed, fenugreek, flax seed, fo ti, forsythia fruit, foxnut barley, fringe bark tree, fumitory herb, gential root, ginseng, glaborous greenbrier rhizome, glehnia, gloryvine stem, goats rue, goldenseal, green clay, guduchi root powder, gypsum, hawthorne berry, helichrysum flowers, hibiscus, homalomena rhizome, honeysuckle vine, horsetail, houttuynia cordata, hydrangea root, hylocerus flower, hyssop, isatis leaf, jasmine flower, kadsura stem, kelp, knotweed grass, kola nut, kombu, kudzu root, kukicha twig, laminaria (kelp), lemon, lobelia, lotis leaf or root, lungwort, lycii berries, lycium bark, lycopodium japonicum vine, marshmallow root, melilot herb, mica-schist, milk thistle seed, mother-of-pearl, motherwort, msm, mugwort, muirapuama, mettle root, noni juice, onion powder, orange, pagoda tree fruit, paprika, parsley leaf or root, passion flower, peppermint, perilla leaf or root, periwinkle, pigeon pea root, pivet fruit, plantain leaf, pleurisy root, poke root, poppy seed, psylliam seed, puff-ball/lasiophaera, purnarnava herb, pyrrosia leaf, red clover, reed rhizome, rehmannia root, rooibos tea, rosehips, safflower threads, saffron, scrophularia root, scutellaria barbata herb, self heal, shank pushpin herb, shephards purse, skull cap, slippery elm, soloman seal, spearmint, speranskia herb, spilanthes, spirulina, stone lotus seed, swalloeort root, tonka bean, tribulus, uncaria vina with hooks, vanilla root, vasak leaves powder, vasma rochna leaves, watercress, wheat grass, white oak bark, white peony root, white pine powder, woolly grass rhizome, yellow mustard seed, yohimbe bark, yucca root, zedoary rhizome.

Nutrition Requirement of Anticancer Herb Active Components in Human Use Approved by U.S. Food and Drug Administration

The U.S. Food and Drug Administration (FDA) has given guidelines for recommended daily requirements (RDA) of bioactive foods and their use among humans.

- Isothiocyanates from broccoli (RDA 300 mg/day)
- Ubiquinone and derivatives (RDA 300 mg/day; 3 % wt fraction)
- Coenzyme Q and derivatives (RDA 300 mg/day; 3% wt fraction)
- Riboflavin and derivatives (RDA 300 mg/day; therapeutic dose 1000 mg; 3% wt fraction): Riboflavin
- 2′,3,4′,5,7-Pentahydoxyflavone (therapeutic dose 1000 mg)
- Polyphenolic flavonoid and derivatives (RDA 800 mg/day; therapeutic dose 2000 mg; 9% wt fraction): 2-3-dimethoxy-5-methyl-1,4-benzoquinone (therapeutic dose 2000 mg/day; 31% wt fraction)
- Alkaline compounds (RDA 750 mg/day; 18% wt fraction)
- Antiproliferative bioactive herbs (RDA 200 mg/mL; 5% wt fraction): Speranskia or goldenseal herb powder (therapeutic dose 2000 mg)
- Balm of Gilead buds, red sandalwood, rosemary (therapeutic dose 1500 mg/day; 58% wt fraction) with myrrh gum (therapeutic dose 500 mg/day); black walnut (therapeutic dose 2000 mg/day, 17% wt fraction) to inhibit LDH.
- FMN, FAD, 5-amino-6-(5′-phosphoribitylamino) uracil, 6,7-dimetyl-8-(1-D-ribityl)lumazine, ribitol, 5,6-dimethylbenzimidazole (therapeutic dose 300 mg/day of each).

Biochemical Mechanisms of Tumor Treatment by Nutraceuticals from Bioactive Foods

The authors propose the concepts of biochemical changes that occur step by step in cancer cells leading to arrested growth or cancer cell killing.

- The effect of nutraceuticals is measured by tumor volume, animal weight, tumor size shrinkage, inhibited cell growth, percentage loss of cell viability, inhibited biomarkers (enzymes).
- What do herbs do on host cells? The aerobic glucose oxidation in normal cells leads to enhanced mitochondrial oxidative phosphorylation in presence of oxygen. High concentration of CO_2 suffocates the normal cells and anerobic glucose oxidation results with a halt in mitochondrial energy. So, it does not favor the host normal cells.
- Cancer cells demonstrate high glucose consumption, high glycolytic rate, rapid cell proliferation, lactic acid accumulation, extracellular acidic low pH, low glucose available, oxygen deprivation, or hypoxia. Overall, it resists the chemotherapeutic action.

Concept 1: Tumor cells starve by blocking anerobic glucose utility by nutraceuticals: The glucose utility block augments the aerobic energy metabolism in the host. The block leads the lactate dehydrogenase inhibition and cytosolic TCA cycle [2,3-dimethoxy-5-methyl-1,4-benzoquinone (DMBQ)] in coenzyme Q10. The blocked glycolysis also favors mitochondrial oxidative function through complex I–IV (example riboflavin, FAD, FMN derivatives).

Concept 2: Oxygen depletion and hypoxia in tumor: High oxygen is not good for tumor cells. High oxygen in tumor cells causes high mitochondrial respiration (where oxygen is a substrate for mitochondrial complex IV) and high alkalinity.

Concept 3: Inhibition of lactate dehydrogenase (LDH-V M4): The LDH plays active role in the development of malignancy as enzyme LDH generates product NAD^+ and 2 pyruvate molecules from one glucose. Immediately NAD^+ is consumed as cofactor of glyceraldehydes-3-phosphate dehydrogenase to push ATP more production through anaerobic metabolism catalyzed by phosphoglycerate/pyruvate kinase, while pyruvate molecule gets into acetylCoA by pyruvate dehydrogenase in aerobic metabolism.

Biochemical Basis of Anticancer Activity of Bioactive Foods

Biochemical reactions in the body maintain metabolism. Different specific enzymes participate and regulate the intermediary metabolism in the body (Colic and Pavelic, 2000). The enzymes alter in cancer condition or other specific diseases (Aggarwal and Shishodia, 2006). Some lead examples recently reviewed or investigated are cited to evaluate the anticancer activity of bioactive foods (Pavelic et al., 2005). The examples are limited to end-point metabolic or biochemical changes at the cellular level. Other molecular mechanisms of cancer prevention or anticancer activity are immunomodulation, senescence, ongogenes, inhibitory cascades of cancer pathways. The focus is the identification of molecular targets and measurable biomarkers to indicate the cancer prevention effect of dietary bioactive foods as outlined in Figure 9.4. However, some of the following biomarkers may not true representatives of the cancer preventive effect. Most of experiments in animal models or cancer cells appear with incomplete information or with suggestion of further research.

1. *Inhibition of pyruvate kinase:* The elevated puruvate kinase to cause lactate accumulation in cancer cells is inhibited by herbs as shown in Figure 9.1. Examples: Green tea, clove, myrrh, morin, walnut (Mazzio and Suleman, 2006).
2. *Inhibition of cytochrome oxidase enzymes:* In the presence of oxygen, the mitochondrial complex I and IV subunits function together as dependent on each other. Their catalytic binding with active herb component (nutraceutical like riboflavin, FMN, FAD) provides the measure of cancer cell status. The flavins enhance the oxygen utilization by cytochrome oxidase and keep low the aerobic glucose utilization to generate ATP (substrate level phosphorylation). The enhanced V_{max} and reduced K_m value for reduction of

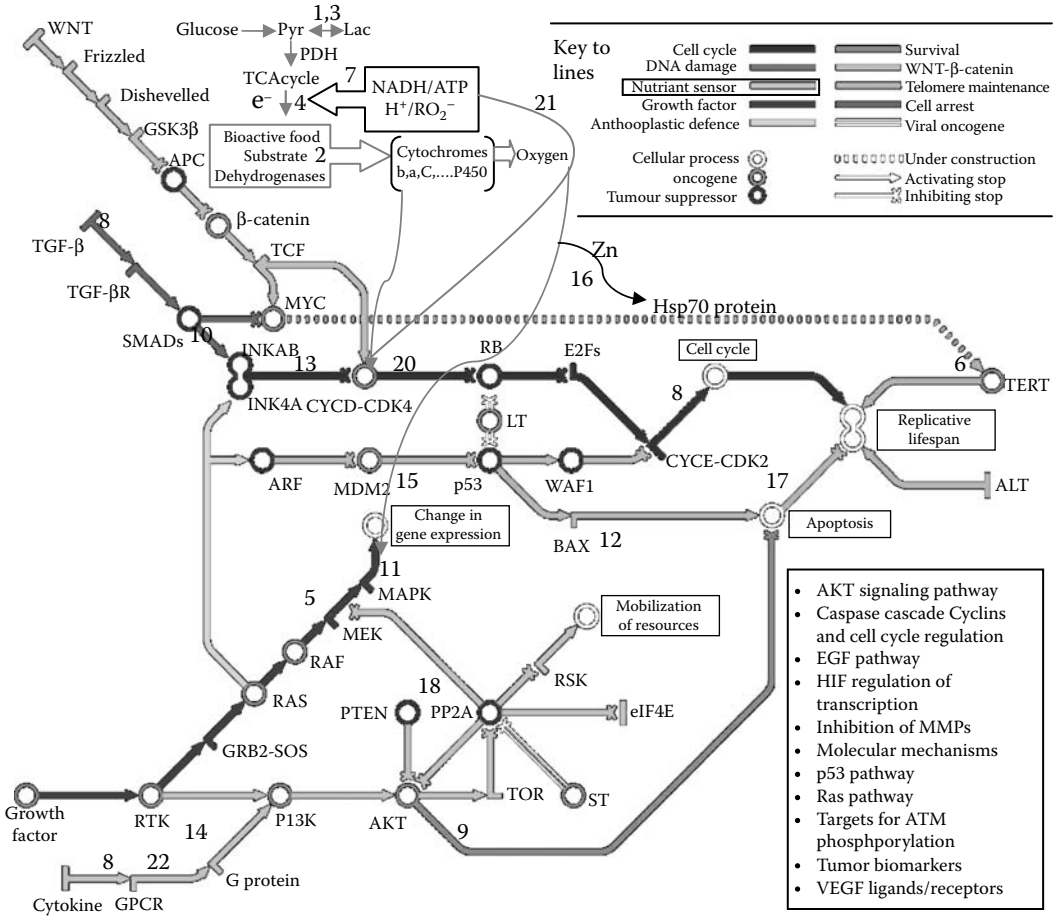


FIGURE 9.4 The biochemical basis of different cancer pathways. Intersections show the posible targets of bioactive food constituents (shown in numbers 1–24 with details in text). Different abbreviations are used: Eukaryotic translation initiation factor 4E (etF4E); simian virus 40 strain Br54 small T antigen gene (ST); FK506 binding protein 12-nutrient associated protein 1(TOR); phosphatase and tensin homolog (PTEN); v-raf-1 murine leukemia viral oncogene homolog 1 (RAS); receptor signaling protein tyrosine kinase (RTK); G-protein coupled receptor (GRCR); glycogen synthase kinase 3 beta; adenomatous polyposis coli; cadherin-associated protein, beta 1, 88kDa; transcription factor 4; cyclin-dependent kinase inhibitor 2B; cyclin-dependent kinase inhibitor 2A; Mdm2 p53 binding protein homolog; retinoblastoma 1; Simian virus 40 strain A2895 large T antigen gene (LT); tumor protein p53; cyclin-dependent kinase inhibitor 1A (WAF1); BCL2-associated X protein (BAX). (Adapted from NIH State-of-the-Science Conference Statement on Multivitamin/Mineral Supplements and Chronic Disease Prevention. *NIH Consens State Sci Statements* 2006; 23(2):1–30.)

mitochondrial complexes I and IV are indicators of mitochondrial function in cells. In cancer cells, in presence of oxygen, cytochrome oxidase competes with anerobic glucose utilization) and substrate level phosphorylation to generate ATP. Example: Isothiocyantes (Ioannides and Lewis et al., 2006).

3. *Lactate dehydrogenase enzyme inhibition and enhanced energy equivalents:* In cancer cells, high LDH enzyme levels (indicator of anaerobic metabolism) and reduced ratio of equivalents NADH/FADH (during acetyl-CoA utilization by TCA cycle) indicate the low

availability of NADH to electron transport chain in making ATP. Morin, an anticancer inhibits LDH-V M4. Examples: Ubiquinones (Mazzio and Suleman, 2006).

4. *Anaerobic cytosolic carboxylation (TCA cycle status and ETS enzyme battery):* Inhibition of TCA cycle enzymes aconitase, isocitrate lyase, malate synthase. It directly affects the NADH and ATP supply. The bioactive food may have inducing effect on the maintenance of constant energy supply. Example: Withania somnífera (Mazzio and Suleman, 2006).
5. *High mitogen/stress pathway kinase activity:* In cancer cell cultures, enzyme activity was elevated. Example: Morin (Brown and O'Prey, 2003).
6. *Tropoisomerase enzyme inhibition:* Herbs have shown the enzyme inhibition in cell lines. Example: Frankincense (Boswellia carteri) (Boege et al., 1996).
7. *Enhanced p-glycoprotein ATP efflux:* The depleted p-glycoprotein ATP efflux was reversed by herb extract in cell cultures. Example: Flavanoids (Ikegawa et al., 2000).
8. *Arrested G2/M, downregulation of NF-kappa B, Akt, cyclin D, c-myc:* In cancer cells, the herbs have shown the possibility of arrested G2/M, downregulation of NF-kappa B, Akt, cyclin D, c-myc to reduce the PARP cleavage and DNA fragmentation. Examples: Vitex, wild yam (Dioscorea villosa) (Shishodia and Agrawal, 2006).
9. *Antiproliferation and induced apoptosis (Caspase-3/8/9) and PARP cleavage:* The PARP cleavage followed by activation of cathepsins is enhanced. Example: Garcinia cambogia (garcina fruit), Mace (Hostanska et al., 2002).
10. *Blocked leukotriene/5-lipoxygenase pathway:* The immunomodulatory cytokines including leukotrienes and oxigenase pathways get blocked by herbal extracts. Example: Hunteria zeylanica bark (Sun et al., 2006).
11. *Inhibition of histone acetyltransferase p300 and PCAF:* Garcinia cambogia (garcina fruit) inhibits nuclear histone acetyltransferase p300 and PCAF, as indicator of pro-apoptosis (beginning or initiation) after cancer cells indicate cell proliferation, migration, cell adhesion, and viability. Other biomarkers are inhibited MAP kinase, ER kinase, P13K/Akt, membrane adhesion kinase with activated cytochrome *c* release and PARP-1 cleavage. Example: Garcinia fruit extract (Liao et al., 2005).
12. *Oxidative stress induced pro-apoptosis:* Vitex showed the reduced gene expressions of BCL-2, Bcl-XL and Bid protein by caspase 3,8-9-OH oxidase with negative effect on enhanced Bad gene expression and induced DNA fragmentation. Example: Vitex (Ohyama et al., 2005).
13. *Downregulation of cyclin D1 expression/apoptotic effects:* After herb intervention, a reduced proliferation, a pro-apoptosis process initiated to cause cyclin D1 expression. Example: Wild cherry bark (Yamaguchi et al., 2006).
14. *Modulation of p-glycoprotein:* Due to phytochemical-mediated modulation of *p*-glycoprotein process initiates anticancer behavior of herbs. Example: Goldenseal and kava kava (Gurley et al., 2005).
15. *Expression of gene:* Downregulated gene expression of silbinin synthase enzyme causes the activation of nerves and brain activity with enhanced immunity. Example: Vitamin E, JIVA™ (Claycombe and Meydani, 2001; Condori et al., 2009).
16. *High shock proteins (hsp70) protection and downregulation of zinc transporters:* Zinc supplementation upregulates hsp70 and higher binding activity of HSF-1 that compensates an age-dependent delay in HSF-1 phosphorylation (Rishi et al., 2003;Ambra et al., 2004; Sun et al., 2007).
17. *Induced apoptosis and chemoprevention:* Dietary polyphenolics and flavonoids induce endonucleases and cathepsins to lead chemoprevention. The hydroxychalcones also partly depleted hepatocyte GSH and oxidized GSH to GSSG. Example. Dietary polyphenols, polyethylene-glycol (Galati et al., 2000; Galati and O'Brien, 2004; Roy et al., 2004; Moon et al., 2006).

18. *Induction of CYP2E1 and CYP2C19 enzymes:* The liver cells showed enhanced induction of enzyme as chemoprevention mode of cells with increased activity of CYP3A4 and 2D6 and CYP1A2 and 2D6 Example: several trade herbal products St John's wart, gingko biloba. Both PCNA and p53 were highly expressed in the liver but inhibited by natural food. Example: Spirulina (Hellum et al., 2009; Ismail et al., 2009).
19. *Transcription inhibition:* The beta-carotene intervention showed that cell lines display a widely variant behaviour, which hampers translation to the *in vivo* situation in the body. Example: Beta carotenes (Russell, 2004; Keijer et al., 2005).
20. *Protein Rb and induction of apoptosis via the release of cytochrome c:* Equiguard suppresses androgen-dependent LNCaP prostate cancer cell proliferation by targeting cell cycle control via down regulation of the retinoblastoma protein Rb and induction of apoptosis via the release of cytochrome *c*. Example: Equiguard (Lu et al., 2004).
21. *Induced antioxidant, oxidative defense, and xenobiotics gene expression:* Vitamin E upregulates detoxifying enzymes against free-radical peroxidation. The malondialdehyde and hydroxynonenal, generated by heating a mixture of linoleic and linolenic acid, caused DNA adduct formation as a result of reduced xenobiotics gene expression. Selenates are peculier in enhanced gene expression of antioxidant and xenobiotic metabolising enzymes proteins in lymphocytes. Example: Vitamin E, selenium-rich yeast (Kim and Milner, 2001; Lunec et al., 2004; Mariappan et al., 2006; Pagmantidis et al., 2008; Ravn-Haren, 2008a,b).
22. *GCP-mediated growth inhibition and induced apoptosis:* Garlic genestein combined polysaccharides (GCP) inhibited the proliferation of androgen-dependent LNCaP and androgen-independent LNCaP-p53(GOF) and 22Rv1 cell lines in a dose-dependent manner and cells were more responsive in the presence of androgen. GCP markedly suppressed mTOR-p70S6K signaling while Akt and p53 were only modestly modulated. GCP significantly attenuated androgen signaling as evidenced by diminished AR protein levels and a consequent reduction in transcriptional activity and prostate-specific antigen (PSA) expression. Example: Garlic genistein, daidzein, and glycitein (Tepper et al., 2007).
23. *Inhibition of orthotopic growth and metastasis:* The isoflavone depleted soy protein, soy phytochemical concentrate (SPC), and genistin showed produced a significant increase in tumor p53 expression androgen-sensitive human prostate cell line LNCaP. Example: Soyabean (Zhou et al., 2002).
24. *Antioxidant free radical scavenger and cyt P450/lipid peroxidation inhibition:* Curcumin acts as a free radical scavenger and antioxidant, inhibiting lipid peroxidation and oxidative DNA damage. Curcuminoids induce glutathione-*S*-transferase and are potent inhibitors of cytochrome P450. Example: Indian yellow turmeric (Aggrawal and Shishodia, 2006; Kita et al., 2008).
25. The efforts are in progress to explore new mechanisms of bioactive foods as possible anticancer agents involving new pathways such as AKT signaling pathway; caspase cascade; cyclins and cell cycle regulation; EGF pathway; HIF regulation of transcription; inhibition of MMPs; molecular mechanisms of cancer; p53 pathway; Ras pathway; targets for ATM phosphporylation; tumor biomarkers; VEGF family ligands and receptors.

Concepts on Biochemical Role of Cytochromes in Cancer Prevention

The efficacy of any nutrient in cancer prevention depends on the bioactivation of nutrient specific metabolizing enzymes and partly it depends on the reduced detoxication of chemical carcinogens by nutrient in the body (see nutrient sensor pathway in Figure 9.4). How nutrients choose their target intermediate molecules? Answer lies in nutrient driven defence mechanisms. In the body major genotoxic intermediate metabolites are formed by "bioactivation" as a result of carcinogenesis

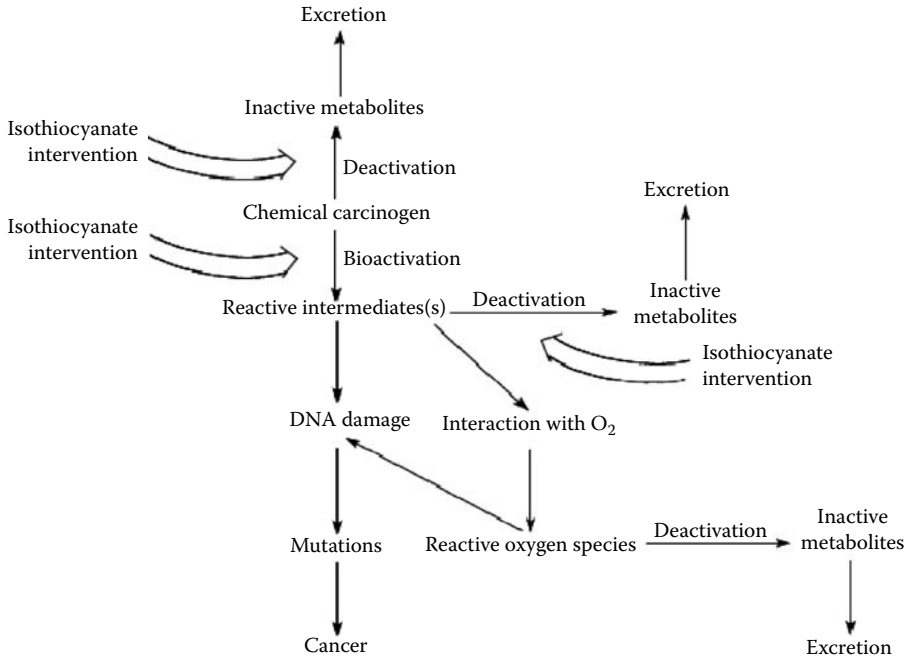


FIGURE 9.5 An anticancer mechanism is sketched out for the action of isothiocyanate-containing herbs or active components. (Adapted from Ioannides C, Lewis DFV. *Curr Top Med Chem* 2004; 4:1767–88.)

caused by cancer. These reactive carcinogen intermediates cause biotransformation, DNA adducts, and cytochromes by following enzyme reactions:

- The electrophiles interact with DNA to make adducts by biotransformation "metabolic activation" or "bioactivation" to cause mutation (Figure 9.5).
- The hydroxyl radical cause DNA damage and mutation. The nutrients may repair the DNA damage (Mathers et al., 2007).
- In defence, cytochromes P450 through *N*-hydroxylation makes genotoxic nitrenium ion (through ring-hydroxylation at the 5-position) as glucuronide and excreted (Figure 9.5). The cytochrome P450 is a super family on the basis of primary sequence homology and participates in xenobiotic metabolism of chemical carcinogens such as CYP1 to CYP3 and others shown in Table 9.1.
- As a defensive mechanism of CYP in cell, it produces hydrophilic, biologically inactive metabolites (detoxification) that can be readily excreted (Figure 9.6) to keep them away from DNA. Examples of detoxification are conjugated metabolites with endogenous glutathione (glutathione conjugates and mercapturates) excreted into the urine and bile.
- Other defensive mechanism is quinone reductase prevents quinines and aromatic hydrocarbons to make semiquinone radicals or superoxide anion interacting with DNA (Dietz et al., 2005). Quinone reductase converts the quinone or aromatic hydrocarbons or reactive oxygen species to hydroquinone through a two-electron reduction (Figure 9.7).

Dietary Chemicals as Modulators of Carcinogen Metabolism

The golden rule is, suppressing the bioactivation of chemicals and/or stimulating the detoxication pathways. The diets can modulate the balance of activation/detoxication to facilitate detoxication at

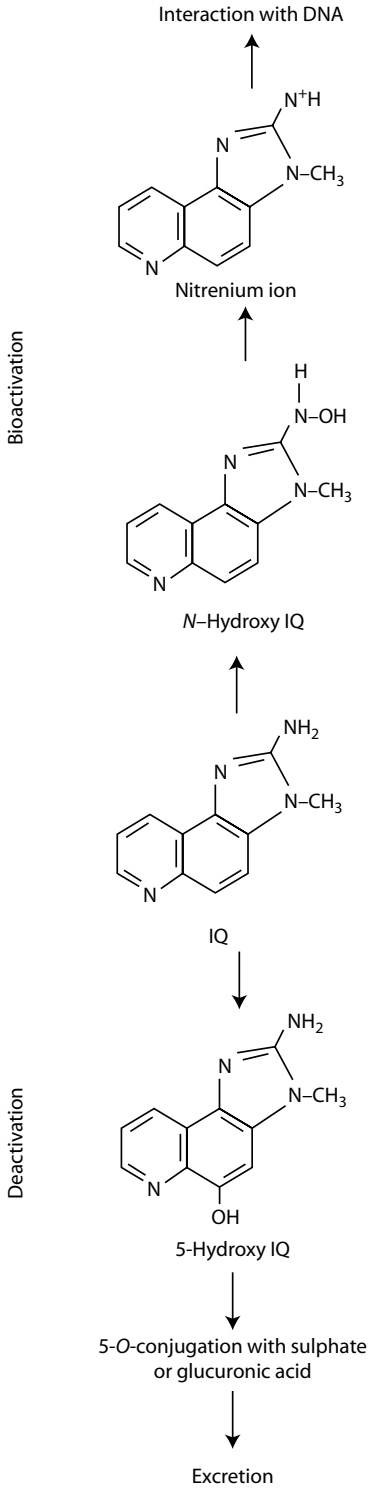


FIGURE 9.6 The deactivation/bioactivation reactions are shown for the quinone ring. (Modified from Guo Z, et al. *Carcinogenesis* 1992; 13:2205–10.)

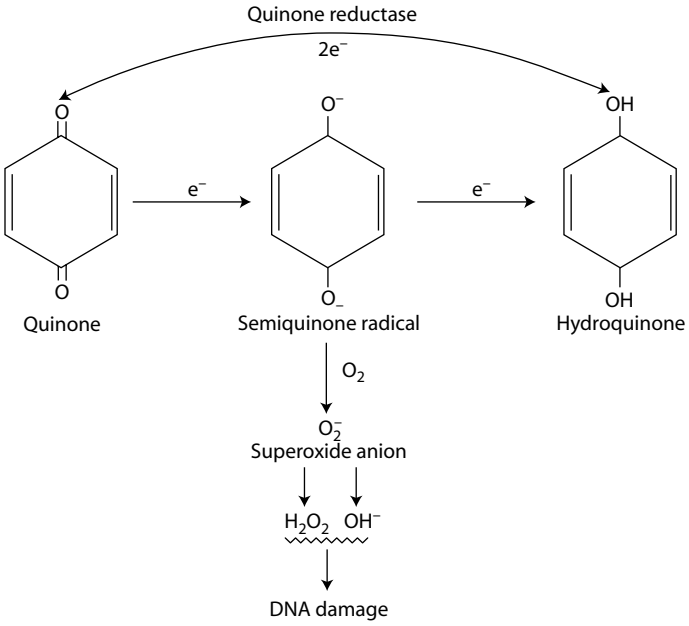


FIGURE 9.7 The quinine ring participates in superoxide anion induced DNA damage. (Modified from Talalay P, Fahey JW. *J Nutr.* 2001; 131:3027S–33S.)

the expense of bioactivation. Biphenyl, low-protein diets, organosulphates in garlic, indoles and isothiocyanates in cruciferous vegetables, caffeine in coffee, bergamottins in grapefruit juice and methylenedioxyphenyl compounds in spices, ascorbic acid, cruciferous vegetables, such as cabbage and Brussels sprouts modulate the carcinogen-metabolising enzymes, xenobiotics; lightly cooked broccoli upregulated CYP1A2 (a P450 enzyme); watercress (100 g/day) for a week stimulated CYP2E1; cruciferous Brussels sprouts and broccoli stimulated cytochrome P450 and glucuronosyl transferase activities (Hanlon et al., 2008, 2009).

Isothiocyanates in Bioactive Foods as Example

The dietary glucosinolates, sulphur-containing precursors show chemopreventive activity in lung, oesophagus, small intestine, colon, pancreas, liver, prostate, bladder, and mammary gland in humans. The bioactive vegetable enzyme myrosinase (β-thioglucoside glucohydrolase) converts glucosinolate to harmless isothiocyanates (Figure 9.5). The chemopreventive mechanism of isothiocyanates is to protecting the DNA and isothiocyanates limit the availability of the reactive intermediates of chemical carcinogens (detoxication) (Figure 9.5).

Inhibition of the Cytochrome P450-mediated Bioactivation of Chemical Carcinogens by Isothiocyanates

Three isothiocyanates, sulforaphane, erucin, phenethyl isothiocyanate (PEITC) (Table 9.2) are common in broccoli. Steaming the broccoli or cooking it using microwave makes isothiocyanates less available due to inhibition of the activity of the hepatic CYP1A, CYP2B, CYP2E and CYP3A (to modulate cytochrome P450 activity or increased CYP1A expression) (Hanlon et al., 2008, 2009) The PEITC isothiocyanate impaired CYP2E1 activity but elevated CYP2B1 (Ishizaki et al.,1990; Guo et al., 1992).

TABLE 9.2
Rat and Human Xenobiotic-Metabolising Cytochromes P450 and their Role in the Bioactivation of Chemical Carcinogens

Cytochrome P450 Subfamily	Human Proteins	Role in Chemical Carcinogenesis	Major Classes of Activated Carcinogens
CYP1A	1A1, 1A2	+++++	AA, AAB, HA, MC, NNK, PAH
CYP1B	1B1	+++++	AA, AAB, HA, MC, NNK, oestrogens, PAH
CYP2A	2A6, 2A7, 2A13	+++	NA, OP
CYP2B	2B6	+++	NA, OP
CYP2C	2C8, 2C9, 2C18, 2C19	++	PAH
CYP2D	2D6	+	NNK
CYP2E	2E1	++++	HH, NA
CYP2F	2F1	??	–
CYP2G		??	–
CYP2J	2J2	??	–
CYP3A	3A4, 3A5, 3A7	+++	MC, PA, PAH
CYP4A	4A9, 4A11	None	–
CYP4B	4B1	++	AA

Source: Adapted from Ioannides C and Lewis DFV. *Curr Top Med Chem.* 2004; 4:1767–88.
PAH, polycyclic aromatic hydrocarbons from bioactive foods: AA, aromatic amines; HA, heterocyclic amines; MC, mycotoxins; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; AAB, aminoazobenzenes; OP, oxazophosphorines; HH, halogenated hydrocarbons; PA, pyrrolizidine alkaloids. The role of carcinogen is shown as: +++++ very extensive; ++++ extensive; +++ moderate; ++ minor; +poor; ?? unknown.

Detoxication of Reactive Intermediates: Glutathione Transferase and Quinine Reductase Chemopreventive Activity

Isothiocyanates cause detoxication of the reactive intermediates of chemical carcinogens (chemoprevention) as glutathione-*S*-transferases and quinone reductase disable reactive epoxides and quinones (Talalay and Fahey, 2001). Quinone reductase is upregulated by erucin and sulforaphane, PEITC. The isothiocyanates stimulate the glutathione-*S*-transferase activity (chemopreventive activity). The raw broccoli florets or broccoli increase the glutathione-*S*-transferase activity.

The cruciferous vegetables reduce cancer incidence. The isothiocyanates show chemopreventive activity. The bioactivation of carcinogens results in the formation of quinines, semiquinones, and reactive oxygen species. Isothiocyanates upregulate the quinone reductase (quinones to hydroquinones) and suppress polycyclic aromatic hydrocarbon-induced cancers. The isothiocyanates also modulate the glutathione-*S*-transferases and cytochromes P450 enzyme in chemoprevention. Other enzymes sulphotransferases, acetylases and glucuronosyl transferases may also modify carcinogen metabolism (Figure 9.8).

In bioactive cruciferous vegetables, modulators of carcinogen metabolism may be a synergistic and antagonistic nature.

Pharmaceutical Approach of Herbal Formula with Anticancer Properties

A simple plan is suggested for nutraceutical mixture including active biochemicals, herbs, roots in cancer therapeutics.

Group 1: Herb group: (40–90% w/w): Wild yam root (Dioscorea villosa), teasel root (Dipsacus asper), balm of gilead bud (Populus balsamifera), bakuchi seed (Cymopsis psoralidoides),

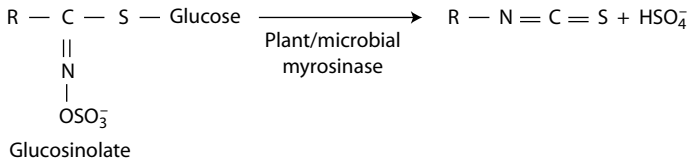


FIGURE 9.8 Bioconversion of glucosinolates into isothiocyanates. (Adapted from Talalay P, Fahey JW. *J Nutr.* 2001; 131:3027S–33S.)

dichroa root (Dichroa febrifuga), kochia seed (Kochia scoparia), kantakari (Solanum xanthocarpum), bushy knotweed rhizome (Polugonum Cuspidatum), arjun (Terminalia arjuna), babul chall bark (Acacia Arabica), sweet myrrh (Opopanax) and bhumy amalaki (Phyllanthus nirur), garcinia fruit (Garcinia Cambogia), vitex (Vitex agnus-castus), dragons blood (Calamus draco), mace (Myristica fragans), white sage (Salvia apian), red sandal wood (Pterocarpus santalinu).

Group 2: Ubiquinone with precursor: (40–90% w/w): 2,3-Dimethoxy-5-methyl-1,4-benzoquinones(5-45), hydroqunolenes, ubichromenols, ubichromanols, and ubiquinols in form of *p*-hydroxybenzoate, *p*-hydroxycinnamate, *p*-hydroxyphenylpyruvate, *p*-hydroxyphenyllactate.

Group 3: Mitochondrial respiration suppressors: (40–99% w/w): Ribitol, riboflavin, flavin adenine dinucleotide, 5-amino-6-(5′-phosphoribitylamino)uracil, 6,7-dimethyl-8-(1-D-ribityl)-lumazine, 5,6-dimethylbenzimidazole with multivitamin mixture.

Group 4: Inhibitor of LDH (10–15% w/w): 2′,3,4′,5,7-Pentahydroxyflavone, epigallocatechin galate, quercetin, rosemary (Rosmarinus officinalis), black walnut (Juglans nigra), clove (Syzygium aromaticum), nutmeg (Myristica fragans), licorice root (Glycyrrhiza glabra), coriander (Coriandrum sativum), cinnamon (Cinnamomum cassia), ginger root (Zingiber officinale), myrrh gum (Commiphora molmol) and green tea (Camellia sinensis).

Group 5: alkalinating agent: (18–20% w/w): Aloe vera (Aloe barbadensis), chlorella (Chorella pyrendoidosa), wheat grass (triticum aestivum) in solution of sodium or potassium carbonate.

Group 6: antiproliferative herbs: (3–5% w/w): Speranskia herb (Speranskia tuberculata) and goldenseal (hydrastis Canadensis).

Carriers of different nutraceutical components: Water, saline, starch, sugar, gel, lipids, waxes, glycerol, solvents, oils, liquids, proteins, glycols, electrolyte solutions, alcohols, fillers, binders, emulsifiers, preservatives, buffers, colorants, emollients, sweeteners, surfactants, additives and solvents used as solid, liquid, powder, paste, gel, tablet, foam, pack, aerosol, solvent, diluent, capsule, pill, liposome, syrup, solution, suppository, emulsion, suspension, biodelivery agents. The nutraceutical mixture may be delivered through oral, injectable or external administration for treatment of cancer of: skin, breast, breast, colon, kidney, bone, blood, lymph, stomach, gastrointestinal, ovary, prostate, liver, lung, head and neck, adrenal, brain, bronchial, hypothalamus, parathyroid, thyroid, pancreas, pituitary, sinus, endometrium, bile duct, leukemia, AIDS, astrocytoma, glioma, lymphoma.

Controversial status: Several bioactive compounds such as CoQ2,4,6 and CoQ10 and herbs such as knotwood rhizome, elecampane root, Turkey rhubarb are less understood and their anticancer properties remain controversial.

Alternative Approaches of Bioactive Foods in Cancer Prevention Formulation

Functional foods were recently reported in health promotion with possibility of disease prevention (Shahidi, 2004). Now a new concept is emerging on the use of bioactive foods in formulation as combined herbs, enzyme or cancer pathway inhibitors, vitamins, and minerals (nutraceutical

formulation). The idea was to follow the pharmaceuticals if nutraceuticals or herbiceuticals act in the same way (Hardy et al., 2002). For diabetes, the use of herbs and nutraceuticals is established as health protective, promotive, and disease preventive but in cancer prevention it is making slow awareness. However, the acceptance of bioactive foods and nutraceuticals in the main stream of cancer prevention supplements is the practical challenge of stability testing on nutraceuticals (Mehta, 2007). Phenethylisothiocyanates have been recently reported as ingradients in many bioactive foods useful in general health (Ribnicky et al., 2002). Nutraceutical formulations of PEITC are available for general health and may be found in common sources like watercress. The glucosinolates constitute a homogeneous family of more than 100 metabolites with high value nutraceutical formulations to prevent cancer in humans (Getahun and Chung, 1999). The bioactive foods were reported to prevent colon cancer, lung cancer, skin cancer, and blood cancer by the use of whey protein typically formulated into bakery foods as a powder. The U.S. company Genetic Services Management, Inc. (GSM) has formulated a bioactive formula with the efficacy of cancer-risk modulation (Block, 2001). Morinda citrifolia combined with methylsulfonylmethane, LDH inhibitors, and active vitamins was reported in breast cancer treatment (Pawlus, 2007). The center of nutraceutical studies reported nutraceutical formula for treatment of cervical cancer (PSP Project, 2007). Different food companies have advocated new formulations to prevent or fight cancer.

PRESENT STATE OF ART ON BIOACTIVE FOODS IN CANCER PREVENTION

The UNISCI article, "Diet Called Most Important Breast Cancer Risk Factor," discusses the relationship between breast cancer and vitamin D, and between breast cancer and animal-derived vs. plant-derived foods (Grant, 2002). The bottom line is, diet and environment exposure are two major risks. The major question is whether bioactive foods can reduce the chance of getting cancer through dietary modifications? The answer is not clear. The recommendation of the National Cancer Institute (NCI) is "to eat at least 5 servings of brightly colored fruits and vegetables a day, to restrict ingestion of animal products (excluding farm-bred fish) while upping vegetable sources of protein (e.g., beans), to consume cooked tomato sauces, and to insure that we get, perhaps, 200 mcg a day of selenium (found in Walmart's OneSource supplement)" (NCI Internet, 2009). FDA requires appropriate scientific evidence regarding safety of any bioactive food use as daily prescription. However, new recommendations suggested that daily diet must contain 6.25 g of soy protein per serving, microcompound allicin (a small component of garlic) ad libitum amount, ecosapentanoic acid/docosaheaxanoic acid as polyunsaturated fatty acids (PUFAs) from fish or fish oils. The complementary medicine and alternative medicine approach is emerging as regulated tool to prescribe the norms of bioactive foods as daily supplements in cancer and other diseases (Nutraceuticals International internet document, 2000).

Insurance and Prescription

National and federal agencies such as NCI and FDA need evidences and established data in large trials to approve the wider use of bioactive foods or nutraceuticals in clinical practice. Due to a lack of such evidences and databases, bioactive foods and nutraceutical practice remains at the door steps as nonprescription or self-prescription available on counter. As a result, insurance companies still shy to accept any bioactive food as prescription. However, nutrition companies continue to promote the success of bioactive natural and bioengineered foods in diseases (NCI Statement, 2007).

Criteria of Nutrition Supplement Practice of Bioactive Foods in Cancer Prevention: A Government Policy

The use of complementary and alternative medicine (CAM) is increasing rapidly in developed countries (NCI Internet, 2007). Functional foods and nutraceuticals in cancer prevention were

highlighted as tomato, dietary fibers, soy, phystoesterogens, herbs, isothiocynates in cruciferous vegetables (Fullerton et al., 1991).

NTP-2000 and NIH-07 bioactive foods in diets were reported to be rich in nutraceuticals to meet recommended daily allowances. NTP-2000 diet has lower protein, higher fiber, and higher fat than the *NIH*-07 diet. Both diets were suggested as preventive in cancer. The main causative factors of cancer were free radicals, deficiency of vitamins C, D, E, and selenium, loss of cellular immunity in daily diet (Bames, 2007).

Recently, the National Cancer Institute put forth efforts on alternative ways of cancer prevention in the cause of public awareness to mainly focus on lifestyle, prevention and control care measures, eating habits, hazardous contaminants, with several successful attempts at using antioxidants, garlic, and vitamins (Huang et al., 2006).

Recent Advances on Bioactive Foods in Cancer

In recent years, the major focus was on more evidence-based wider use of multivitamin-multimineral combined with isolated bioactive components from plants and functional foods in various cancer types. In last four years, maximum efforts were devoted on reviews and compilation of evidenced experimental results on bioactive food constituents in reducing cancer progress and identification of associations of active food components in diet with reduced cell proliferation, necrosis, and apoptosis. However, NCI of the view that the sequential events during the bioactive food-induced or nutraceutical-treated cell growth or arresting cancer are controversial (Overcash, 2008). Literature suggested major information for the following: (1) direct link of vitamins and minerals in cancer prevention; (2) new bioactive food components with new mechanisms of arresting cell growth; (3) more controlled trials and regulated studies under federal support; (4) new awareness of unpopular foods in cancer prevention; and (5) new federal and statuary guidelines on the use of bioactive foods or their nutraceutical-recommended allowances and marketing.

The following information is grouped based on literature on bioactive foods in cancer management with major focus on controlled randomized trials on cancer in experimental setups and clinical cancer subjects. The description is divided into three sections.

BIOACTIVE FOODS IN CANCER PREVENTION: A QUICK SURVEY 2000–2009

Mechanism of Cancer Prevention by Herbs

Several approaches have been reported to investigate the role of nutraceuticals from bioactive foods on reduced cell damage in the normal cells of the body and possibility of delayed apoptosis, DNA interaction, reduced necrosis, cell proliferation, signaling, and maintaining metabolic integrity in the cancer tissue as cancer prevention mechanisms (Johnson, 2004; Hayes, 2005; Lin et al., 2006a; Sandur et al., 2007; Walfisch et al., 2007; Fazeli et al., 2009). The biomarkers of cancer such as metalloproteinases (Katiyar, 2007) vitamin D hydroxylase (Cross and Kállay, 2005), interleukins (Dijsselbloem et al., 2004; Powell et al., 2008), omega-3 fatty acids (Siddiqui et al., 2003; Nakamura et al., 2005; Nemes-Nagy et al., 2008) induced neutropenia (Branda et al., 2004; Anraku et al., 2009), DNA adducts (Mooney et al., 2005; Gaube et al., 2008), DNA methylases (Fang et al., 2007; Balunas et al., 2008), polymorphism (Roupe et al., 2006), superoxide dismutase (Clauson et al., 2008), UV damage (Grant, 2002; Gonzalez et al., 2007), and angiostatic therapy (McCarty and Block, 2005) have been discovered as potent indicators of nutraceutical chemopreventive mechanism. Still the action of phytochemicals and role of bacteria is not understood (Deng et al., 2008; Overk et al., 2008a,b). Recently mechanisms of nutraceuticals were reviewed thoroughly (Aggarwal and Shishodia, 2006; Overk et al., 2008a,b). Sharma et al. (2005) established the mechanism of intracellular sodium as a major player in breast and prostate tumors to induce delayed apoptosis. The MRI and PET techniques evidenced the coexisting mechanism

of reduced glycolysis and intracellular sodium release in tumors as chemosensitivity assay (Sharma et al., 2005).

The present focus is to divert attention to the identification of new food constitutents and their potential anticancer properties. In continuing attempts, the newly identified aflatoxin, alkaloids, cimifugic acid, and serotonin derivatives sources showed potentials of anticancer properties (Boyer, 2005; Balunas et al., 2008; Farnsworth, 2008; Kim et al., 2008; Overk et al., 2008a,b). The investigators attempted to calibrate the potencies of these active substances in roots. Other important antioxidant properties of dietary supplements were reported as aromatase enzyme inhibitors (Angley, 2007; Jordan and Haywood, 2007; Oritz, 2007; Wickett et al., 2007). The investigators demonstrated gene expression profiling assays as useful markers. New reports provided the information of pharmacometrics, safety issues, and associated chemical mechanisms of relative new herbs such as red clover, angelica, garcinia (Piersen et al., 2004; Booth et al., 2006; Balanaus et al., 2008; Deng et al., 2008; Overk et al., 2008a,b). The other major focus was the mechanism based evaluation of botanicals, extracts, and antiproliferation inhibitory properties in isolated cancer cell lines under the supervision of NCI to solve the issues for their clinical use (Bomeman and Field, 2006; Huang et al., 2006; Lee et al., 2006; NCI Internet, 2009; NCI Statement, 2009). Still now, the positive anticancer results are not perceived as practical in health care. On the other hand, several nutraceutical products have become commonly useable in general health and cosmetics with high propaganda (Hercberg et al., 2007; Kanaze et al., 2007; Rana et al., 2008). Silibinin, tannins, herbals dietary sources have been investigated as cytotoxic to cancer tissue in patients and emerged as potential anticancer formula (Gu et al., 2005; Raina and Agarwal, 2007; Reddy et al., 2007). In recent study, breast cancer survivor women were on complementary alternative medicine (Boon et al., 2007). However, risks and concepts of none or incomplete anticancer effects were addressed to alert the public for any unforeseen side effects of new products (Dvorkin et al., 2006; Michaud et al., 2007; Clauson et al., 2008). The new information of herbal evidence-based analysis has become available to standardize and calibrate new products such as some flavones, polyphenols, and ginseng (Boege et al., 1996; Chen 2004a; Chen et al., 2004b; Fang et al., 2007; Hofseth and Wargovich, 2007; Guy et al., 2008). The investigators and manufacturers put forth the idea to harvest the maximum prior knowledge of new food products and implement the benefits in cancer prevention trials. On the other hand, it remains to establish the potential interaction of dietary supplements and prescription medicines in cancer patients if taken togather (Boyer, 2005; Jordan and Haywood, 2007; Oritz, 2007; Famsworth et al., 2008). In this direction, several reports of the xenobiotics and cytochrome P450-3A activity, P-glycoprotein inhibition, estrogenic activities and quinine reductase have come to light as mechanisms of anticancer properties of flavonoids, black cohosh, trifolium pretense, and xanthohumol (already in phase II or III stage) to decide the preference between herb and medication (Gurley et al., 2004, 2005, 2006; Chung et al., 2005; Mahady, 2005; Overk et al., 2008a,b; Gödecke et al., 2009a,b). In general, the clinical safety of dietary products and beneficial effects of natural products has become priority in clinical trials (Dvorkin and Song, 2002; Barnes, 2003a,b; Burdette et al., 2003; Zhang and Morris, 2003; Piersen, 2003; Piersen et al., 2004; Chen and Kong, 2004; Chen et al., 2004; NIH Statement, 2006; Lawson et al., 2007; Chlebowskil et al., 2008).

The ample literature documenting the use of herbs in cancer prevention in the last five years is reviewed in following section. New information was investigated on successful intake of bioactive foods supplements in present day life style, affluence and daily nutraceutical rich diet reduced cancer prevalence in these studies. Major minerals as magnesium, zinc, micronutrients were evidenced in cancer prevention. Other new concepts emerged on the role of dietary vitamins as anitioxidants in primary and secondary cancers in meta-analysis, randomized trials and epidemiological evidences with established metabolic and biochemical mechanism of these bioactive dietary constituents. However, risks and concepts of none or incomplete anticancer effects were addressed and still remain unresolved. Soy phytoesterogens and isoflavones emerged as single potent chemopreventive agents to reduce the cancer risk. Randomized and double blind control trials indicated the increased

importance of vitamins, herbs and fresh vegetables as likely protective supplements (Walsh, 2005; Pham and Plakogiannis, 2005; Lewis et al., 2006; Wright et al., 2007a,b; Ambrosini et al., 2008; Van Patten et al., 2008; Zhang et al., 2008), tomato (lycopene) in prostate cancer prevention (Bunker et al., 2007; Curtis Nickel et al., 2008; Demark-Wahnefried, 2008), nutritional intervention in different cancers of different body organs in the body (Pan et al., 2004; Sartippour et al., 2004; Baron et al., 2005; Hernáandez et al., 2005; Taylor and Greenwald, 2005; Cho et al., 2006; Gallicchio et al., 2008).

Cancers in the Human Body and Bioactive Foods

The awareness of cancer prevention by use of bioactive foods and nutraceuticals began in late part of the last decade. Complementary and alternative medicine began a new era of harmless nonprescribed drugs with rampant success of self-prescription and on-counter sale of bioactive foods and nutraceuticals. The last five years evidenced wider acceptance of natural or bioengineered bioactive foods and nutraceuticals in both public and federal agencies. The major health hazards were identified as breast, prostate, colorectal, ovarian, pancreatic, skin cancers (Sharma, 2009a,b,c).

Breast cancer was identified as a single major health hazard three decades ago and still it remains a major risk among women. Different bioactive foods and nutraceuticals have been reported in reducing breast cancer risk at both self-medication at homes and health centers. Of special mention, tomato lycopene, and phytoesterogen were reported as preventive principles (Makarinec, 2005; Vantyghem et al., 2005; Lajous, 2006; Ericson, 2007; Dorjgochoo, 2008; Ishitani, 2008; Messina and Wood, 2008; Powell, 2008; Speers and Brown, 2008; Tomar and Shiao, 2008; Velentzis et al., 2008). Still it remains to establish the value to nutraceuticals in clinical prescription at health centers.

The prostate cancer was still recognized as a single major health hazard among men and remains as the main focus of nutraceutical intervention to reduce cancer risk by randomized control clinical trials. The major examples of cancer protective bioactive principles are multivitamin antioxidants, soy isoflavones (Demark-Wahnefried, 2008; Guy et al., 2008), soy-tomato combo products (Moyad et al., 2001; Brawley, 2002; Chang, 2004; Shukla and Gupta, 2005; Sonn et al., 2005; Bemis et al., 2006; Santillo et al., 2006; Weinstein et al., 2006, 2007; Molokwu, 2007; Syed et al., 2007, 2008; Dhillon et al., 2008; Grainger et al., 2008a,b; Guy et al., 2008; Kristal et al., 2008). The majority of experimental animal cancer studies supported the reduced prostate cancer by nutraceutical supplementation. Still it remains to establish the value of nutraceuticals in clinical prescription at health centers. In this direction, a lot of academic and global federal efforts are going on to establish the long-term benefits of nutraceuticals in prostate cancer risk (Moyad et al., 2001; Brawley, 2002; Weinstein et al., 2006; Molokwu, 2007; Syed et al., 2007; Kristal et al., 2008). The increased awareness and self-prescription of nutraceuticals among public for prostate cancer benefits is the present major concern of health authorities (Chang, 2004; Shukla and Gupta, 2005; Sonn et al., 2005; Bemis et al., 2006; Santillo et al., 2006; Weinstein et al., 2007; Wright et al., 2007a,b; Dhillon et al., 2008).

Lung cancer and esophageal cancer remained as the ignored health hazard perhaps due to other responsible environmental factors as main causative determinants of respiratory diseases with possible cancer risks. Recently, few reports suggested the possible increased benefits of vitamins A and E in protection against lung cancer (Meyer et al., 2005; Byers, 2008; Gallicchio et al., 2008; Mahabir et al., 2008).

Colon and colorectal cancers have been identified as health hazards of modernization in food processing, artificial foods, and affluent eating lifestyle in metro cities and fast-pace society (Franco et al., 2005). The increased incidence of colon, colorectal cancers have shown the processed food diet as main source of cancer. The colorectal and colon cancer is widely reported as reduced by use of bioactive food contituents such as folate (Bingham, 2006; Kim, 2007; Sanderson et al., 2007; Hubner and Houlston, 2008; Jaszewski et al., 2008), calcium (Benamouzig and Chaussade, 2004; Kripke,

2004; Flood et al., 2005; Weingarten et al., 2008), tomato-soy diet (Ryan-Harshman and Aldoori, 2007; Vrieling et al., 2007; Walfisch et al., 2007), fiber (Ishikawa et al., 2005; Das et al., 2007; Ryan-Harshman and Aldoori, 2007; Vrieling et al., 2007) and vitamins (Harris and Go, 2004).

However, there are hypes and controversies in risk assessment of nutraceutical in esophageal and gastrointestinal cancer management (Dong et al., 2008). The several reports highlighted the tradeoff between increased neoplasia in gastric cancer and the limits of nutraceuticals to reduce cancer growth (Bjelakovic et al., 2008; Dong et al., 2008). Still efforts are in the direction of antioxidant nutraceuticals to prevent or arrest the gastric cancer growth (Fock et al., 2008).

The ovarian and endometrial cancers are on the increase among privileged women population. Vitamins A and D, antioxidants, calcium, folate from bioactive foods still remain a choice as cancer preventive supplements (Tung et al., 2005; Schumann and Ewigman, 2007; McCullough et al., 2008).

Still efforts are in progress to observe more and more use of bioactive food and nutraceuticals in less known cancers. Recently, less reported and newly investigated cancer protection by nutraceuticals were evidenced for lymphoma (Russell et al., 2007), skin cancer (Hercberg et al., 2007), pancreatic cancer (Larsson et al., 2007; Sandler et al., 2008). In other recent reports the investigators showed a positive response of different food supplements and foods in cancer prevention of different organs in the body (Table 9.3).

TABLE 9.3
Documented or Approved Use of Nutraceuticals in Prevention or Management of Cancer in Different Organs

Cancer	Bioactive Food/Nutraceutical	Reference
Bone cancer	Soy isoflavones	Rackley et al., 2006
Breast cancer	Lycopene, phytoesterogen	Dorjgochoo, 2008; Ericson, 2007; Ishitani, 2008; Lajous, 2006; Maskarinec, 2005; Messina and Wood, 2008; Powles, 2008; Speers and Brown, 2008; Tomar and Shiao, 2008; Vantyghem et al., 2005; Velentzis et al., 2008
Common cancer	Cruciferous vegetables	Fullerton et al., 1991; Sandler et al., 2008
Colon cancer	Walnuts, fibers	Campbell et al., 2003; Benamouzig and Chaussade, 2004; Bingham, 2006; Das et al., 2007; Flood et al., 2005; Franco et al., 2005; Harris and Go, 2004; Hubner and Houlston, 2008; Ishikawa et al., 2005; Jaszewski et al., 2008; Kim, 2007; Kripke, 2004; Ryan-Harshman and Aldoori, 2007; Vrieling et al., 2007; Sanderson et al., 2007; Weingarten et al., 2008
Gastric cancer	Common herbs	Dong et al., 2008; Bjelakovic et al., 2008; Fock et al., 2008
Intestinal cancer	Sphingolipids	Borek, 2004; Bjelakovic et al., 2004; McCullough et al., 2008
Liver cancer	Silbinin, citrous, flavonoids	Fullerton et al., 1991
Lung cancer	Vitamins A and E	Byers, 2008; Gallicchio et al., 2008; Mahabir et al., 2008; Meyer et al., 2005
Ovary cancer	Vitamin A/D, antioxidants	McCullough et al., 2008; Pan et al., 2004; Schumann and Ewigman, 2007; Tung et al., 2005
Pancreatic cancer	Vitamins and isoflavones	Larsson et al., 2007; Mayo New, 2007; Sandler et al., 2008
Prostate cancer	Lycopene, phytoesterogen	Meyer et al., 2005; Bemis et al., 2006; Brawley, 2002; Chang, 2004; Demark-Wahnefried, 2008; Dhillon et al., 2008; Grainger et al., 2008a,b; Guy et al., 2008; Kranse, 2005; Kristal et al., 2008; Molokwu 2007; Moyad et al., 2001; Santillo et al., 2006; Shukla and Gupta, 2005; Sonn et al., 2005; Syed et al., 2007, 2008; Weinstein et al., 2006, 2007; Wright et al., 2007a,b

Challenges, Hypes, Hopes and Futuristic Role of Bioactive Foods

The nutraceuticals are common in bioactive foods. The safe levels of these bioactive compounds in bioactive foods is a challenge to keep them on the shelf within safety levels approved by FDA. Other challenges are the large R&D budgets, stringent experience in safety and efficacy testing, proprietary technology, access to multiple distribution channels—such as healthcare professionals—or high value-added products. The success of bioactive food in cancer therapy depends on cancer-preventing products tasting good, and being nutritious—such as oat bran added to cereals. Conceptually, a multiarm, double-blind, placebo-controlled, randomized study with a well-defined end point and a large study population represents the "gold standard" for clinical trials. At the opposite end of the spectrum would be nonhuman studies such as *in vitro* or animal studies. Bioactive foods with strong scientific evidence demonstrated by definitive clinical findings in humans—such as significant improvement on a health-related end point—that are accepted and published in peer-reviewed journals would be highly rated. Other factors of bioactive foods include patent protection, source of supply or availability, stability and other technical formulation issues, and cost. Some bioactive foods have physical and chemical properties that make them more versatile and suitable for a variety of food products. For example, stanol esters are fat soluble (in margarines, dressings, bars, and other fatty foods).

The nutraceuticals in bioactive foods must provide good flavor, texture, refreshment, satiety, and so on, are greatest challenges in nutraceutical product development. Nestlé's M&M VO2Max energy antioxidant-packed energy bar for serious athletes, but failed in market. The FDA requires appropriate scientific evidence regarding safety before a nutraceutical product containing a bioactive compound can be marketed as a food product. Bioactive compounds and bioactive-containing products also have some regulatory and manufacturing challenges—such as it must contain 6.25 g of soy protein per serving. In the case of garlic, for example, the microcompound allicin in garlic are often extremely sensitive to heat, light, and other conditions such as pH and metal ions. The polyunsaturated fatty acids (PUFAs) in fish oils—menhaden oil are extremely sensitive to heat, light, and metal ions—all of which cause rapid oxidation in the food system and off-flavors.

Most of the success of nutraceuticals is based on self-prescription and one's individual word of mouth experiences. Still it is far to realize the miraculous benefits of nutraceuticals unless controlled clinical trials support the evidences and facts of nutraceutical preventive therapeutic efficacy. The major challenge is early detection of premalignancy and timely effective evaluation of intact cancer tumors or isolated cancer cells after exposure of bioactive food active component(s) or nutraceutical formula as oncological treatment. In spite of all tools available, cancer is a major health hazard. The vast available data on use of nutriceuticals in cancer comes from epidemiological health and population statistics experienced with bioactive foods. The reduced cancer incidence due to bioactive foods or nutraceuticals seems a hype but greater hopes are anticipated with advancements in food science. However, cancer still remains a major threat because of high mortality compounded with incomplete success of chemotherapy, oncotherapy, and surgical intervention. In future, bioengineered bioactive foods and nutraceuticals will play a significant role in cancer prevention as alternative oncotherapeutics.

CONCLUSION

Bioactive foods are rich in nutraceuticals. They are slowly emerging as acceptable dietary lifestyle among cancer survivors. The bioactive foods still are growing in number, and investigations suggest high hopes of new nutraceuticals from bioactive foods in cancer prevention. The role of governments and globalization will certainly support the health risks and clinical trials on nutraceuticals. The nutraceuticals from bioactive foods and herbs are becoming popular as they are harmless and natural food constituents participating in natural body metabolism. The nutraceuticals are still food supplements and the last five years demonstrated enormous change in the perception of nutraceuticals as cancer preventive and therapeutic supplements in cancers of different organs.

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10 Curcumin-Mediated Cellular Responses in Chemical Carcinogenesis
In Vivo Studies

Girish B. Maru, Asha G. Ramchandani, Gaurav Kumar, and Rachana Garg

CONTENTS

INTRODUCTION

Cancer accounts for more than 12% of deaths globally. It is a silent disease characterized by unscheduled and uncontrolled cell proliferation. Almost any mammalian cell can succumb to oncogenic transformation. Majority of human cancers are caused, mediated, or modified by environmental factors. Causative agent(s)/factor(s) may also act simultaneously or in sequence to initiate or promote carcinogenesis that often has a long latency period of 20 years or more. Although dose and duration of exposure to exogenous/endogenous carcinogen(s) is one of the determining factors, that alone is not sufficient to explain the exposure-related outcome, as majority of cancers result from complex interactions between environmental exposure(s) and genetic/acquired susceptibility or protective host factors. Epidemiological studies have successfully demonstrated that certain well-defined exposures (e.g., tobacco, alcohol, ionizing radiation, occupational carcinogens, viruses, etc.) increase the risk of cancers at specific sites. Causal relationships have been established for some cancers, for example, tobacco use and oral/lung cancer. However, for other cancers (breast, colon, stomach, prostate, esophagus) causative factor(s) still need to be determined. Elimination of known and established carcinogens from the environment, that is, primary prevention of cancer has proven to be rather difficult due to social, economic, and political reasons (e.g., tobacco). Moreover, despite the tremendous advancement in understanding molecular and cellular basis of cancer and in current treatment modalities including surgery, radiation and chemotherapy, the mortality rates (age-adjusted) for cancer have not declined in the past 50 years.

Based on the experience with some infectious diseases and the recent progress in cardiology, prevention of diseases appears to be one of the attractive, cost effective and achievable approaches. Cancer chemoprevention envisages the use of natural or synthetic compounds to prevent, suppress, or delay the development of clinically detectable cancer. This is a "prescription" approach and may form an adjunct to other cancer control and prevention strategies. The scientific rationale for the use of cancer chemoprevention is based on the multistep nature of carcinogenesis.

CARCINOGENESIS AND CHEMOPREVENTION

Carcinogenesis is a multifactorial, multistep and long evolving process characterized by three major steps viz. initiation, where DNA of the cells in normal tissue is damaged by exposure to exogenous/endogenous carcinogens and subsequently damage is fixed; promotion, which is characterized by selective expansion of initiated cells to form an actively proliferating premalignant tumor cell population; and progression, which is an irreversible event forming clone of tumor cells with increased proliferative capacity, invasiveness, and metastatic potential. Several of these steps are susceptible to modulation by a variety of environmental agents and thus, inhibition of any stage of carcinogenesis can potentially delay/prevent cancer (Sporn and Suh, 2002). Chemopreventive agents have been classified into two groups based on their mode of action: blocking agents, compounds that inhibit the initiation process either by inhibiting the formation of carcinogen from pro-carcinogens or preventing the carcinogen from reaching/reacting with critical cellular targets, and suppressing agents, compounds that inhibit/suppress tumor promotion/progression (Figure 10.1).

Putative chemopreventive agents are subjected to rigorous *in vitro* and *in vivo* screening assays to determine their efficacy against different stages of carcinogenesis in defined model systems and to investigate the mechanism(s) of their chemopreventive action(s). At present a number of

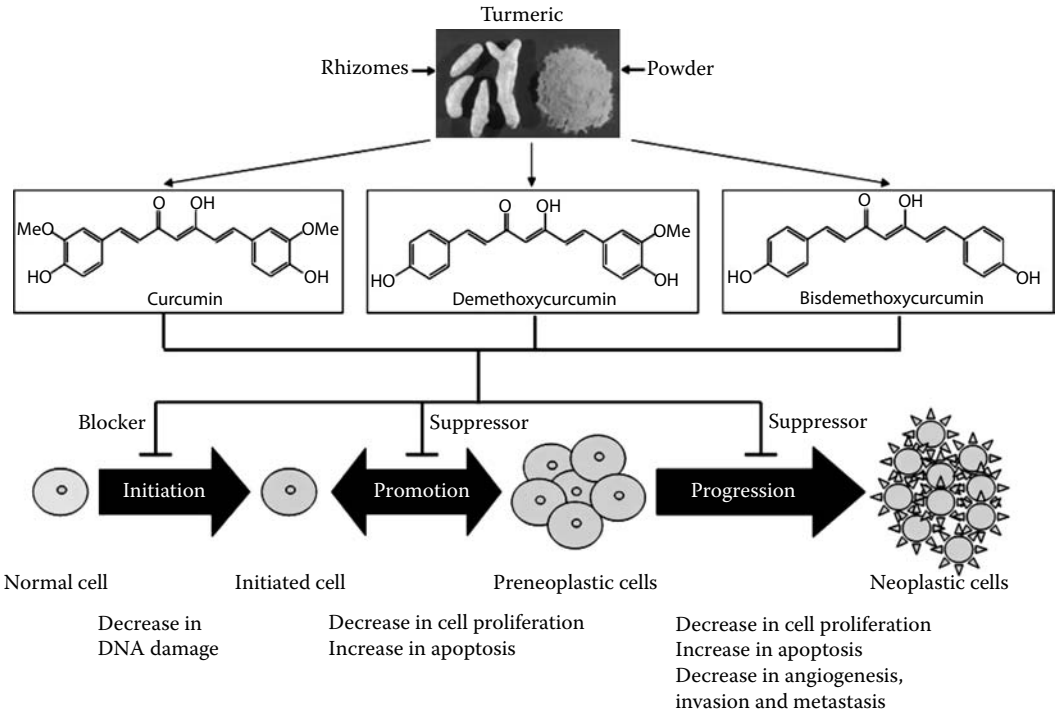

FIGURE 10.1 Schematic diagram showing multistep process of carcinogenesis, steps at which curcumins exhibit their biological effects in experimental systems *in vivo*.

chemopreventives are being considered for clinical trials. Among them, plant-derived antioxidants and dietary agents are receiving increasing attention due to their high tolerability and low toxicity (Surh, 2003; Patel et al., 2007), and are currently under clinical investigations. However, most of the agents that have emerged as highly promising after preclinical safety and efficacy studies, have failed in human clinical trials (Seifried et al., 2003). These failures can be attributed to lack of (a) ability to replicate the conditions of exposure (route, dose, sequence, and frequency, duration, etc.) and other host factors (genetic/acquired) between the two systems, and (b) knowledge about the mechanism(s) of action and toxicity of the agent on normal physiological processes in different organ systems. These failures have channelized rest of the preclinical studies on putative chemopreventive agents in understanding their mechanism(s) of actions, rather than focussing only on endpoint of tumor development (decrease in incidence/multiplicity, increase in latency period).

Curcumin (diferuloylmethane), the active polyphenolic component of turmeric is one such molecule currently under clinical investigations in human trials. Voluminous data are available in literature *in vitro* (in cell lines) demonstrating the chemopreventive potential of curcumin as well as its effects on various signalling pathways (Aggarwal et al., 2003; Campbell and Collett, 2005). However, reports on the mechanism(s) of curcumin-mediated chemoprevention *in vivo* are limited. Therefore, this review focuses mainly on describing some of the mechanism(s) of anti-initiating, antipromoting and antiprogressor action(s) of turmeric/curcumin employing *in vivo* experimental model systems.

TURMERIC

Turmeric, the powdered rhizome of the plant *Curcuma longa* Linn has been widely used as a coloring agent, a food additive, preservative, and in the treatment of inflammatory conditions and diseases (Ammon and Wahl, 1991). The three main curcuminoids isolated from turmeric are curcumin, demethoxycurcumin and bisdemethoxycurcumin. Curcumin has been shown to possess anti-inflammatory, antioxidant, anticlastogenic, antiviral, antifungal properties. Additionally, the hepato-protective, nephro-protective, thrombosis suppressing, myocardial-infarction protective, hypoglycaemic, antirheumatic effects of curcumin are also well documented (Strimpakos and Sharma, 2008). Studies employing different *in vivo* systems have demonstrated anti-initiating/antipromoting/antiprogressor activity of turmeric/curcumin in both chemically induced and genetic models (Tables 10.1 and 10.2). Oral and topical administration of turmeric/curcumin has been shown to modulate incidence/multiplicity/latency period or defer degenerative changes at various organ sites in experimental animals (Table 10.1). The dose levels employed in evaluation of *in vivo* chemopreventive effects of curcumin were: topical (0.001–10 μmol), gavage (50–600 mg/kg BW), dietary (0.01–2.0%) and dietary turmeric (0.2–5.0%). None of the studies covered in Table 10.1 have reported turmeric/curcumin exposure-related toxicity. Most of these reports have not measured the circulating or tissue levels of free curcumin, its conjugates and metabolites. Available pharmacokinetic evaluations in rodents show low nanomolar levels of free curcumin in plasma probably due to poor systemic bioavailability/rapid metabolism and excretion (Strimpakos and Sharma, 2008).

Mechanism(s) of Chemopreventive Actions of Curcumin

Curcumin has been shown to inhibit carcinogenesis at initiation, postinitiation as well as promotion stages, thereby acting as both "blocker" and "suppressor" of carcinogenesis.

Mechanism(s) of Anti-Initiating Action

Xenobiotic metabolising phase I and II enzymes play a crucial role in metabolic activation and detoxification of xenobiotics entering into the cellular environment and thus in the initiation process of carcinogenesis. These metabolising enzymes thus could be one of the plausible targets for cancer chemoprevention.

TABLE 10.1
***In vivo* Chemopreventive Effects of Turmeric/Curcumin in Different Rodent Models**

S. No.	Animal Model	Organ	Carcinogen Dose/Route/Duration	Curcumin/Turmeric Dose/ Route/Duration	Observations	Reference
1	CD-1 mice	Skin	DMBA–TPA 200 nmol, topical, single – 5 nmol, topical, 2×/wk – 20 wks	Curcumin – 1, 3, 10 µmol or 1–100, 3000 nmol; DMC, BDMC, THC–1, 3, 10 µmol; topical, during promotion	Dose-dependent inhibition of TPA-induced promotion (tumor multiplicity/volume) especially with pure curcumin and DMC	Huang et al., 1988, 1995, 1997
2	CD-1 mice	Skin	i) B[*a*]P–TPA 20 nmol, topical, 1×/wk – 10 wks – 15 nmol, topical, 2×/wk – 21 wks; ii) DMBA–TPA 2 nmol, topical, 1×/wk – 10 wks – 15 nmol, topical, 2×/wk – 15 wks	Curcumin i) and ii) 3 or 10 µmol, topical, during initiation	Decrease in the multiplicity/ incidence of tumors	Huang et al., 1992
3	Swiss mice	Skin	i) DMBA 100 nmol, topical, 2×/wk – 8 wks; ii) DMBA TPA; 100 µg, topical, single – 2.5 µg, topical, 2×/wk – 26 wks	i) Turmeric, 2–5%, diet, during initiation, postinitiation and until end; ii) Curcumin, 1%, diet, during initiation and until end	Decrease in tumor volume, multiplicity/incidence	i) Azuine and Bhide, 1992 ii) Limtrakul et al., 1997
4	i) Swiss mice ii) A/J mice	Forestomach	B[*a*]P i) 1 mg, gavage, 2×/wk – 4 wks; ii) a) and b) 1.5 mg, gavage, 1×/wk – 4 wks	i) AqTE (3 mg/day), curcumin (1 mg/day), gavage; ETE (0.01–0.25%), Turmeric (0.2–5%), diet; during initiation, postinitiation and until end; ii) Curcumin; a) 0.5–4.0%, diet, during initiation, postinitiation and until end; b) 2%, diet, during initiation	Significant decrease in multiplicity/incidence of forestomach tumors	i) Azuine and Bhide, 1992; Azuine et al., 1992; Deshpande et al., 1997 ii) a) Huang et al., 1994; b) Singh et al., 1998
5	Wistar rats	Stomach	MNNG + NaCl, 100 ppm, drinking water, 8 wks – 5%, diet, 8 wks	Curcumin, 0.05–0.2%, diet, during promotion	Decrease in incidence/ multiplicity of glandular stomach hyperplasia and adenocarcinomas	Ikezaki et al., 2001

6	C57BL/6 mice	Duodenum	ENNG, 120 mg/L, drinking water, 4 wks	Curcumin (commercial grade) 0.5–4.0%, diet, postinitiation until end	Decrease in tumor size, multiplicity/incidence	Huang et al., 1994
7	CF-1 mice	Colon	AOM, 10 mg/kg BW, s.c., 1×/wk – 6 wks	Curcumin (commercial grade); 0.5–4.0%, diet, during initiation, postinitiation and until end	Decrease in tumor size, multiplicity/incidence	Huang et al., 1994
8	F344 rats	Colon	AOM i) a), b) and c) 15 mg kg BW, s.c., 1×/wk – 2 wks, after curcumin; ii) 30 mg/kg BW, s.c., single	Curcumin, i) a) and b) 2000 ppm, diet, during initiation and until end c) 0.2–0.6%, diet, during initiation, postinitiation and promotion until end; ii) 8–16 g/kg diet, 45 wks	Decrease in tumor volume, multiplicity/ incidence of preneoplasia and invasive/ non-invasive colon tumors	i) a) Rao et al., 1995; b) Samaha et al., 1997; c) Kawamori et al., 1999 ii) Pereira et al., 1996
9	B6C3F1 mice	Colon	DMH, 20 mg/kg BW, s.c., 2×/wk – 3 wks	Curcumin, THC 0.5%, diet, postinitiation	Decrease in preneoplastic aberrant crypt foci	Kim et al., 1998
10	Wistar rats	Colon	DMH, 20 mg/kg BW, s.c., 1×/wk – 15 wks	Curcumin, BDMC 80 mg/kg BW, i.g., during initiation and postinitiation	Reduction in number/size of colon tumors	Devasena et al., 2002
11	C57BL/6J-(Min/+) mice	Colon	Spontaneous intestinal tumors	Curcumin, 0.1–0.5%, diet, 15 wks	Reduction in multiplicity of colon adenomas	Perkins et al., 2002
12	C57BL/6J-(Min/+) mice	Intestine	Spontaneous intestinal tumors	Curcumin, 0.1%, diet, started at 5 wk of age up to 110 days of age	Decreased intestinal adenomas	Mahmoud et al., 2000
13	F344 rats	Mammary gland	DMBA, 12 mg/rat, gavage, single, 1 wk after start of curcumin	Curcumin, 10–20 g/kg diet, during initiation and until end	No alterations in incidence/ multiplicity of mammary tumors	Pereira et al., 1996
14	Sencar mice	Mammary gland	DMBA, 1 mg, gavage, 1×/wk – 5 wks	i) Curcumin – 2%; ii) Dibenzoyl methane (β-diketone analogue of curcumin) – 1% diet, during initiation and postinitiation	i) Little or no effect on incidence of mammary tumors; Reduction in the incidence of lymphoma/ leukemia; ii) Decrease in multiplicity/incidence of mammary tumor	i) Huang et al., 1998 ii) Lin et al., 2001

continued

TABLE 10.1 (continued)
***In vivo* Chemopreventive Effects of Turmeric/Curcumin in Different Rodent Models**

S. No.	Animal Model	Organ	Carcinogen Dose/Route/Duration	Curcumin/Turmeric Dose/ Route/Duration	Observations	Reference
15	Sprague Dawley rats	Mammary gland	DMBA i) 15 mg, gavage, single on day 55; ii) 30 mg/kg, i.g., single, at 50 days of age	i) Turmeric (1%), ETE (0.05%), diet, during initiation and post initiation ii) Curcumin, 1%, diet, 2 wk preinitiation	i) Significant reduction in tumor multiplicity/incidence/burden; ii) No significant effect on mammary tumor development	i) Deshpande et al., 1998 ii) Singletary et al., 1998
16	Wistar-MS rats	Mammary gland	γ-rays (2.6 Gy) day 20 of pregnancy – DES pellet implantation after weaning until end (0.38 µg/day)	Curcumin, 1%, diet, during initiation	Significant reduction in incidence/multiplicity of mammary tumors, Increase in latency period	Inano et al., 1999, 2000
17	I a, b, c) Wistar rats II) C3H/ HeN mice	Liver	I a) DEN – 150 mg/kg, i.p., 1×/wk – 4 wks I b) DEN, 200 ppm, drinking water, 4 wks; I c) DEN – PB, 200 mg/kg, i.p., single – PB, 0.05%, drinking water, 13 wks; II) DEN-20 µg/g BW, i.p., single	Curcumin Ia) 200–600 mg/kg, gavage, preinitiation; Ic) 100 mg/kg/day, gavage, preinitiation and until end; II) 0.2%, diet, during initiation and until end; Ib) Turmeric, 0.2–5%, diet, during initiation and postinitiation	Ia) Suppression of DEN-induced inflammation and hyperplasia; Ib) Decrease in incidence of focal dysplasia and HCC; Ic) Prevention of hepatic hyperplastic nodules; II) Reduction in multiplicity/ incidence of HCC	Ia and II) Chuang et al., 2000a, b; Ib) Thapliyal et al., 2003; Ic) Sreepriya and Bali, 2005
18	LEC rats	Liver, kidney	Copper, ~300 µg/rat/day, diet and drinking water, lifespan	Curcumin, 0.5%, diet, life span	No decrease in copper-induced liver or kidney tumor incidence; Reduction in overall cancer formation and metastasis	Frank et al., 2003
19	A/J mice	Lung	B[*a*]P + NNK, 3 µmol each, gavage, 1×/wk – 8 wks	Curcumin, 2000 ppm, diet, postinitiation	No effect on lung tumor multiplicity	Hecht et al., 1999
20	CCSP-rtTA/ Ki-ras TG mice	Lung	DOX – BHT, 500 µg/mL, drinking water – 150 mg/kg, i.p., 1×/wk – 6 wks	Curcumin, 8000 ppm, diet, postinitiation	No change in tumor multiplicity and progression to later lesions	Dance-Barnes et al., 2009

21	Syrian golden hamsters	Cheek pouch	DMBA, 0.5%, topical, 3×/wk – i) 6 wks; ii) 12 wks; iii) 14 wks	i) Curcumin, 10 µmol, topical, postinitiation; ii) Turmeric, 1%, diet, during initiation iii) Curcumin, 80 mg/kg BW, gavage, 14 wks, during initiation	i) Decrease in incidence of SCC and tumor volume, inhibited angiogenesis in papillomas and SCC ii) Decrease in tumor burden/multiplicity, increase in latency period iii) Prevented formation of oral carcinoma, but showed hyperplasia and dysplasia	i) Li et al., 2002 ii) Garg et al., 2008c iii) Manoharan et al., 2009
22	F344 rats	Esophagus	NMBA, 0.5 mg/kg BW, i.p., 3×/wk – 5 wks	Curcumin, 500 ppm, diet, during initiation and postinitiation	Reduction in incidence/ multiplicity of esophageal tumors and preneoplastic lesions	Ushida et al., 2000
23	F344 rats	Prostate	i) DMAB, 50 mg/kg BW, s.c., 10 × 2wk intervals – 20 wks; ii) PhlP, 100 mg/kg BW, i.g., 2×/wk – 20 wks	Curcumin i) 500 ppm, diet, during initiation and postinitiation; ii) 500 ppm, diet, postinitiation	Inhibition of DMAB induced prostate carcinomas, no effect on PhlP-induced tumors	Imaida et al., 2001

AqTE = aqueous turmeric extract; AOM = azoxymethane; B[a]P = benzo[a]pyrene; BDMC = bisdemethoxycurcumin; BHT = butylated hydroxytoluene; DEN = diethylnitrosamine; DES = diethylstilbestrol; DMAB = 3,2′-dimethyl-4-aminobiphenol; DMBA = 7,12-dimethylbenz[a]anthracene; DMC = demethoxycurcumin; DMH = 1,2-dimethylhydrazine; DOX = doxycycline; ENNG = *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine; ETE = ethanolic turmeric extract; HCC = hepatocellular carcinoma; MNNG = *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; NaCl = sodium chloride; NMBA = *N*-nitrosomethylbenzylamine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NQO = 4-nitroquinoline-1-oxide; PB = phenobarbitone; PhlP = 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; SCC = squamous cell carcinoma; THC = tetrahydrocurcumin; TPA = 12-*O*-tetradecanoylphorbol-13-acetate.

TABLE 10.2
***In vivo* Antiprogressor Activity of Turmeric/Curcumin in Rodent Models Bearing Transplanted Tumors/Xenografts**

S. No.	Animal Model	Tumor Cells/Growth Factor; Dose/Route	Curcumin/Turmeric; Dose/Route/ Duration	Observation	Reference
1	Swiss mice	Ehrlich ascites tumor cells, 1×10^6, i.p.	Curcuminoids (liposomally encapsulated), 12.5 mg/kg, i.p., 10 days	Increased lifespan of animals compared to appropriate controls	Anto et al., 1996
2	C57BL/6 mice	B16F 10 melanoma cells 1×10^6, i.v. tail vein	Curcumin, 200 µmol/kg BW, gavage, 10 alternate days	Significantly inhibited the invasion of B16F10 melanoma cells/lung tumor metastasis; increase in life span of animals; lung collagen hydroxyproline and serum sialic acid levels lowered; inhibition of MMP	Menon et al., 1999
3	C57BL/6 mice	βFGF, 80 ng/pellet, intrastromal implantation	Curcumin/analogs, 44 µg/pellet, intrastromal implantation, single	Inhibition of βFGF-induced neovascularization in the mouse cornea	Arbiser et al., 1998
4	Wistar rats	Yoshida AH-130 cells 1×10^8, i.p.	Curcumin, 20 µg/kg BW, i.p., postinoculation, 6 days,	Inhibition of tumor growth; little effect as anticachectic compound	Busquets et al., 2001
5	Nude mice	LNCaP prostate cancer cells, 1×10^6, s.c.	Curcumin, 2%, diet, 6 wks	Marked decrease in the extent of cell proliferation, microvessel density; significant increase in apoptosis	Dorai et al., 2001
6	Swiss mice	Ehrlich ascites tumor cells, 5×10^6, i.p.	Curcumin, 10 mM/mouse, i.p., after 5 days of cell growth, alternate day for up to 10 days	Effectively decreased the formation of ascites fluid; inhibition of peritoneal angiogenesis; downregulation of production of VEGF, βFGF, EGF; decreased expression of angiopoietin genes	Gururaj et al., 2002
7	B6C3F1	Murine HCC cells (CBO140C12), 1×10^7 Orthotopic implantation into left lobe of liver	Curcumin, 100–200 mg/kg, p.o., daily, postimplantation, 20 days	Suppression of intrahepatic métastasis mediated by inhibition through matrigel and production of MMP-9 adhesion and haptotactic migration to fibronectin/laminin	Ohashi et al., 2003

8	C57BL/6 mice	B16F-10 melanoma cells, 2×10^6, i.v.	Curcumin, 50 μM/48 h in B16F-10 melanoma cells, 2×10^6, i.v.	Fewer lung metastasis; reduction in binding to extracelluar matrix proteins, expression of integrin, cadherin, adhesion, MMP activity	Ray et al., 2003
9	Athymic nude mice	Breast cancer cells (MDA-MB-435LVB), 2×10^6, inj. into mammary fat pad	Curcumin, 2%, diet, from day 5 after tumor removal, 5 wks	Decrease in incidence of breast cancer metastasis to the lung; suppression of expression of NF-κB, COX-2, MMP-9	Aggarwal et al., 2005
10	Athymic nu/nu mice	Pancreatic carcinoma cells (B × PC-3, MiaPaCa2), 5×10^6, s.c.	Liposomal curcumin, 40 mg/kg BW, i.v. tail vein, when tumor masses established, 3×/wk	Suppression of pancreatic carcinoma growth and tumor angiogenesis; downregulation of NF-κB machinery	Li et al., 2005
11	Balb/c nude mice	HepG2 cells, 2×10^6, inoculated in upper skin surface chamber	Curcumin, 3000 mg/kg BW, oral, daily for up to 7–14 days	Increase of tumor neocapillary density attenuated significantly	Yoysungnoen et al., 2005
12	SCID mice	Prostate cancer cells (DU-145) 1×10^6, s.c. foot pads	Curcumin + placebo 5 mg/kg, gavage, 3×/wk – 10 wks	Marked reduction of tumor volume, MMP activity; induction of apoptosis; fewer metastatic nodules in lung	Hong et al., 2006
13	Athymic NCr-nu mice	Human ovarian cancer cells (Hey A8, SKOV3ip1), 2.5×10^5, i.p.	Curcumin, 500 mg/kg, gavage, daily, 4 wks	Decrease in mean tumor growth; proliferation/microvessel density; increased tumor cell apoptosis	Lin et al., 2007
14	SCID mice	Human lung adenocarcinoma cells (CL1-5), 5×10^5, i.v. tail vein	Curcumin, 1 g/kg, p.o., daily, 5 wks	Inhibition of lung cancer cells invasion and metastasis, increased HLJ1 expression	Chen et al., 2008

AH = ascites hepatoma; βFGF = beta fibroblast growth factor; EGF = epidermal growth factor; HLJ1 = heat shock protein 40; MMP = matrix metalloproteinases; VEGF = vascular endothelial growth factor.

Effects on Carcinogen-Activating Phase I Enzymes

Phase I enzymes, predominantly cytochrome P450s (CYP) (super family of heme-thiolate enzymes), play a major role in the first step of metabolism, where xenobiotics are processed to more electrophilic moieties by addition of functional groups, making them more water soluble, thus facilitating further detoxification by phase II enzymes. Electrophilic intermediates in turn can react with cellular macromolecules to form adducts, thereby marking the process of initiation. CYP1A, which are primarily involved in the metabolic bioactivation of polycyclic aromatic hydrocarbons, in general, are regulated by a basic helix-loop-helix cytosolic protein, aryl hydrocarbon receptor (AhR). Upon ligand binding, AhR translocates to the nucleus, where it heterodimerizes with aryl hydrocarbon receptor nuclear translocator (ARNT) protein and binds to the xenobiotic response element (XRE) flanking CYP1A1 gene, thereby activating its transcription (Kawajiri and Fujii-Kuriyama, 2007).

Gavage administrations of turmeric (4 g/kg BW) or curcumin (0.4 g/kg BW) to lactating mice for 14/21 days have been shown to increase the levels of hepatic cytochrome b5 and cytochrome P450s in mothers and suckling pups (Singh et al., 1995). Exposure of mice to dietary curcumin (2%, 14 days) has been reported to decrease activity of hepatic ethoxyresorufin *o*-deethylase (Singh et al., 1998). Gavage administration of curcumin (200–400 mg/kg BW, one to two weeks) showed decrease in hepatic CYP1A3 activity and protein levels and decrease in activity of CYP1A1 without alterations in the level of protein in mice (Valentine et al., 2006). Dietary pretreatment with turmeric (1%) or curcumin (0.01%, 0.05%) significantly decreased the benzo[*a*]pyrene [B[*a*]P]-induced activity of CYP1A1 and CYP1A2 in liver, lungs and stomach/forestomach of rats/mice (Thapliyal et al., 2001; Thapliyal and Maru, 2001; Garg et al., 2008a) and phenobarbitone (PB)-induced activity of CYP2B1 in liver of rats (Thapliyal and Maru, 2001), although the extent of decrease was different. Notably, turmeric or curcumin diet alone did not alter the basal activity of CYP isozymes in the controls. Parallel to the decrease in B[*a*]P-induced isozyme activity of CYP, pretreatment with chemopreventive doses of dietary curcumin (0.01%, 0.05%) could significantly inhibit the carcinogen-induced CYP1A1/1A2 protein and mRNA expressions in tissues of mice, although it did not alter the basal protein/mRNA levels, suggesting that curcumin mediates transcriptional regulation of CYP1A. Further study has shown that pretreatment with dietary curcumin abrogated the B[*a*]P-induced binding of AhR to DNA in nuclear extracts from mouse liver and lungs. This in turn is accountable for the decrease in B[*a*]P-induced transactivation of CYP1A gene. Furthermore, it was shown that curcumin pretreatment resulted in decrease in B[*a*]P-induced protein levels of total AhR, although curcumin alone did not affect the basal levels when compared with vehicle-treated controls. Additionally, it was shown that curcumin pretreatment led to significant and dose-dependent decrease in B[*a*]P-induced AhR nuclear translocation in liver and lungs of mice (Garg et al., 2008a). Phosphorylation of AhR is one of the crucial events required for transformation of liganded AhR to a DNA-binding form (Carrier et al., 1992) and the process may be modulated by mitogen activated protein kinases (MAPKs)/protein kinase C (PKC). Recent study has shown it for the first time that dietary curcumin decreased the B[*a*]P-induced phosphorylation of AhR (Garg et al., 2008a).

Taken together, dietary curcumin modulates AhR *in vivo* by decreasing carcinogen-induced phosphorylation/activation, nuclear uptake, and DNA binding and thereby decreased the carcinogen-induced transactivation of CYP1A1 resulting in decreased CYP1A1 activity (Figure 10.2). Considering the above observations and preliminary reports on effects of curcumin on PB-induced CYP2B1 and acetone-induced CYP2E1, role of curcumin in modulation of activation of specific receptor-mediated gene expression of phase I metabolizing enzymes involved in the metabolism of diverse xenobiotics needs further investigation.

Effects on Detoxifying Phase II Enzymes

Phase II enzymes play a crucial role in the detoxification of activated carcinogen, and hence in the elimination of reactive intermediates from cellular environment by preventing their interaction with DNA. Activated carcinogens are conjugated with endogenous biomolecules such as glutathione

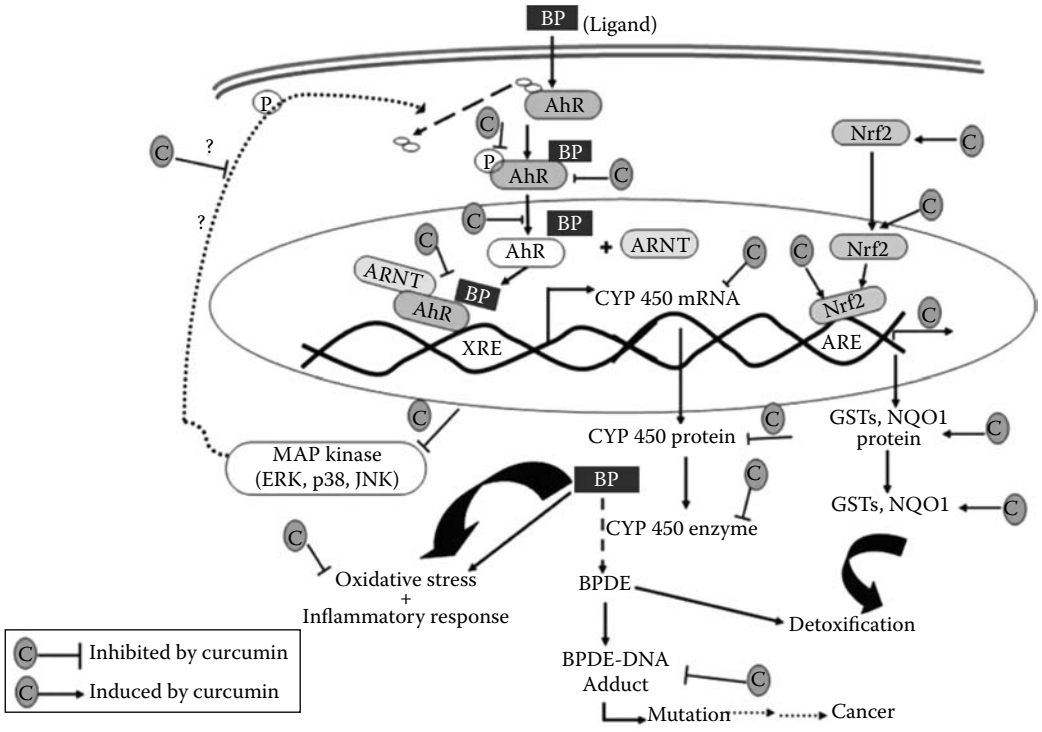

FIGURE 10.2 Schematic presentation of possible steps at which curcumin exhibits anti-initiating effects in tissues of benzo[*a*]pyrene-treated animals. AhR = aryl hydrocarbon receptor; ARE = antioxidant response element; ARNT = aryl hydrocarbon receptor nuclear translocator; BP = benzo[*a*]pyrene; BPDE = benzo[*a*] pyrene diol epoxide; CYP 450 = cytochromes P450; ERK = extracellular signal-regulated kinase; GSTs = glutathione-*S*-transferases; JNK = c-Jun N-terminal kinase; MAP Kinase = mitogen activated protein kinase; NQO1 = NADPH (nicotinamide adenine dinucleotide phosphate, reduced)-quinone oxidoreductase-1; Nrf2 = nuclear transcription factor erythroid 2 p45-related factor2; P = phosphorylation; p38 = p38 protein kinase; XRE = xenobiotic response element.

(GSH), glucuronic acid by phase II enzymes rendering them less toxic and more water soluble. Therefore, enhancement in the activity of detoxifying enzymes by chemopreventives would play an important role in blocking the cancer initiation process. The common detoxifying enzymes are glutathione-*S*-transferases (GSTs), UDP-glucuronosyl transferase (UDPGT), NADPH-quinone oxidoreductase-1 (NQO1), epoxide hydrolase (EH), glutamate cystine ligase, heme-oxygenase-1 (HO-1), γ-glutamyl transpeptidase, and so on. Unlike phase I enzymes, phase II enzymes are regulated in part, by nuclear transcription factor erythroid 2 p45-related factor2 (Nrf2), member of the cap "n" collar family of basic region leucine b-zip transcription factor via an antioxidant response element (ARE). Nrf2 under normal un-induced cellular environment is sequestered in cytoplasm by kelch-like ECH-associated protein 1 (Keap1), which in turn is bound to actin cytoskeleton. Upon activation by ARE inducers, Nrf2 dissociates from Keap1 and translocates to nucleus where it binds to ARE after heterodimerizing with other leucine zipper proteins to transcriptionally activate the downstream genes (McMahon et al., 2001).

Curcumin (250 mg/kg BW, oral, 15 days) has been demonstrated to increase the activity of GST in liver, but its effect on activity of GST in extra-hepatic tissues was not significant (Susan and Rao, 1992). Administration of dietary turmeric (5–10%, four weeks) resulted in enhancement of activity of hepatic GST and UDPGT in rats (Goud et al., 1993). Gavage administration of turmeric (4 g/kg BW) and curcumin (0.4 g/kg BW) for 14/21 days resulted in significant increase in hepatic GST and

acid soluble sulfhydryl, both in lactating mothers and pups (Singh et al., 1995). Dietary curcumin (2%, 14 days) has been shown to increase the activity of hepatic EH and GST in mice and rats with maximum induction observed in pi class of GST isozyme (Singh et al., 1998; Sharma et al., 2001). Studies using gavage administration of curcumin (1–500 mg/kg BW, 14 days) in mice have shown biphasic response. An increase in hepatic GST activity has been observed at 20–50 mg/kg dose of curcumin and decrease at higher dose (500 mg/kg) (Piper et al., 1998), while in other study curcumin (400 mg/kg) was shown to increase the hepatic GST activity (Valentine et al., 2006). Male F344 rats fed curcumin by gavage over 5 days exhibited an increase in total GST levels and activity of GST-mu in the prostate (Jones and Brooks, 2006). In C57BL/6J wild type and Nrf2 knockout mice administered curcumin (1000 mg/kg BW, single oral dose), 822 and 222 curcumin-regulated Nrf2-dependent genes were identified in the liver and small intestine, respectively (Shen et al., 2006). Consistent with these findings, dietary turmeric (1%)/curcumin (0.05%) have shown significant enhancement in the activity of GST in mouse liver and lungs (Thapliyal et al., 2002; Garg et al., 2008a) and dietary curcumin (2%) enhanced the activity of GST and quinone reductase in liver and kidney in mice (Iqbal et al., 2003). Increase in the activity of isoforms of hepatic GST has been reported with dietary curcumin (0.01%/0.05%) (Garg et al., 2008a). However, there were differences in the relative induction of isoforms studied. Additionally, curcumin-mediated induction of isoforms of GST (alpha, mu, pi) in extra-hepatic tissues, (lungs) and of another phase II enzyme, NQO1 in both liver and lungs of mice has been demonstrated. It is noteworthy that mice pretreated with dietary curcumin and subsequently challenged with B[*a*]P also showed enhanced activity of GST, its isoforms and NQO1, suggesting increased detoxification of B[*a*]P by dietary curcumin-induced phase II enzymes *in vivo*. Significant increment, mediated by dietary curcumin, in the protein and mRNA expressions of GSTs and NQO1 suggest the role of curcumin in transcriptional regulation of phase II enzymes (Garg et al., 2008a).

Oral administration of curcumin (200 mg/kg/day ×4) to rats led to enhancement in nuclear translocation and ARE binding of Nrf2, thereby also regulating the HO-1 expression (Farombi et al., 2008). In agreement with these observations, a recent study highlighted dietary curcumin-mediated increase in Nrf2 protein levels and enhancement in its nuclear translocation and DNA-binding in hepatic and pulmonary tissues of mice. Additionally, it was shown that curcumin-mediated enhanced binding of nuclear extracts from liver and lungs of mice to GST Ya-ARE paralleled the increased transcription of GSTs (Garg et al., 2008a).

In essence, curcumin treatment enhanced the Nrf2 protein levels, its nuclear accumulation, and DNA-binding to augment the transcription of phase II enzymes, resulting in increased enzyme activity, which in turn plays an important role in carcinogen detoxification (Figure 10.2).

Effects on Xenobiotics-Induced DNA Damage

Subsequent to xenobiotic exposure, metabolic processing of compounds may occur. Ultimate genotoxic forms of most carcinogens are electron-deficient in nature and form chemical adducts with nucleophilic moieties in DNA, RNA, and proteins. In addition, changes like DNA single/double strand breaks, cross linkages, depurination/depyrimidation, dimerization of pyrimidines and structural modifications of DNA bases/deoxyribose, and so on, may also result. DNA adduct levels measured at any point in time, reflect tissue-specific rates of adduct formation and removal, which depends upon carcinogen activation, DNA repair, adduct instability and tissue turnover. Associations have been observed between DNA adduct formation and mutagenesis. Furthermore, quantitative relationships between DNA adduct levels and mutagenicity/carcinogenicity has been established in a number of short-term tests, transformation assays and animal carcinogenicity studies.

Pretreatment(s) with dietary turmeric (0.1%, 0.2%, 0.5%, 1%, 3%) and curcumin (0.01%, 0.03%, 0.05%) through diet/topical (3–10 μmol)/i.p. (50–200 mg/kg BW/day) prior to administration of radiolabelled/unlabelled B[*a*]P/dimethylbenz[*a*]anthracene (DMBA) have shown significant decrease in the levels of carcinogen-derived DNA adducts in mouse skin, liver, lungs, and mammary gland of mice, rats, and hamster buccal pouch (HBP) (Huang et al., 1992; Lahiri et al., 1992;

Mukundan et al., 1993; Singletary et al., 1996; Krishnaswamy et al., 1998; Thapliyal et al., 2002; Garg et al., 2008a). Administration (i.p.) of curcumin (10–50 mg/kg BW) for five consecutive days showed marked dose-dependent decrease in nicotine-DNA adducts in liver of mice treated with nicotine (18.2 μg/kg BW, i.p., single) (Cheng et al., 2003). Dietary curcumin (2%, 14 days) has also been shown to prevent carbon tetrachloride-induced increase in the levels of malondialdehyde-derived DNA adducts in rat colon (Sharma et al., 2001). Curcumin given in drinking water to mice has also been shown to decrease oral B[*a*]P-induced DNA single strand breaks (Lahiri et al., 1992). Exposure of rats and mice to dietary turmeric (0.5%)/curcumin (0.015%) did not have significant effect on the frequency of micronucleated cells and chromosomes in bone marrow (Vijayalaxmi, 1980). Although dietary curcumin (0.2%) has been shown to prevent copper (69–390 ppm, drinking water, 48 h)-induced DNA single strand breaks and micronucleated cell frequency in mouse blood (Corona-Rivera et al., 2007), it has been observed to enhance the lipid peroxidation-induced etheno-DNA adducts in liver of LEC rats (susceptible to developing chronic hepatitis and liver tumors due to accumulation of copper and induced oxidative stress) (Nair et al., 2005). Topical curcumin treatment has been shown to decrease 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced oxidative DNA damage measured in terms of levels of 8-hydroxy-2′-deoxyguanosine (8-OH-dG) in mouse epidermis (Wei and Frenkel, 1991; Garg et al., 2008c). Turmeric (8–16 mg/kg BW, single i.p./oral 7 days) or curcumin (2–8 mg/kg BW, single i.p./oral 7 days) did not show protection against cyclophosphamide (20 mg/kg BW) or mitomycin C (1.5 mg/kg BW)-induced clastogenicity in mice (Mukhopadhyay et al., 1998).

The observed turmeric/curcumin-mediated decrease in the levels of carcinogen-derived DNA adducts could be due to inhibition of phase I enzymes/enhancement of phase II enzymes (decreased formation of DNA adducts); enhanced repair of adducts; turnover of adduct containing cells; direct scavenging of reactive species by turmeric/curcumin; or dilution of adducted DNA by newly synthesised nonadducted DNA in response to chemopreventives. Studies so far have shown curcumin-mediated inhibition of carcinogen-induced phase I enzymes, while it enhances the phase II enzymes. However, convincing evidence on other factors if responsible for decreased DNA adduct levels needs attention.

Mechanism(s) of Antipromoting Action

The tumor promotion phase of multistep carcinogenesis involves the clonal expansion of initiated cells to give rise to tumor comprised of preneoplastic cells. This stage is largely characterized by two important cellular events viz. cellular proliferation and apoptosis. Evidence suggest that in response to various extracellular stimuli, cellular kinases including PKC, PI3 Kinase, MAPKs [extracellular signal-regulated kinase (ERK), p38, c-Jun N-terminal kinase (JNK)] are activated, which in turn, regulate the transcription factors [jun, fos, nuclear factor kappa B (NF-κB)] thereby modulating the downstream effector molecules associated with various cellular responses such as cell proliferation, inflammation, differentiation, apoptosis, and so on. This therefore suggests that modulation of these signalling effector molecules to either suppress cell proliferation or to induce apoptosis would be one of the important strategy by which a potential chemopreventive could inhibit promotion/progression phase of carcinogenesis (Figure 10.3).

Effects on Cellular Kinases

Curcumin has been shown to suppress MAPKs *in vitro* (Strimpakos and Sharma, 2008). Protein kinase C, the major intracellular receptor for tumor-promoting phorbol esters, for example, TPA, plays a crucial role in modulation of target proteins associated with inflammation, cell proliferation, differentiation, survival, apoptosis, invasion, epidermal tumor formation, and malignant progression. PKCs have also been implicated in the promotion/progression phase of carcinogenesis. A recent study demonstrated the abrogation of TPA-induced cell proliferation (decreased hyperplasia and expression of cyclin D1), inflammation [decreased levels of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2)], oxidative damage (levels of 8-OH-dG) but increased apoptosis (increased

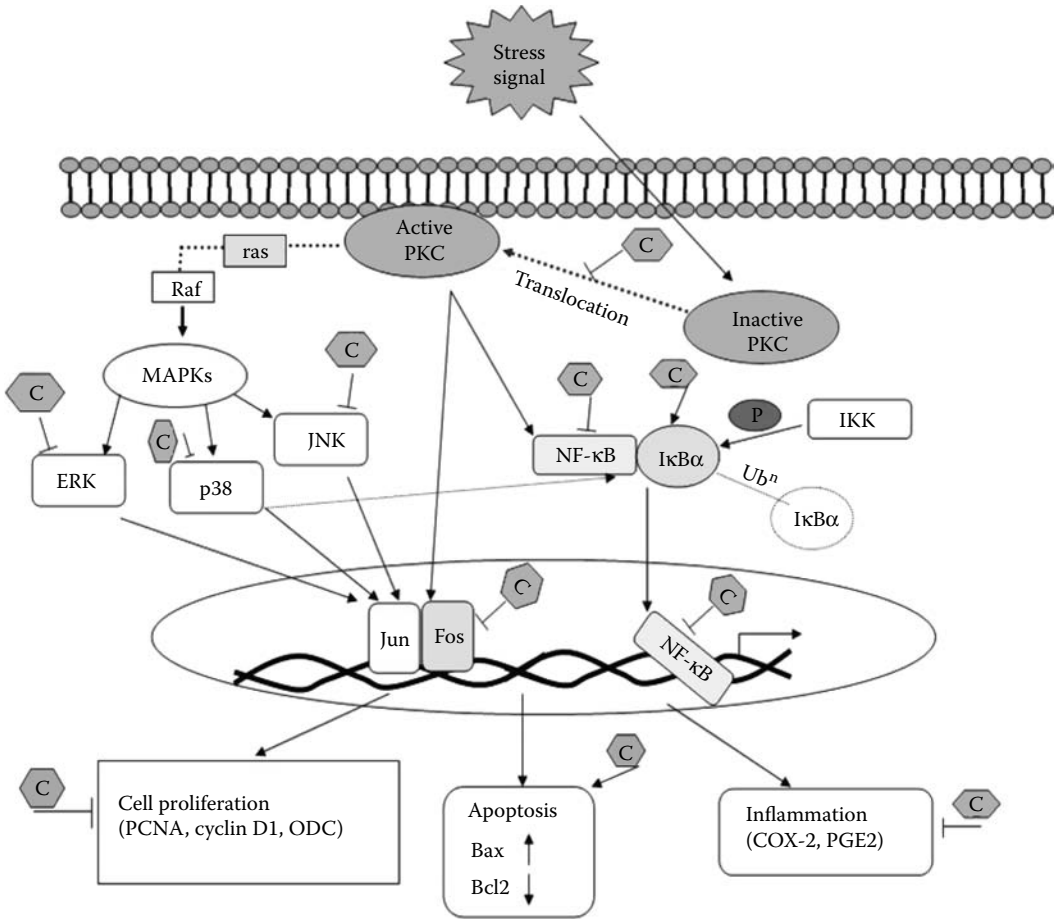

FIGURE 10.3 Schematic presentation of possible steps at which curcumin exhibits antipromoting effects in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-treated mouse skin. COX-2 = cyclooxygenase 2; ERK = extracellular signal-regulated kinase; IκBα = inhibitor of NF-κB alpha; IKK = IκB kinase; JNK = c-Jun N-terminal kinase; MAPKs = mitogen activated protein kinases; NF-κB = nuclear factor kappa B; ODC = ornithine decarboxylase; PCNA = proliferating cell nuclear antigen; p38 = p38 protein kinase; PGE2 = prostaglandin E2; PKC = protein kinase C; ub^n = ubiquination.

ratio of Bax/Bcl2) in mouse skin upon pretreatment with a specific PKC inhibitor, Ro-31-8220 (Garg et al., 2008c). These *in vivo* observations thus demonstrate the crucial involvement of PKC in TPA-induced tumor promotion events. In the same study, topical pretreatment of curcumin (10 μmol) to mouse skin resulted in a decrease in TPA-induced translocation of protein of PKC isozymes (alpha, beta, gamma, epsilon, eta) from cytosol to membrane. However, curcumin alone did not influence the PKC activity as well as the protein levels in either compartment. Furthermore, in mouse skin curcumin has been shown to inhibit the catalytic activities of ERK and p38 MAPK, which are upstream of NF-κB (Chun et al., 2003). Pretreatment of mouse skin with curcumin has been shown to decrease the TPA-induced phosphorylation of MAPKs (ERK, p38, JNK) (Garg et al., 2008c). Sequential evaluation of dietary turmeric (1%)-mediated chemoprevention in DMBA-induced HBP tumorigenesis showed significant decrease in DMBA-induced levels of p-ERK and p-p38 during tumor development (2–12 weeks), while expressions of total ERK and p38 were not altered in any of the treatment groups throughout the observation period (Garg et al., 2008b). These findings thus suggest that curcumin modulates cellular kinases *in vivo* to exert its chemo-protective effects.

Effects on Transcription Factors and Oncogenes

Transcription factors are known to participate in the control of genes that regulate various cellular responses including cell proliferation, differentiation, inflammation, cell survival, cell death, angiogenesis, and so on. The activator protein-1 (AP-1) family of transcription factor is composed of homo- and hetero-dimers of the Fos (c-Fos, FosB, Fra-1, Fra-2) and Jun (c-Jun, JunB, JunD) families. After dimerization, they bind to TPA-responsive elements (TRE) in the promoter and enhancer region of target genes that have been shown to be important mediators in oncogenic transformation. Likewise, Rel/NF-κB family of transcription factors is involved in many cellular functions. In an inactive state, NF-κB remains sequestered in the cytoplasm by an inhibitory protein, IκBα. Upon stimulation by agents such as cytokines, phorbol esters, mitogens, bacterial products, oxidative stress, ultraviolet light, and so on, IκB kinases (IKKs) mediate the phosphorylation of IκBα, leading to its proteosomal degradation, and facilitating the release of NF-κB into the nucleus (Aggarwal et al., 2003).

Topical application of curcumin to mouse skin prior to TPA treatment has been shown to inhibit the TPA-induced mRNA expression of proto-oncogenes (c-jun, c-fos, c-myc) (Kakar and Roy, 1994). Studies have also shown curcumin to inhibit the TPA-induced protein levels of c-jun, c-fos and NF-κB in mouse epidermis (Lu et al., 1994; Chun et al., 2003). Furthermore, curcumin pretreatment blunted the TPA-mediated increase in p-IκBα protein expression, which in turn accounted for the decrease in TPA-mediated degradation of IκBα protein, and decrease in nuclear translocation of NF-κB p65 subunit (Chun et al., 2003; Garg et al., 2008c). Besides, curcumin pretreatment abrogated the TPA-induced AP-1 and NF-κB DNA-binding ability in nuclear extracts obtained from mouse epidermis (Garg et al., 2008c). Sequential overexpression of c-Ha-ras gene has been demonstrated in DMBA-induced HBP carcinogenesis. Dietary turmeric (1%) decreased the DMBA-induced p21ras expression during tumor development, while turmeric diet alone did not affect the p21ras expression throughout the experimental period (Garg et al., 2008b). Similar decrease in p21 protein has also been observed in DMBA-initiated and TPA-promoted mouse skin from animals receiving dietary curcumin (0.2–1.0%) (Limtrakul et al., 2001).

Applications of DMBA to HBP increased the m-RNA expression of c-jun, c-fos, and NF-κB when compared with vehicle-treated animals. Although turmeric diet (1%) alone did not alter the basal levels, it significantly decreased the DMBA-mediated increase in c-jun, c-fos, and NF-κB m-RNA. Similarly, significant and sequential increase was observed in the nuclear protein levels of c-jun, c-fos, and NF-κB p65 in DMBA-treated buccal pouches during tumor development, while significant decrease in these parameters has been demonstrated upon turmeric treatment. In contrast, the levels of cytosolic NF-κB inhibitory protein, IκBα progressively declined in DMBA-treated buccal pouches and dietary turmeric significantly protected against DMBA-mediated decreases in IκBα expression demonstrating agreement with the observed decrease in DMBA-induced NF-κB p65 nuclear accumulation. Moreover, dietary turmeric attenuated the DMBA-induced DNA-binding ability of AP-1 and NF-κB in nuclear extracts from HBP (Garg et al., 2008b). Overall results suggested that dietary turmeric decreased the DMBA-induced activation of ras protein product p21 and transcription factors, AP-1 and NF-κB during HBP tumorigenesis (Figure 10.4). Together, these studies suggest that curcumin decreased the TPA-induced activation of cellular kinases (PKC, MAPKs) and transcription factors (AP-1, NF-κB) that modulate the cellular pathways associated with cell proliferation, apoptosis, inflammation, and differentiation in TPA-induced tumor promotion events (Figure 10.4).

Effects on Cellular Response Markers

Cell Proliferation

Uncontrolled cell proliferation is a hallmark of malignancy and may arise from stepwise increase in mitogenic stimuli that are otherwise involved in normal cell growth. Histopathological observations showed that although topical application of curcumin alone did not induce any epidermal hyperplasia in mouse skin, similar pretreatment with curcumin markedly reduced the TPA-induced

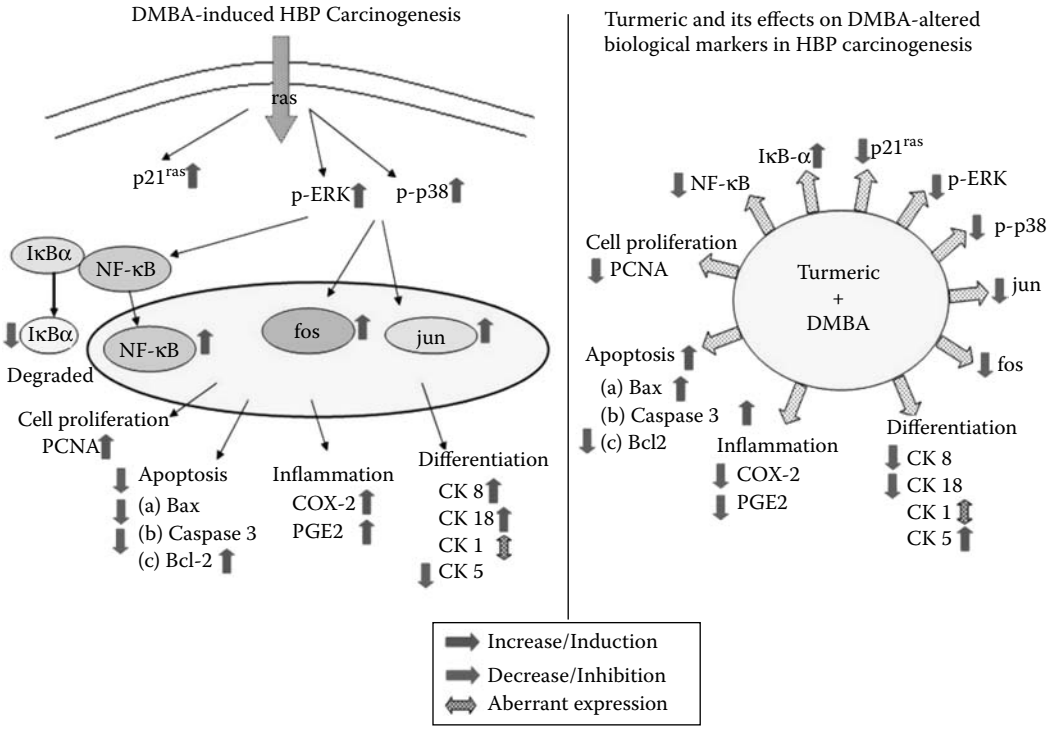

FIGURE 10.4 Summary of modulatory effects of dietary turmeric on DMBA-induced oncoproteins and associated cellular response markers in hamster buccal pouch (HBP) carcinogenesis. COX-2 = cyclooxygenase 2; CK = cytokeratin; DMBA = 7,12-di-methylbenz[*a*]anthracene; IκBα = inhibitor of NF-κB alpha; NF-κB = nuclear factor kappa B; PCNA = proliferating cell nuclear antigen; p-p38 = phosphorylated p38 protein kinase; p-ERK = phosphorylated extracellular signal-regulated kinase; PGE2 = prostaglandin E2.

hyperplasia. This has further been complemented with immunoblot analyses of cell proliferation marker, cyclin D1 wherein curcumin pretreatment has been shown to decrease the levels of TPA-induced cyclin D1 in mouse epidermis (Garg et al., 2008c). Similarly, DMBA-induced hyperplasia/dysplastic changes were deferred in hamsters receiving pretreatment and concurrent treatment with dietary turmeric. Buccal pouches of DMBA-treated animals showed a significant and sequential increase in the protein levels of proliferating cell nuclear antigen (PCNA) during tumorigenesis. Dietary turmeric significantly decreased DMBA-induced PCNA levels in HBP measured by Western blotting and immunohistochemical analysis (Garg et al., 2008b).

Ornithine decarboxylase (ODC) is the rate-controlling enzyme in polyamine biosynthesis, and has been associated with tumorigenesis process. Curcumin has been shown to inhibit TPA-induced ODC activity as well as protein levels in mouse epidermis (Huang et al., 1997; Garg et al., 2008c). Decrease in TPA-induced expression of ODC has also been observed in curcumin pretreated (5–25 μmol, topical) mouse skin through suppression of MAPKs and NF-KB (Ishizaki et al., 1996). Dietary curcumin (2%) has also been shown to decrease ferric nitrilotriacetate-induced ODC, oxidative damage and modulated the glutathione and antioxidant enzymes in mouse kidney (Okazaki et al., 2005). Dietary turmeric modulated DMBA-mediated aberrant expressions of cytokeratins known to be associated with cell proliferation/differentiation (Garg et al., 2008b).

Inflammation

Chronic inflammation is closely linked to tumor promotion and substances with anti-inflammatory activities are anticipated to exert chemopreventive effects particularly during the promotion stage. COX-2 and inducible nitric oxide synthase are important enzymes that mediate inflammatory

processes and are upregulated during tumorigenesis. Curcumin has been shown to inhibit COX-2 expression in transformed cells or other cell lines upon exposure to various extracellular stimuli *in vitro* (Zhang et al., 1999). Topical application of curcumin has been reported to markedly reduce TPA- and arachidonic acid-induced epidermal inflammation (ear edema) in mice (Huang et al., 1988). Furthermore, curcumin when applied topically prior to TPA led to significant decrease in TPA-induced COX-2 protein in mouse epidermis (Ishizaki et al., 1996; Chun et al., 2003; Garg et al., 2008c). It was also shown that curcumin inhibited TPA-induced expression of COX-2 through suppression of ERK activity and NF-κB activation (Chun et al., 2003). Recent observations have shown that curcumin could significantly decrease the TPA-induced COX-2 and PGE2 levels via a PKC-dependent pathway (Garg et al., 2008c). Dietary turmeric decreased the levels of DMBA-induced COX-2 and PGE2 during HBP tumorigenesis (Garg et al., 2008b). Overall, curcumin decreases carcinogen/promoter-induced inflammation and oxidative stress which have been closely linked to promotion phase of carcinogenesis.

Apoptosis

Apoptosis facilitates the elimination of damaged cells under physiological and pathological situations. It has been well documented that p53 protein increases during apoptosis induced by DNA-damaging agents thereby participating in the elimination of aberrant cells and/or preventing the replication of damaged DNA (Levine, 1997). It is to note that all of these investigations with curcumin have been made in immortalized or transformed cells *in vitro*; however, information on its effects on malignant and nonmalignant cells in animal models *in vivo* is limited. It has been observed that diet containing 2000 ppm curcumin administered to rats treated with azoxymethane (15 mg/kg BW, 1×/week – 2 weeks) during initiation and postinitiation significantly increased apoptotic index (17.7%) in colonic tumors as compared with those in the control diet (8.3%) (Samaha et al., 1997). Curcumin enhanced 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced apoptosis (4% curcumin + PhIP) when compared with PhIP alone (2.1%) and inhibited PhIP-induced intestinal tumorigenesis in Apc min mice (Collett et al., 2001). In a recent study with Swiss mice administered B[*a*]P (1 mg/mouse, single gavage), posttreatment with dietary curcumin (0.05%) resulted in enhancement in the disappearance of benzo[*a*]pyrene diol epoxide (BPDE)-DNA containing stained nuclei and B[*a*]P-induced apoptosis related parameters in liver and lungs (Garg and Maru, 2009). In another study, pretreatment with curcumin has been shown to abrogate the TPA-induced expression of antiapoptotic protein, Bcl2, and increase the expression of Bax, pro-apoptotic marker, in mouse skin. Curcumin-mediated inhibition of TPA-induced antiapoptotic response was also reflected in increased Bax/Bcl2 ratio (Garg et al., 2008c). Sequential increment in DMBA-induced Bax and caspase-3, and sequential diminution of DMBA-induced levels of Bcl2 upon turmeric treatment in HBP has been reported (Garg et al., 2008b). This in turn accounts for the turmeric-mediated progressive increase in Bax/Bcl2 ratio in DMBA-treated hamsters. Bax/Bcl2 ratio has been documented to be an important factor determining the fate of the cell toward death or survival. Taken together, the observations suggest that chemopreventive interventions offered during early stage is likely to be effective in decreasing carcinogen-induced cell proliferation while augmenting the probability of initiated cell getting apoptosized.

Evaluation of some of the above-referred biomarkers is likely to be helpful in monitoring clinical trials with curcumin and evaluating the drug effect measurements.

Mechanism(s) of Antiprogressor Activity

Experimental studies covering antiangiogenic, anti-invasive, and antimetastatic effects of curcumin *in vivo* have been summarized in Table 10.2. Curcumin has been shown to inhibit the growth of transplantable tumors in different animal models and increase the life span of tumor-bearing animals.

Angiogenesis, the growth of new capillary blood vessels, is crucial for tumor growth and expansion. Curcumin has been shown to affect angiogenesis by downregulating transcription factors such as NF-κB, pro-angiogenic factors such as vascular endothelial growth factor (VEGF), beta fibroblast growth factor (βFGF) and COX-2; decreasing cell adhesion molecules and extracellular proteolysis

and inhibiting cell motility, endothelial cell migration and invasion (see references in Table 10.2). As previously mentioned, curcumin also exhibits antiproliferative and pro-apoptotic effects on tumor cells. All these observations *in vivo* suggest antiangiogenic activity of curcumin.

Metastasis is the process by which cancer cells migrate from the tissue of origin to distant sites to form new malignant lesions in other organs. During metastasis, the invasive tumor cells might have to penetrate the basement membrane. A group of proteolytic enzymes, namely matrix metalloproteinases (MMPs), play a key role in cancer invasion and metastasis. Curcumin inhibits the metastasis of tumor cells *in vivo* and the possible mechanism is through the inhibition of MMPs. As shown earlier, curcumin suppresses the expression of COX-2, VEGF, and intercellular adhesion molecule-1 (ICAM-1), and elevates the expression of antimetastatic proteins, the tissue inhibitor of metalloproteases-2 (TIMP-2), nonmetastatic gene 23, and E-cadherin. In addition, curcumin also acts as an immunomodulator. It augments the natural killer cell activity and inhibits the production of cytokines (see references in Table 10.2).

SUMMARY AND CONCLUSIONS

The mechanisms implicated in the inhibition of tumorigenesis by curcumin are summarized (Figure 10.5) and involve modulation of signaling kinases or xenobiotic-induced activation/translocation of kinases or modulation of tumor-induced responses ultimately leading to effects on genes and cell signalling pathways at multiple levels. Considering the reported biological effects of

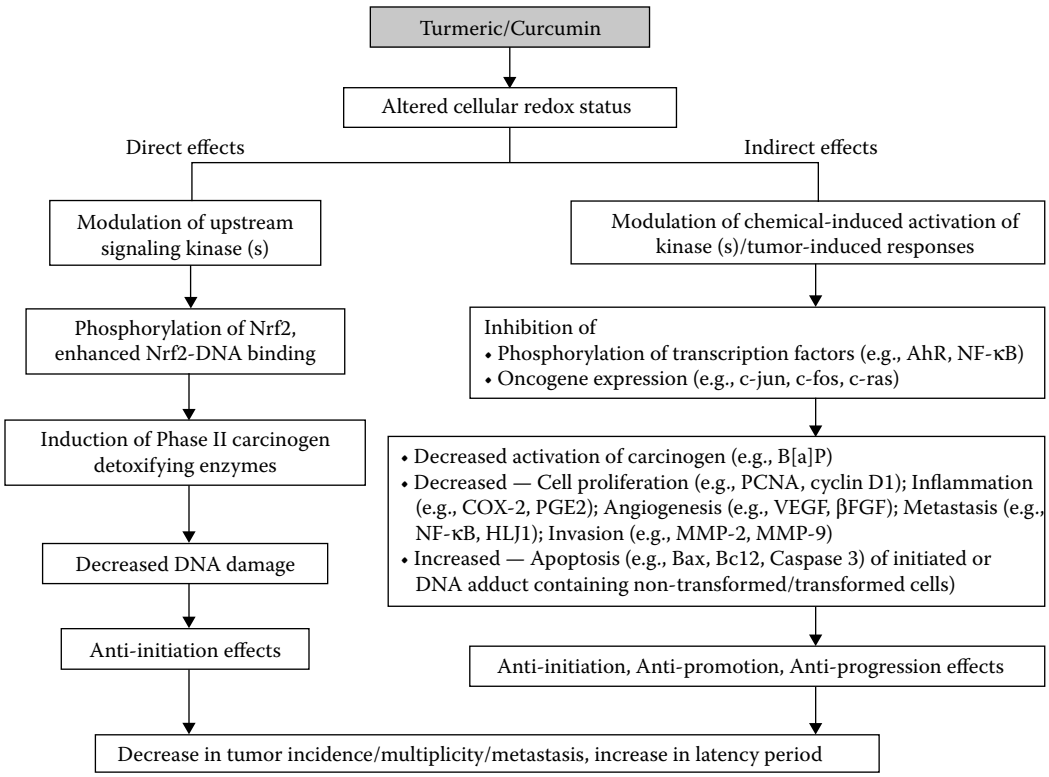

FIGURE 10.5 Summary of *in vivo* chemopreventive effects of turmeric/curcumin and underlying mechanisms. AhR = aryl hydrocarbon receptor; B[*a*]P = benzo[*a*]pyrene; βFGF = beta fibroblast growth factor; HLJ1 = heat shock protein 40; MMP = matrix metalloproteinases; Nrf2 = nuclear transcription factor erythroid 2 p45-related factor2; NF-κB = nuclear factor kappa B; PCNA = proliferating cell nuclear antigen; VEGF = vascular endothelial growth factor.

both turmeric and curcumin *in vivo* and the potential for the development of modern medicine, further investigations in well-designed experimental and human intervention studies are needed. On-going studies are likely to help in determining the usefulness of curcumin in prevention and therapy of specific diseases including cancer.

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11 Prunes and Plums in Health Promotion

Felina Marie Cordova and Ronald Ross Watson

CONTENTS

INTRODUCTION

The plum fruit has been grown specifically for consumption for over 2000 years, in many diverse regions worldwide and has a wide range of physical characteristics. Plum (*Prunus domestica* L.) and its dried counterpart—prune—are fruits that can create many health-related benefits when ingested. Plums are known to have been utilized in the past and the present for their ability to alleviate many medical conditions. Due to their high fiber and antioxidant contents, plums and prunes are being further researched for their ability to prevent and treat medical conditions such as cardiac related problems, osteoporosis, and cancer.

BOTANICAL DATA

Plums of and descending from *Prunus domestica* L. and also the Japanese plum (Prunus *salicina*) belong to the family Rosacaseae and are in the genus Prunus. Plums are found in numerous cultivars, some that are naturally occurring and some that are grown for human consumption as well as for laboratory research. The number of varieties of plums reported varies; some researchers list approximately 200 while others have listed as high as between 1000 and 2000 (LaRue and Johnson, 1989). With so many different cultivars CDC reports that consumers in the United States have more than 140 different plums to choose from when purchasing (CDC, 2009). New types of plums still continue to be created with researchers looking for ways to genetically engineer plums to be stone and seedless (Durham, 2009).

ORIGIN AND DISTRIBUTION

The fruit now known as *Prunus domestica* L. was created in the western region of Asia as the result of the crossbreeding of other antiquated fruits and then later appearing in the European countries

and then further west (Stacewicz-Sapuntzakis et al., 2001; Kayano et al., 2003). The genetic combination that brought the modern plum into existence was through the fruits *Prunus cerasifera* (cherry plum) and *Prunus spinosa* L. (blackthorn) (Stacewicz-Sapuntzakis et al., 2001). The Japanese plum is thought to have also originated in Asia–China specifically and the modern version was brought into existence through breeding of *P. salicina*, *P. simonii*, and a species that was indigenous to the continent of North America (Harmann and Neumuller, 2009). Today, plums are known to grow in many countries, but it is in the region of Asia where most are produced, followed by Europe and North America (Harmann and Neumuller, 2009). As for prunes, it is in the state of California in the United States where more than half of all prunes are produced for consumption by the entire world (Stacewicz-Sapuntzakis et al., 2001). In the United States, the majority of plums and prune consumers are infants and the older populations (Stacewicz-Sapuntzakiset et al., 2001). In 2005 there were 9,843,000 tons of plums produced for consumption (Harmann and Neumuller, 2009). Of all the fruits produced in the world for consumption, plums are the 10th most grown and shipped fruit (Ramming and Cociu, 1991).

DESCRIPTION AND HABITAT

The exact phenotype of plums depends upon the species being looked at. Plums run a wide gambit in terms of their shape (uniform and spherical to nonuniform spheroid) and size (small, medium, or large) (Ensminger and Konlande, 1993). Plums larger than 50 g are recommended for consumption as fresh fruit and smaller plums (less than 40 g) are recommended for being processed into its components for use in the food industry or into prunes (Harmann and Neumuller, 2009). The skin of plums has been shown to vary from being very dark (blackish) in color to lighter colors (yellow) (Harmann and Neumuller, 2009). The fruit, in addition to having flesh color, comes also commonly in red, yellow, orange and white (Harmann and Neumuller, 2009). As for the taste of plums they range from being bitter to sweet (Harmann and Neumuller, 2009). Prunes, the dried version of plums, have been found to be sweeter in taste than most plums (Stacewicz-Sapuntzakis, 2001).

Plums are grown in many countries of the world, including the Untied States of America, France, Australia, Africa, Turkey, Italy, Japan, China, Argentina, Bosnia, Chile, Korea, Germany, Russia, Spain, Iran, Poland, Russia, Ukraine, but they are native to locations where there are no extremes of cold or hot temperatures (LaRue and Johnson, 1989; Stacewicz-Sapuntzakist et al., 2001; Harmann and Neumuller, 2009). Due to the wide varieties of environments that plums grow in, there is no set soil composition required, but high calcium levels in the soil are important in plum ripening (Rato et al., 2008). To adapt to local soil and growing conditions some plum cultivars are grafted onto rootstocks (Ramming and Cociu, 1991).

HISTORY, FOOD USES, AND NUTRITIONAL FACTS

Plums have been grown for more than 2000 years, but were introduced into the United States through early inhabitants during the settling period of the 1800s. It is believed that the modern prunes that are produced come from the introduction of a European plum into the United States back in the 1850s (LaRue and Johnson, 1989; Stacewicz-Sapuntzakis et al., 2001). The Japanese plum (*Prunus salicina*) has its origins in China and arrived in the United States during the 1800s where it was called the "Kelsey" plum during its production (Harmann and Neumuller, 2009). In modern times, the Japanese plum is the preferred variety to be used sold to consumers as a whole fruit, while the European plum is used to be broken into its constituents for food products and to be made into prunes (Harmann and Neumuller, 2009).

Plums have a wide variety of uses in food products. Prune juice is used in its pure form or as an additive to other types of juices, puree, jelly/jam, paste, powder, canning, and so on (LaRue and Johnson, 1989; Stacewicz-Sapuntzakis et al., 2001). Due to its sugar content, sorbitol, plums are commonly used in baking products to keep baked products moist (Stacewicz-Sapuntzakis et al.,

2001). Plums can also be found as additives in meat products (Stacewicz-Sapuntzakis et al., 2001). Plum and its extracts are also important in making liquor and, in particular, various types of brandies in Europe (Harmann and Neumuller, 2009).

Plums are made up mostly of flesh. As for nutrient composition, plums in both its fresh and dried forms contain different levels of water, carbohydrates, sugars, protein, fat, fiber, amino acids, various minerals, potassium, boron, vitamins C, K, B1, and B6, and niacin (Stacewicz-Sapuntzakis et al., 2001; Harmann and Neumuller, 2009; Franklin et al., 2006). Although nutrition values vary according to plum varieties, as per the Consumer Nutrition Center, 100 g of plums have higher amounts than prunes of water (85.20 g vs. 32.39 g), fat (0.62 vs. 0.52) and absorbic acid (9.5 mg vs. 3.3 mg) (Ramming and Cociu, 1991). Prunes have higher content than plums of the majority of nutrient constituents: calories (239 cal vs. 55 cal), protein (2.61 vs. 0.79), carbohydrates (62.73 g vs. 13.01 g), fiber (2.04 vs. 0.60) (Ramming and Cociu, 1991). Prunes also have higher proportions of all minerals: calcium (51 mg vs. 4 mg), iron (2.48 mg vs. 0.10 mg), potassium (745 vs. 172), magnesium (45 vs. 7), zinc (0.53 vs. 0.10) and sodium (4 mg vs. 0 mg) (Ramming and Cociu, 1991). Additionally, prunes have higher vitamin levels than plums: vitamin A (199 RE vs. 132 RE), thiamin (0.081 mg vs. 0.043 mg), riboflavin (0.162 mg vs. 0.096 mg), niacin (1.961 vs. 0.500), panthothenic acid (0.460 mg vs. 0.182 mg), vitamin B6 (0.264 mg vs. 0.081 mg), and folacin (3.7 μg vs. 2.2 μg) (Ramming and Cociu, 1991).

Traditional Medicine Uses

Prunes have been and continue to be used as a natural laxative for constipation. In literature, this usage can be traced to AD 1345 among the French (Piirainen et al., 2007). Prunes were also used in medieval times in the treatment for mouth ulcers (Stacewicz-Sapuntzakis et al., 2001). In India, prunes have been used to help regulate female menstrual cycles as well as to aid in recovery after a miscarriage (Kayano et al., 2003). The medicinal effects of the prune are attributed to its high fiber content, sugars, and phenols which aid in bowel movement (Lucas et al., 2004; Piirainen et al., 2007).

Constituents

Plums of both the dried and fresh variety contain phenols which act as antioxidants. Dried plums contain 394 mg more of free phenols and 560 mg more total phenols than fresh plums (Vinsone et al., 2005). Due to the large amount of plum varieties the exact number of phenols will vary by type of plum. Factors that can alter the amount of phenols are soil composition, season, age picked at, as well as methods of storage after being picked, and for prunes the method of drying (Kim et al., 2008; Yu et al., 2009). The polyphenols levels in plums/prunes affect the color of the fruits as well as their taste (Kim et al., 2003a). Plums with higher concentrations of phenols can be identified visually as those having a red or purple appearance, as was found through analysis of 53 plum types (Byrne et al., 2004). Carotenoids are also found in fresh plums in their peel at low levels of 83–231 μg (Gil et al., 2002). Absorbic acid at high levels is also a component of plums at 27.4–61.1 mg (Gil et al., 2002). Chlorogenic acid is also present in plums as well as flavonoids such as quercetin-3-rutinoside (Fang and Prior, 2002; Chun et al., 2003a). Other constituents of plums are hydroxycinnamic acid and anthocyanin (Yu et al., 2009).

Pharmacological Properties

All of the positive health effects of plums and prunes are attributed to their high ability to eliminate free radicals through their antioxidant activity that is higher than all other fruits and vegetables (Piga et al., 2003; Kim et al., 2003b). Plum and prune research is diversified in the field of medicine. Research has shown the positive effects of the fruit in reducing cardiac conditions through animal

models. Gallaher and Gallaher (2009) have shown prune powder to be effective in reducing atherosclerosis lesions in mice when it comprised 4.75% of mice diet (Gallaher and Gallaher, 2009). In this study, lower plasma cholesterol at 15 weeks (22.7 mmol/L) vs. 4 weeks (25.1 mmol/L) was observed when prune powder comprised 9.5% of the diet. These findings are believed to be due to the antioxidants in the plum in the form of polyphenols (Gallaher and Gallaher, 2009). In addition, its antioxidants can reduce the unhealthy form of cholesterol known as low density lipoprotein (LDL) and therefore improve cholesterol-related cardiac complications (Chun et al., 2003b). A study by Tinker et al. (1991) also confirms these findings as human participants with hypocholesterolemia that consumed 100 g prunes per day in their study experienced statistically significant lower LDL levels than controls (309 mmol/L vs. 4.1 mmol/L).

Prunes have also been shown to be a future natural treatment to stop bone loss in patients with osteoporosis. In laboratory research working with a rat model, prunes were able to increase bone levels in numerous studies with one study showing an 11% increase in bone density (Deyhim et al., 2005; Bu et al., 2007). In a recent *in vitro* study looking at polyphenol extracts from dried prunes and their effects on osteoporosis it was found that osteoclasts were inhibited as well as inflammation and TNF-α secretion which could lead to less bone deterioration (Bu et al., 2008). For human studies, one study of postmenopausal females saw a 12% increase in bone formation, while another study indicated elevated levels of important bone building components (bone-specific alkaline phosphatase and insulin growth factor-1) when participants of both studies consumed dried plums (Arjmandi et al., 2002; Hooshmand and Arjmandi, 2008).

In addition to improving the condition of osteoporosis, plums can also be utilized for the prevention and treatment of cancer. Plums that have not completely matured have been recognized as being able to inhibit the activity of proteins that are implicated in the tumor progression process (Yu et al., 2009). Such is the case for the metalloproteinase-9 (MMP-9) whose activity has been shown to be inhibited by immature plums in an *in vitro* cellular model (Yu et al., 2009). Studies have also been conducted to show the effects of dried plums on colon cancer prevention. In a rat model it was found that dried plums at a low diet concentration of 4.75% reduced the risk of colon cancer by decreasing fecal bile acids (Yang snd Gallaher, 2005). In human males the same effect has been observed with lower fecal bile acids (0.95 mg vs. 1.20 mg) being produced in the experimental group consuming 100 g of prunes a day over 4 weeks vs. 360 mL of grape juice consumed each day by controls (Tinker et al., 1991).

Safety Profile

No cases of continuous intake of prunes causing toxicity or safety issues have been found in literature. In addition, when increasing plum and prune consumption daily for 3 months no reports of major problems have been found (Lucas et al., 2004). With prunes being ingested up to 12 per day, no negative effects related to bowel problems are seen even at this level in both men and women (Tinker et al., 1991; Lucas et al., 2004). The only type of plum that has been identified theoretically as posing a health safety problem is the red fresh plum. Problems could occur in those with asthma if they ingest more than 15 mg of salicytes which does not appear to be a direct problem, as the red plum contains only 0.11 mg/100 g of salicytes (Perry et al., 1996).

CONCLUSIONS

The plum comes in various shapes, sizes, skin colors, flesh colors, and tastes. Since their introduction and cultivation, plums, prunes, and their products have been beneficial to the health of humans. From ancient peoples using prunes to aid in digestion and menstruation process to modern times, scientific research has illustrated heart, bone, and oncological benefits. Plums and prunes not only improve health and fight diseases but do so with little to no risk to humans. Their positive health effects have been attributed in large part to their high antioxidant levels.

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12 Cancer Preventive Phytochemicals from Southeast Asian Countries

In Vivo Activities and Underlying Molecular Mechanisms

Akira Murakami

CONTENTS

INTRODUCTION

According to a recent report published by the World Health Organization, more than 11 million people are diagnosed with cancer and 7 million die from the disease every year, making cancer one of the leading causes of death among humans worldwide (Stewart and Kleihues, 2003). More than 10 years are required for dormant tumor cells to become malignant tissues, which are then detected and diagnosed in clinical specimens. It has been well established by experimental data that the process of malignant cell conversion from normal cells has multiple stages, which involve genetic and epigenetic changes. In addition to inherited and acquired gene mutations, hormonal status, age, sex, and immune conditions of a given individual are all critical factors for cancer development.

Prevention is the ideal means to reduce the death rates of most, if not all, lifestyle-related diseases including oncogenesis. In particular, dietary compounds have some advantages in terms of their high availability from natural sources, and relatively low toxicity and side effects, as long as their administration dosages are within daily limits. In addition, recent clinical trials of cancer prevention with food phytochemicals have demonstrated their marked potentials. For example, Stoner et al. (2008) recently published an excellent review article on the effectiveness of a berry

preparation toward cancer prevention. Berries contains several classes of bioactive polyphenols, including anthocyanins, flavonols, flavanols, proanthocyanidins, hydroxycinnamic and hydroxybenzoic acids, stilbenoids, and ellagitannins. In a clinical trial, 23 subjects with colorectal cancer and/or polyps consumed 20 g of freeze-dried lyophilized black raspberry powder three times per day (60 g total), usually for two to four weeks, which led to a reduction in proliferation and angiogenesis biomarkers, with apoptosis enhancement also noted in their biopsy specimens. In support of these findings, the effects of topical applications of 10% black raspberry gel to oral intraepithelial neoplastic lesions in 17 patients and normal tissues in 10 patients, four times per day for six weeks, were assessed, with significant attenuation of the expression pro-inflammatory genes confirmed by the results.

Goel et al. (2008) presented extensive evidence showing that curcumin, the major yellow pigment in turmeric, is one of the most advanced phytochemicals for clinical use. One of the great advantages of this compound is its "non-toxic or extremely less toxic" characteristic, as a single administration of a standardized powder extract of uniformly milled curcumin to 24 healthy volunteers (ranging from 500 to 12,000 mg) caused minimal, non-dose-related toxicity in only 7 subjects. In addition, a number of laboratories have shown that curcumin can modulate biomarkers of colorectal cancer and polyp formation in familial adenomatous polyposis patients, in whom combination treatment with curcumin (480 mg) and quercetin (20 mg) orally three times a day for six months significantly decreased polyp number and size without producing any noticeable side effects.

The above-mentioned successful examples have encouraged scientists engaged in this field of research to further explore other candidate compounds. Although natural products have great potential as cancer chemopreventive agents for clinical trials, their precise mechanisms of action remain to be fully elucidated. In contrast, synthetic agents are designed to act on specific molecular targets, though their safety should be intensively assessed using various models. In addition, they have serious disadvantages in terms of availability, because the synthetic steps are often numerous and complicated. Of note, plants are known to have the potential to biosynthesize a number of bioactive phytochemicals. In addition, untold numbers of synthetic drugs with optimized pharmacological activities and safety have been developed by focusing on such natural chemicals for their precursors and/or key materials.

Interestingly, phytochemicals, secondary metabolites in particular, are biosynthesized when plants are exposed to environmental stress stimuli such as ultraviolet (UV) exposure, invading insects, and microbes (Figure 12.1). These responses are apparently related to their survival. For instance, antioxidants are produced to quench or scavenge free radicals derived from UV light exposure. Also, there is evidence that plants are known to have certain chemicals called phytoalexins, which are considered to be "chemical weapons" against harmful microorganisms such as fungi and bacteria. In addition to the physical barrier provided by the cell wall of plants, such chemicals are produced in both constitutive and inducible manners by sensing the proteins and nucleic acids specific to microbes (e.g., toll-like receptors and CpG). Based on these self-protective mechanisms, we have hypothesized that plants exposed to harsher stress produce more biologically active phytochemicals in terms of quality and quantity. On the other hand, plants produce these secondary metabolites for their own protection, not for humans, and such chemicals are basically biosynthesized for maintaining homeostasis and providing protection from environmental stress. Nonetheless, it has been established that certain plant extracts have noted potentials for cancer prevention, and it may be possible to isolate and identify the active constituents that are functionally novel and have marked cancer preventive activity.

This chapter describes the results of screening tests of unique extracts from vegetables and fruits obtained from Southeast Asian countries, which demonstrated the chemopreventive efficacy of some of their active principles, as observed in rodent carcinogenesis models, and also discusses their plausible action mechanisms.

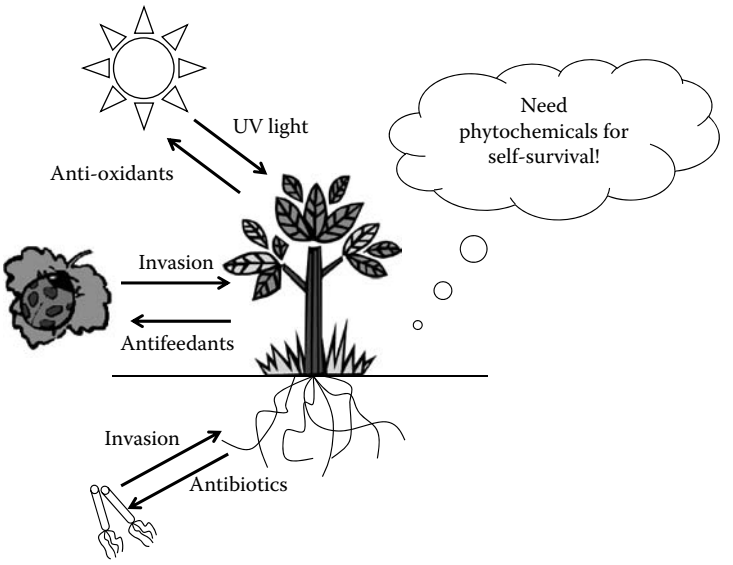

FIGURE 12.1 Possible roles of phytochemicals in plants.

SCREENING TESTS FOR CANDIDATE PHYTOCHEMICALS FOR CANCER PREVENTION

In vitro assay systems provide rapid and reliable means for identifying cancer preventive compounds by screening a wide range of pure, natural, and synthetic compounds that have biological and biochemical relevance to human carcinogenesis. Alternatively, a natural product chemistry approach is also useful for discovering structurally and functionally novel compounds. In that process, bioactive crude extracts are subjected to activity-guided separation using column chromatography and the purified compounds are identified by spectroscopic methods, including nuclear magnetic resonance and mass spectrometry.

The phorbol ester class of tumor promoters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) have been used in a great number of studies that attempted to identify novel candidates for cancer prevention. For instance, TPA is known to activate oncogenic Epstein–Barr virus in several B-lymphoblastoid cell lines. Our laboratory has conducted extensive screening tests of 500 or more domestic vegetable and fruit extracts, as well as those from Southeast Asian countries such as Thailand, Indonesia, and Malaysia (Murakami and Ohigashi, 2007a). Of interest, the *in vitro* antitumor promoting activities found in those subtropical plants were markedly and significantly higher than those in plants native to Japan in terms of the frequency of bioactive components and activity potency. Their chemopreventive potency and underlying mechanisms related to the activities of their active principles will be discussed in later sections.

Following studies on cancer preventive phytochemicals from Southeast Asian countries, our attention was directed to those native to Okinawa prefecture, Japan, which is known as a distinct region where unique and traditional food habits are maintained, and average life expectancy is extended. To assess the biological activities of Okinawan natural sources, we used a suppressive assay of free radical generation from stimulated leukocytes. In addition to exogenous oxidative stress (UV radiation, heavy metals, lipid peroxides from foods, etc.), endogenous enzymatic systems have been shown to be highly associated with carcinogenesis processes. In immune systems, neutrophils and macrophages are activated in response to exogenous stimuli, including microorganism invasion, to generate free radicals for host protection. In fact, activated leukocyte-derived oxidative damage may be involved in carcinogenesis by activating procarcinogens, participating in malignant transformation,

and modulating DNA bases (Nakamura et al., 2000). The NADPH oxidase system, present in inflammatory cells, consists of multiple components and produces superoxide anion (O_2^-). In addition to its action as a precursor of various reactive oxygen species, O_2^- itself is involved in several signal transduction pathways. On the other hand, there is now ample evidence that excess and/or prolonged generation of nitric oxide (NO) from its inducible isoform iNOS is associated with the onset of carcinogenesis and its suppression by food phytochemicals may be a promising avenue for cancer prevention (Murakami and Ohigashi, 2007b). Together, these findings show that suppression of leukocytic free radical generation may reduce the risks of carcinogenesis. Thus, we examined the suppressive effects of numerous Okinawan vegetables and fruits on O_2^- in differentiated HL-60 cells and NO generation in RAW264.7 macrophages. As expected, the antioxidative and antinitrosative activities of the target plants were remarkably higher than those commonly found throughout Japan, supporting our hypothesis that vegetables and fruits from subtropical areas are promising and interesting sources of cancer preventive agents, because of the differences in environmental conditions under which they are grown. Subsequently, some of the active constituents (chebulagic acid, a resveratrol derivative, and sesquiterpenoids) were identified and their evaluation for cancer prevention in rodent models is now in progress in our laboratory. In addition, similar screening tests performed using edible Thai plants and promising extracts are summarized in Table 12.1.

CHEMOPREVENTIVE ACTIVITY IN RODENT MODELS

Activity-guided fractionation of bioactive extracts using repeated column chromatography resulted in purification of the active principles, which were identified by spectroscopic analyses. Although each showed notable potential for cancer prevention, most of those compounds could not be subjected to rodent experiments on account of plant sample limitation. In contrast, 1′-acetoxychavicol acetate (ACA, from Alpinia galanga, Zingiberaceae), zerumbone (from Zingiber zerumbet, Zingiberaceae), auraptene, and nobiletin (from citrus fruits), and (±)-13-hydroxy-10-oxo-*trans*-11-octadecenoic acid (HOA) (corn germ) are abundantly present in natural sources and/or available by convenient chemical syntheses (Figure 12.2).

Topical applications of each of those five compounds significantly prevented dimethlbenz[*a*] anthracene-initiated and TPA-promoted skin tumorigenesis in mice. In addition, dietary administration of ACA, zerumbone, auraptene, and nobiletin led to notable reductions in the formation of tumor markers, aberrant crypt foci (ACF), and adenocarcinomas in rodent models of colorectal carcinogenesis. For example, auraptene given during the initiation phase reduced the azoxymethane (AOM)-increased incidence of colon adenocarcinoma by 49% at 100 ppm and 65% at 500 ppm, while those concentrations given during the postinitiation phase inhibited adenocarcinoma formation by 58% and 65%, respectively. Also, the multiplicity of colon carcinomas was significantly reduced by initiation feeding at a dose level of 500 ppm, and postinitiation feeding at levels of 100 and 500 ppm (Tanaka et al., 1998). This efficacy was repetitively seen in other colon carcinogenesis mouse models associated with inflammation and obesity (Tanaka et al., 2008). Oral administration of zerumbone at 100, 250, and 500 ppm also significantly inhibited AOM-initiated and dextran sulfate sodium (DSS)-promoted colonic adenocarcinomas, as well as lung adenoma formation induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (Kim et al., 2009). Among those five agents, the most pronounced cancer preventive efficacy was seen in a rat tongue carcinogenesis model fed ACA at 100 and 500 ppm during initiation or postinitiation, which showed significant decreases in the development of 4-nitroquinoline 1-oxide (4-NQO)-induced tongue carcinoma (93–100% reduction) and preneoplasia (43–50% reduction in hyperplasia, 34–48% reduction in dysplasia) (Ohnishi, 1996). Furthermore, nobiletin prevented colon carcinogenesis in rodents in several separate experimental systems (Kohno et al., 2001; Suzuki et al., 2004; Miyamoto et al., 2008). Until now, the chemopreventive effects of HOA have been limited to a skin carcinogenesis model, though induction of a novel tumor suppressor related to programmed cell death 4 (pdcd4) was implicated as involved in its unique action mechanisms (Yasuda et al., 2009).

TABLE 12.1
List of Edible Thai Plants with High Antioxidative Activities

Family/Species	Thai Name	Part Used[a]	O_2^- Suppression[b] (XA/XOD System)			O_2^- Suppression[b] (XA/XOD System)	
			Total	XOD	O_2^-	NO Suppression[c] (%)	O_2^- Suppression[d] (%)
Amaranthaceae							
Amaranthus gracilis	Pak khom	L	+	+	–	76.1	100
Araceae							
Lasia spinosa	Paknham	Sh	–	–	–	65.0	61.8
Bignoniaceae							
Oroxylum indicum	Pekaa	P(young)	+++	+++	–	75.7	54.1
Cucurbitaceae							
Marmodica charantia	Marakeenok	Fr	+	–	+	58.6	94.4
Labitae							
Ocimum basilicum	Horapaa	L	+	+	–	56.7	81.7
Leguminosae					–		
Sesbania glandiflora	Kae	Fl	–	–	–	63.0	98.9
Meliaceae			–	–	–		
Azadirachta indica	Sadao	Sh	–	–	–	78.6	88.1
Nymphaeacea							
Nelumbo lotus	Buasaikao	St	+++	++	+	63.0	64.8
Rutaceae							
Citrus aurantifolia	Manao	Fr	–	–	–	59.1	78.7
Scrophulariaceae					–		
Limnophila aromatica	Pakkayang	L	–	–	–	70.0	100
Nymphoides cristatum	Paktubtao	L	++	++	–	75.2	84.7
Simaroubaceae					–		
Eurycoma longifolia	Plalaiphaek	L	+++	+++	–	74.8	99.3
Umbelliferae					–		
Anetheum graveolens	Pakcheelao	W	++	++	–	69.5	75.7

[a] Fl, flower; Fr, fruit; L, leaf; P, pods; Pis, pistil; R, root or rhizome; Se, seed; Sh, shoot; St, stem; Sta, stamina; W, whole plant.

[b] Rank of percent inhibition: –IR <30%, +IR 30–49%, ++IR 50–69%, +++IR >70%; IR, inhibition rate.

[c] NO_2^- production inhibitory activity was evaluated in RAW264.7 cells by Griess method.

[d] O_2^- inhibitory activity was evaluated in HL-60 cells and expressed by a relative decreasing ratio of UV absorbance at 550 nm as compared with that of the control.

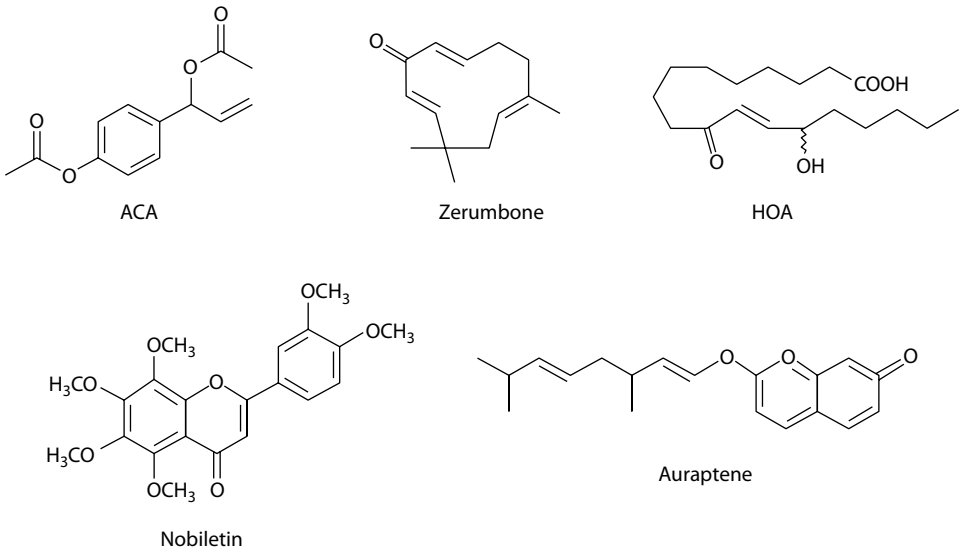

FIGURE 12.2 Structures of cancer preventive food phytochemicals. ACA, 1′-acetoxychavicol acetate; HOA, (±)-13-hydroxy-10-oxo-*trans*-11-octadecenoic acid.

PLAUSIBLE MECHANISMS OF ACTIONS

Synthetic drugs with chemopreventive activity reasonably target specific enzymes, proteins, and others based on the rationale used for their chemical synthesis; thus their modes of action are not generally complicated and can be predicted. In contrast, efforts to dissect the mechanisms of action of cancer preventive agents from natural sources have proven to be difficult and conclusions are occasionally ambiguous. Nonetheless, we have attempted to address the molecular mechanisms by which the above-mentioned phytochemicals prevent experimental carcinogenesis. Consequently, the following two distinct modes of actions have been revealed as probable: (1) modulation of xenobiotics metabolization via phase II enzymes, and (2) attenuation of the expression of pro-inflammatory proteins, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), as described in the following section.

Modulation of Phase II Enzymes

Exogenous and endogenous harmful substances must be metabolized and detoxified by drug-metabolizing enzymes, which consist of three distinct classes of phase I (e.g., cytochrome P450s), phase II (e.g., glutathione-*S*-transferase, GST), and phase III (e.g., *p*-glycoprotein) enzymes. For example, most polycyclic aromatic hydrocarbons with pro-carcinogenic activities are biologically inactive in their native structure, whereas they can be activated by phase I enzymes, leading to the formation of ultimate carcinogens that bind to cellular DNA for triggering mutations. Alternatively, biologically activated carcinogens might be subjected to reactions by phase II enzymes to be inactivated and detoxified. This process is followed by their efflux from cells through the functions of phase III enzymes. Finally, the inactivated metabolites enter the blood stream and urine for excretion from the body. Thus, selective activation of phase II, but not phase I, enzymes is logically ideal for promoting the excretion of carcinogens from the body and thus reducing the risk of chemical carcinogen-induced oncogenesis.

Since most, if not all, natural compounds with cancer preventive properties are xenobiotics, they have more or less potential to activate drug metabolizing systems. It is well known that sulforaphane, an isothiocyanate-related compound found in cruciferous plants such as broccoli, is capable of

selectively activating a set of phase II enzymes without affecting those of phase I (Talalay et al., 1995). Interestingly, there are several lines of evidence showing that increased ingestion of cruciferous plants might be beneficial for cancer prevention in gastric and lung cancer (Kim and Park, 2009). On the other hand, we previously reported that 400 and 800 mg/kg BW (body weight) of auraptene significantly increased GST and NAD(P)H quinone oxidoreductase-1 (NQO-1) activities in the livers of F344 rats (Tanaka et al., 1998). In addition, auraptene significantly elevated GST and NQO-1 activities in the colonic mucosa of those animals. In a mouse model as well, oral administration of auraptene by gavage at 50–200 mg/kg BW induced GST activity in the livers in a dose-dependent manner without affecting cytochrome P-450 activity (Murakami et al., 2000). A chemical characteristic of this coumarin is the presence of the 7-geeranyloxyl group in the coumarin structure. Using 10 different coumarin-related compounds, we found that only those coumarins with a 7-alkyloxyl group, including auraptene, induced GST, but not cytochrome P-450, show activity *in vivo*.

Nakamura et al. (2004) previously reported an interesting finding that exposure of RL34 cells to zerumbone resulted in significant induction of γ-glutamylcysteine synthetase, glutathione peroxidase (GPx), hemeoxygenase-1, and GST, while analogues of zerumbone, α-humulene, and 8-hydroxy-α-humulene, lacking the α,β-unsaturated carbonyl group, were virtually inactive. Therefore, the electrophilic property, characterized by reactivity with intracellular nucleophiles including protein sulfhydryls, of the α,β-unsaturated carbonyl group is indispensable for inducing phase II enzymes. Furthermore, multiple reverse transcriptase-polymerase chain reaction experiments revealed that topical application of zerumbone (2 μmol) enhanced the mRNA expressions of manganese superoxide dismutase, GPx-1, GST-P1, and NQO-1 in mouse epidermis, but not that of cytochrome p450 1A1 or 1B1. Phase II enzyme-selective activation by zerumbone is presumably associated with its effects of marked skin cancer prevention at the initiation stage, in which the activated ultimate carcinogen plays a key role in genetic mutations.

Suppression of iNOS and COX-2

Inflammation is a pathophysiological phenomenon that is involved in numerous diseases and each organ in humans has the potential for certain disease with an inflammatory condition that is essential to its etiology. Of great importance, a considerable proportion of chronic inflammatory diseases display an overlap with the onset and development of cancer, for example, ulcerative colitis reflux esophagitis, Barrett's esophagus, hepatitis, and gastritis. It is also well documented that infection with microorganisms is closely related to inflammation-derived carcinogenesis (Coussens and Werb, 2002). In pro-inflammatory conditions, neutrophils and monocytes mature and are recruited, then infiltrate the inflamed tissue for inducing and producing pro-inflammatory molecules, such as reactive oxygen and nitrogen species, prostaglandins (PGs), inflammatory cytokines, and chemokines, as well as others. NO is released at high levels by iNOS with the formation of stoichiometric amounts of l-citrulline from l-arginine. Furthermore, iNOS-mediated excessive and prolonged NO generation has attracted attention on account of its relevance to epithelial carcinogenesis (Ohshima and Bartsch, 1994). On the other hand, there is a large body of data showing that COX-2 expression is involved in the development of certain cancers (Taketo, 1998). In contrast to COX-1, COX-2 activity is inducible and its elevation enhances the biosynthesis of PGs, including PGE_2, which is one of the physiologically active and stable PGs produced in the pathways downstream of COX enzymes. PGE_2 is known to stimulate bcl-2 activity and thereby prevent apoptosis (Fosslien, 2000). Selective COX-2 inhibition by synthetic drugs resulted in a significant prevention of polyp formation in the colons of familial adenomatous coli patients, though evidence has emerged that such molecular targeting may increase the risk of cardiovascular disease (Moodley, 2008).

The above-mentioned five compounds have notable potency for attenuating the mRNA and protein expressions of iNOS and COX-2 in stimulated macrophages, as well as in *in vivo* models. A diet containing 0.01% or 0.05% zerumbone significantly reduced AOM-induced colonic ACF, and expressions of COX-2 and PGs in colonic mucosa of male F344 rats (Tanaka et al., 2001). Along a

similar line, oral feeding of zerumbone significantly lowered the levels of PGE_2, together with the pro-inflammatory cytokines IL-1β and TNF-α, in a DSS-induced acute colitis mouse model (Murakami et al., 2003). Kohno et al. (2006) reported that male ICR mice given experimental diets containing auraptene at doses of 0.01% and 0.05% showed significant reductions of azoxymethane-initiated and DSS-promoted colonic neoplasms, while the expressions of COX-2, iNOS, and nitrotyrosine were detected in colonic epithelial malignancy by immunohistochemical analyses. In addition, the incidence of colonic adenocarcinoma was 67% in the AOM alone group of male F344 rats, whereas nobiletin in the diet at doses of 0.01% and 0.05% suppressed it in a dose-dependent manner (incidence of 55% and 35%, respectively). Also, nobiletin feeding reduced the levels of PGE_2 in colonic adenocarcinoma and/or colonic mucosa samples, suggesting regulation of COX-2 expression in vivo (Suzuki et al., 2004).

Although the molecular mechanisms underlying iNOS and COX-2 regulation have not been fully elucidated, our *in vitro* studies with RAW264.7 murine macrophages have provided intriguing insight into how they exhibit suppressive effects. Treatment of RAW264.7 cells with lipopolysaccharide (LPS) initiates LPS binding to its membrane receptor, toll-like receptor 4 (TLR4) (Figure 12.3). This step triggers the activation of several adaptor proteins and then numerous protein kinases. It is important to note that mitogen-activated protein kinases (MAPKs) are the major intracellular mediators of LPS signaling, as they control a number of cellular events, including differentiation, proliferation, and death, as well as short-term changes required for homeostasis and acute hormonal responses. To date, at least three major MAPK cascades have been described that involve the activation of ERK1/2, JNK1/2, and p38 MAPK. On the other hand, Akt (protein kinase B) is located upstream of IKK, which phosphorylates IκBα protein, leading to its time-dependent

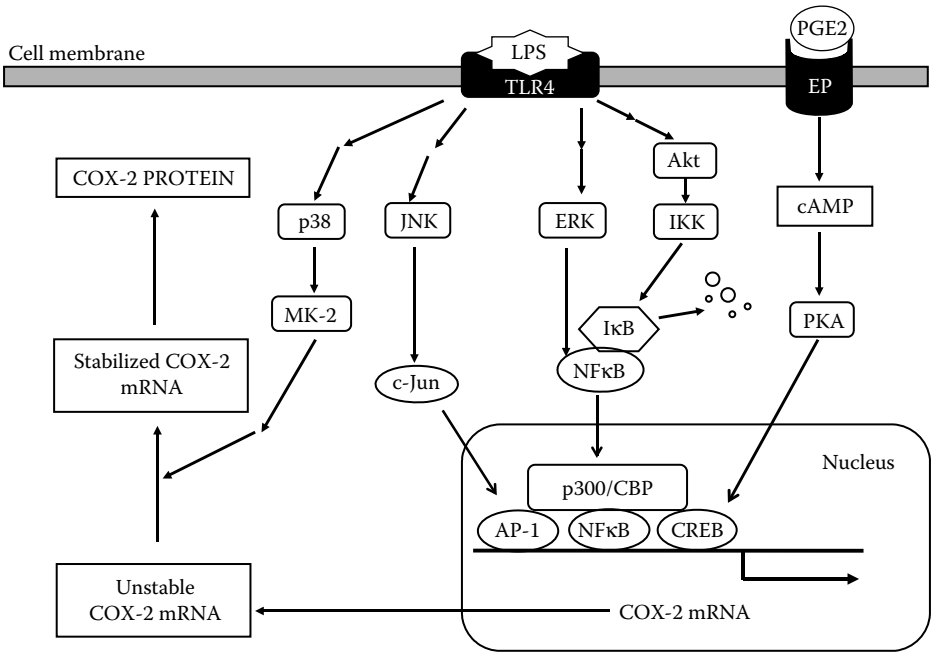

FIGURE 12.3 Possible molecular mechanisms by which LPS-induces COX-2 protein expression in RAW264.7 mouse macrophages. AP-1, activator protein-1; CBP, CREB-binding protein; COX, cyclooxygenase.; CREB, cAMP-responsive element binding protein; ERK, extracellular signal-regulated protein kinase; IKK, IκB kinase; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MAPKAPK-2, MAPK-activated protein kinase 2; NF-κB, nuclear factor κB; PGE_2, prostaglandin E_2; PKA, protein kinase A; TLR, toll-like receptor.

degradation, which results in activation of NF-κB, a master transcription factor of multiple pro-inflammatory genes including iNOS and COX-2. As summarized in Figure 12.4, we found that activation of MAPKs by LPS exposure was not attenuated by nobiletin, zerumbone, or auraptene, whereas both HOA and ACA inhibited it (Murakami et al., 2005). Of note, ACA substantially blocked both JNK1/2 and ERK1/2 activation. None of these compounds, except for HOA, demonstrated any inhibition of Akt activation (at Ser473). In addition, ACA did not allow LPS-induced IκB degradation, whereas zerumbone, nobiletin, and auraptene were virtually inactive. LPS treatment markedly elevated the activities of the transcription factors of NF-κB and AP-1, and it is notable that HOA, ACA and nobiletin significantly suppressed those transcription factors, whereas zerumbone and auraptene had no effects. Furthermore, zerumbone accelerated the degradation of iNOS and COX-2 mRNA, suggesting that it regulates the expression of these enzymes via post-transcriptional mechanisms. Also, auraptene downregulated LPS-induced COX-2 protein expression, whereas it did not affect the mRNA level, suggesting that this phytochemical targets the translation process of that enzyme.

Collectively, ACA targets both JNK1/2 and ERK1/2, while nobiletin may interfere with coactivators, such as CREB-binding protein/p300, which is involved in the full transactivation of NF-κB and AP-1. This notion is supported by a recent report by Choi et al. (2007) which showed that nobiletin inhibited DNA binding of NF-κB in a similar cellular system. Also, zerumbone allowed LPS-induced MAPK/Akt activation and transcriptional activation of those transcription factors, whereas it abrogated iNOS mRNA induction. Thus, it may target MAPK-activated protein kinase (MK)-2 or

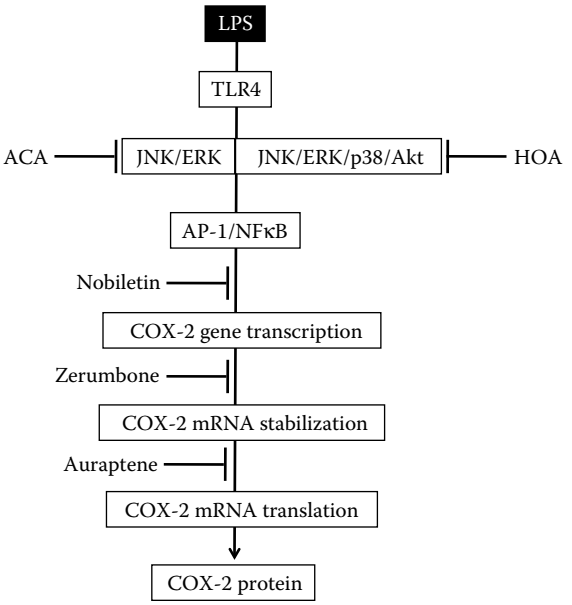

FIGURE 12.4 ACA, HOA, nobiletin, zerumbone, and auraptene attenuate COX-2 protein expression via different mechanisms and at various stages. Both ACA and HOA suppressed JNK/ERK activation; however, p38 MAPK and Akt activation were suppressed only by HOA. Nobiletin did not have effects on the transcriptional activities of either AP-1 or NF-κB, whereas it suppressed the expression of iNOS mRNA. Zerumbone accelerated the degradation of iNOS mRNA, which contains ARE at the 3′-UTR, while auraptene did not attenuate that mRNA expression, but inhibited its protein synthesis, suggesting that it targets the translational step. ACA, 1′-acetoxychavicol acetate; AP-1, activator protein-1; ARE, AU-rich element; COX, cyclooxygenase; ERK, extracellular signal- regulated protein kinase; HOA, (±)-13-hydroxy-10-oxo-*trans*-11-octadecenoic acid; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; UTR, untranslated region.

downstream molecules for destabilizing mRNA. In addition, auraptene might disturb the translation process of iNOS mRNA, since we previously found that it targets the translational step of proMMP-7 by inactivating ERK1/2 in HT-29 human adenocarcinoma cells, whose action mechanism differs from that of rapamycin, an inhibitor of mTOR (Kawabata, 2006). Because auraptene did not affect the level of iNOS mRNA expression but rather suppressed its protein induction, a similar mechanism might be involved. Intriguingly, HOA abolished LPS-up-regulated MAPKs, suggesting that its target molecule(s) are located upstream of the LPS receptor of MAPKs, TLR4. We consider that use of these five dietary factors in combination may lead to synergistic results with higher efficacy and lower toxicity based on their different molecular mechanisms for iNOS and COX-2 protein suppression.

SUMMARY POINTS

- Vegetables and fruits from subtropical countries (Thailand, Indonesia, Malaysia, etc.) are promising sources of bioactive compounds including those related to cancer prevention.
- We have isolated and identified functionally novel compounds with cancer preventive potentials, including 1′-acetoxychavicol acetate (ACA, from Alpinia galanga, Zingiberaceae) and zerumbone (from Zingiber zerumbet, Zingiberaceae), auraptene and nobiletin (from citrus fruits), and (±)-13-hydroxy-10-oxo-*trans*-11-octadecenoic acid (HOA) (from corn germ).
- These compounds were found to markedly suppress experimental carcinogenesis in rodent models (skin, colon, tongue, etc.).
- Both zerumbone and auraptene are suggested to potentiate self-protection systems by promoting the excretion of harmful carcinogens.
- ACA, auraptene, nobiletin, zerumbone, and HOA attenuated the expression of inflammatory biomarkers (iNOS and COX-2) in animal models, and their mechanisms of actions differ.

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13 Mushroom-Derived Substances for Cancer Prevention and Treatment

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CONTENTS

Mushrooms and mushroom-derived substances are recognized for many years for their therapeutic value and for their efficacy in treating a variety of human illnesses including cancer. Historically, hot-water-soluble fractions from medicinal mushrooms were used as medicine in the Far East, where knowledge and practice of mushroom use started primarily (Wasser, 2002). Today, there are approximately 200 species of mushrooms that have been reported to posses therapeutic value including anticancer activity (Yang and Jong, 1989). However, most of these antitumor substances have not been defined yet (Wasser and Weis, 1999).

The interest in mushrooms with potential anticancer activity was initiated by the disclosure by Lucas et al. (1957) who demonstrated antitumor effect of fruiting bodies extracted from the basidiomycete *Boletus edulis* against xenografts of Sarcoma 180. Later, Yohida (1962) isolated an active element, *Lampteromyces japonicas (kowamura) Sing*, that exhibited good antitumor activity against xenografts of Ehrlich carcinoma. Since then increasing scientific reports of mushroom-derived substances with anticancer activity are reported which can be divided into two major categories; the low molecular weight compounds and the high molecular weight compounds (Zaidman et al., 2005). High molecular weight compounds, which are mainly polysaccharides and act as nonspecific immonumodulators such as PSK, Lentina, and others, exerted their antitumor activity not by killing cancer cells directly but by stimulating the body's immune system (Mayell, 2001) which help in combating the cancer.

In general, the anticancer activity of mushrooms can be divided into substances that exhibit preventive activities and others with therapeutic activity. This chapter will review both activities and focus mainly on the activity of mushroom-derived low molecular weight (LMW) entities.

CANCER PREVENTIVE EFFECT OF MUSHROOM SUBSTANCES

It is well established that chronic inflammation could promote cancer development. In fact, chronic inflammation is thought to contribute to about 25% of malignancies worldwide (Hussain and Harris, 2007).

Inflammation is a complex response of the innate and adaptive immune system to infection or tissue injury caused by exogenous or endogenous means, and it involves a variety of pro-inflammatory agents. These include growth factors, cytokines, and chemokines, such as IL-1, IL-6, IL-15, and TNFα as well as reactive oxygen and nitrogen species, all of which dictate the nature of the inflammatory response and lead to tissue repair and to the elimination of pathogens. Whereas in normal inflammation, the production of anti-inflammatory cytokines follows to the production of pro-inflammatory cytokines, thus regulating and limiting the inflammatory response, in chronic inflammation, a sustained generation of these pro-inflammatory agents creates a microenvironment that could promote pathogenesis (Coussens and Werb, 2002).

One of the key molecules that link inflammation and cancer is the nuclear transcription factor kappa-B (NF-κB). NF-κB is a ubiquitous transcription factor involved in the regulation of important cell functions such as cell survival and immune and inflammatory responses. NF-κB complex is maintained in the cytoplasm, in an inactive form, bound to the inhibitory protein kappa B (IκBα), belonging to a family of inhibitory proteins, IκBs. NF-κB pathway is activated in response to various inflammatory stimuli, including infection agents like bacterial lipopplysaccharide (LPS) and viral proteins, pro-inflammatory cytokines such as TNFα and 1L-1, and DNA damaging agents, all of which activate the IκB kinase complex (IKK). After activation, the IKK complex phosphorylates the NF-κB-bound IκBα, leading to IκBα ubiquitination and proteasome-mediated degradation and to the translocation of NF-κB dimers to the nucleus, where they induce the transcription of target genes involved in cellular proliferation, inflammatory responses, and cell adhesion. These target genes include mediators of inflammation like the pro-inflammatory enzymes, cyclooxygenase (COX2) and iNOS, leading to the production of prostaglandin E2 (PGE2) and nitric oxide (NO), respectively, as well as different cytokines and chemokines. NF-κB induces also the transcription of genes involved in cellular proliferation and survival like Bcl-xL, GADD45α, BFL1, cyclin D1 and many others, thus promoting tumor growth and progression (Karin, 2006; Karin and Greten, 2005).

Being a crucial mediator of inflammation and carcinogenesis, NF-κB, and the different molecular pathways it regulates, as well as other pro-inflammatory components, serve as attractive targets for inflammation and cancer prevention and therapy. A large body of evidence point toward the anti-inflammatory properties of mushrooms, thus suggesting a potential use of mushrooms as chemo preventives. Many of these mushrooms exhibit anti-inflammatory activity through interference with various intracellular signal-transduction pathways, in particular the NF-κB activation pathway (Petrova et al., 2008). JNK, together with p38 MAP kinase and the extracellular signal-regulated kinase (ERK), all belong to the mitogen-activated protein (MAP) kinases pathway, known to mediate important regulatory signals in the cell, including inflammation (Hommes et al., 2003), which were also reported to be regulated by mushroom-derived substances.

Many reports demonstrated the ability of whole mushroom extracts, and isolated fractions to exert anti-inflammatory effects. For example, a screening of 242 fungal organic extracts, belonging to different taxonomic and ecological groups, revealed the ability of different extracts to interfere with the NF-κB activation pathway through inhibition of IκBα phosphorylation and degradation (Petrova et al., 2007). In a following study, the crude culture extract of the common edible mushroom, *Marasmius oreades*, was further fractionated leading to the identification of active fractions which caused a significant downregulation of NF-κB activity through strongly inhibiting IκBα phosphorylation and blocking the translocation of p65 to the nucleus. Moreover, the active fractions were shown to inhibit the activity of IKK, suggesting that these fractions modulate NF-κB pathway by acting upstream to IκBα phosphorylation (Petrova et al., 2009).

The methanol extract obtained from *C. pruinosa* suppressed inflammation through *in vivo* and *in vitro* inhibition of inflammatory genes expression, including TNFα, IL-1α, iNOS, and COX2 (Lull et al., 2005).

Park et al. (2005), reported that a methanol extract (MEIO) of the traditional medicinal mushroom, *Inonotus obliquus*, possesses both *in vivo* and *in vitro* anti-inflammatory and antinoiciceptive effects, which seem to be mediated via the inhibition of NF-κB. Due to their central role in the inflammatory response, macrophage cells, stimulated with LPS, are often used to investigate anti-inflammatory effects *in vitro*. MEIO exhibited anti-inflammatory and antinoiciceptive effects in rats and mice, and also strongly inhibited LPS-induced NO and PGE2 production in macrophages, through downregulation of iNOS and COX2 expression, respectively. In addition, MEIO showed an inhibitory effect on LPS-induced TNFα secretion in these cells (Park et al., 2005). Moreover, an ethanol extract of the same mushroom had also exhibited anti-inflammatory effects through the suppression of NO production and iNOS and COX2 expression in LPS-stimulated macrophages, mediated by the inhibition of NF-κB, through the AKT pathway and the inhibition of the c-Jun NH2-terminal kinase (JNK) signaling pathway (Kim et al., 2007a).

Another traditionally used medicinal mushroom, *Phellinus linteus*, was evaluated for its anti-inflammatory influence. Subtraction (*n*-BuOH), prepared from the mushroom's fruiting bodies, strongly inhibited iNOS promoter activity, NO production and LPS-induced iNOS expression in macrophages (Kim et al., 2006a). Kim et al. (2006a) demonstrated the involvement of ROS, generated in macrophages in response to LPS, in the increased production of NO in these cells, and identified the extract's mechanism of action to be mediated via the induction of heme oxygenase (HO)-1, an enzyme with known antioxidative properties. Further study showed that the butanol fraction from *P. linteus* was able to inhibit NO production and iNOS protein and mRNA expression, as well as PGE2 secretion and COX2 protein and mRNA expression in macrophages. The inhibition of these inflammation mediators was suggested to be mediated by the inhibition of the LPS-induced degradation and phosphorylation of IκBα, NF-κB nuclear translocation, JNK and p38 MAPK phosphorylation. Anti-inflammatory effect of *P. linteus* was attributed, in part, to its antioxidant activity, leading to the suppression of ROS increase in LPS-stimulated macrophages (Kim et al., 2007b).

The medicinal mushroom *Daedalea gibbosa*, which was previously reported as possessing anti-cancer activity through inhibiting the growth of CML cell lines (Yassin et al., 2008), also exhibited anti-inflammatory activities (Ruimi et al., 2009). Three active fractions, isolated from the ethyl acetate extract of *D. gibbosa* culture medium, strongly inhibited LPS-induced NO production and iNOS protein expression in LPS-induced macrophage cells. Examining the mechanism of action, through which the fractions exert their effects, revealed that two of the fractions significantly inhibited iNOS transcription, while the third fraction exerted a post-transcription effect. In addition, the iNOS inhibition activity of the three active fractions seemed to be mediated by differential effects on the levels of phospho-p38, phospho-JNK, and phospho-IκBα, and by the inhibition of NF-κB DNA binding activity (Ruimi et al., 2009).

Mushroom-derived chemical compounds were also effective in mediating anti-inflammatory activity. Panepoxydone (Figure 13.1a), a secondary metabolite isolated from the basidiomycetes *Panus rudis, Panus conchatus*, and *Lentinus crinitus*, was examined for its influence on the expression of genes involved in inflammation, and which are related to the NF-κB pathway (Erkel et al., 2007). Using a reporter gene assay in MonoMac6 (acute monocytic leukemia) cell lines stimulated with LPS and phorbolester 12-otetradecanoylphorbol-13-acetate (TPA), Erkel et al. (2007) showed the ability of panepoxydone to significantly inhibit NF-κB, TNFα, and IL-8 reporter gene activity, as well as NF-κB DNA binding activity. Furthermore, panepoxydone also affected NF-κB through the prevention of IκBα phosphorylation. Gene expression microarray analysis revealed that panepoxydone was able to inhibit the expression of 33 pro-inflammatory NF-κB-dependent genes, including cytokines as IL-1α, IL-6, and TNFα, chemokines like IL-8 and CCL3 and pro-inflammatory enzymes such as COX2. Another mushroom-derived compounds with anti-inflammatory activity are sporogen (Figure 13.1b), S14-95 (Figure 13.1c) and S-curvularin (Figure 13.1d) which were isolated

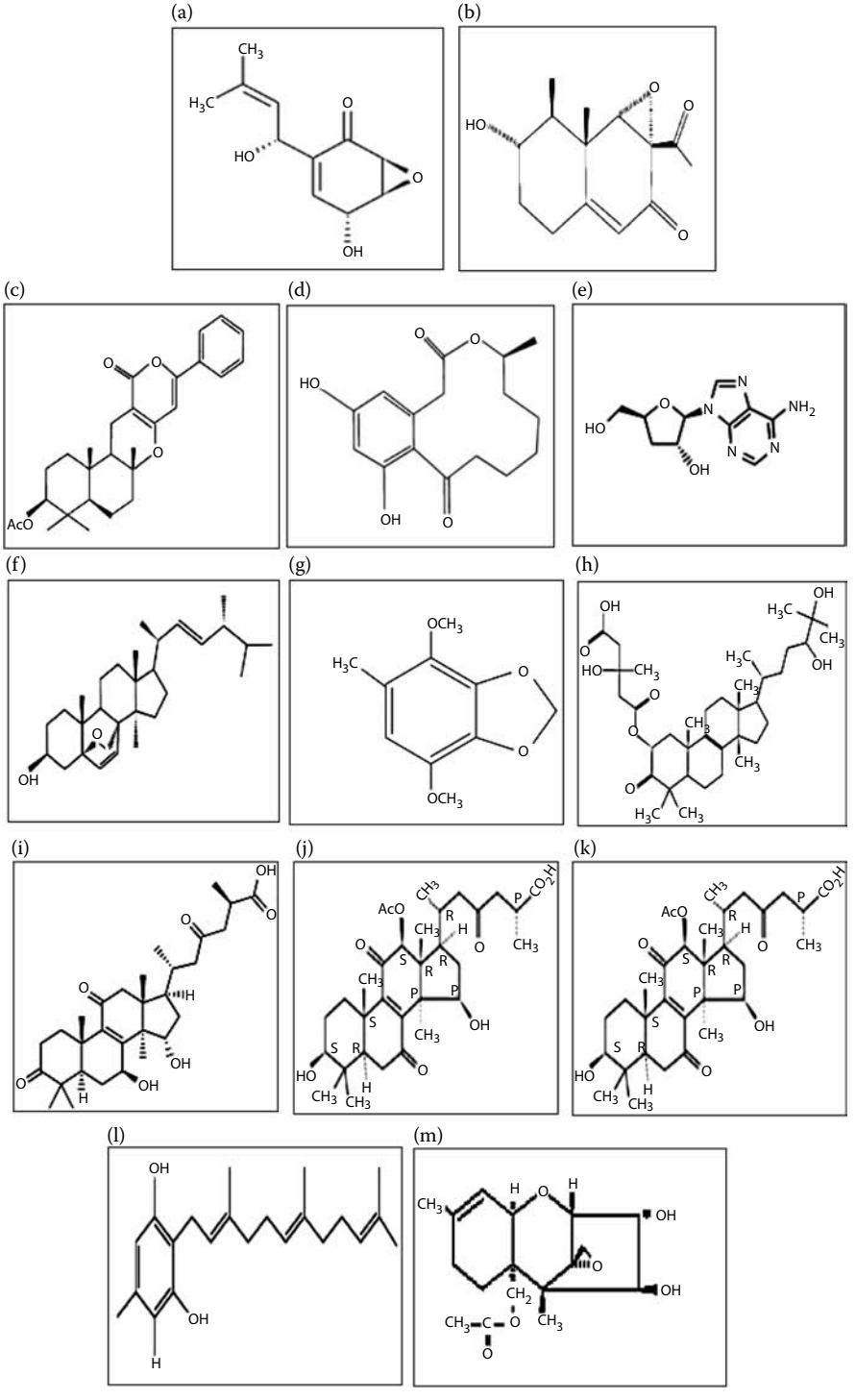

FIGURE 13.1 Low molecular weight fungal substances with anti-inflammatory and anticancer properties: (a) panepoxydone, (b) sporogen, (c) S14-95 (d) *S*-curvularin, (e) cordycepin, (f) ergosterol, (g) 4,7-dimethoxy-5-methyl-l,3-benzodioxole (SY-1), (h) clavaric acid, (i) ganoderic acid A, (j) ganoderic acid F, (k) ganoderic acid H, (l) grifolin, (m) acetoxyscirpenediol.

by Yao et al. (2003) from three different *penicillium* species, with the ability to inhibit NO production and iNOS expression through their suppressive effect on the IFN-γ-JAK-STAT pathway (Yao et al., 2003). Moreover, Cordycepin (3′-deoxyadenosine) (Figure 13.1e), is a component of the butanol fraction that was isolated from *Cordyceps militaris* and exhibited anti-inflammatory properties in LPS-stimulated macrophages. Cordycepin was found to inhibit NO production and iNOS expression, as well as COX2 expression and TNFα expression via its inhibitory effect on NF-κB activation and the suppression of Akt and p38 phosphorylation (Kim et al., 2006b). The fungal sterol, ergosterol (Figure 13.1f), suppressed LPS-induced inflammatory response in macrophages through inhibiting the expression of the inflammation-related genes, IL-1α and 1L-1β, ergosterol also suppressed the production of TNFα in these cells, and inhibited NF-κB DNA-binding activity as well as p38, JNK, and ERK MAPKs phosphorylation (Kobori et al., 2007).

Anti-inflammatory effects were found to be mediated also by mushroom derived polysaccharides. For example, polysaccharides isolated from *Pholiota nameko* (Li et al., 2008) and *Pleurotus pulmonarius* (Smiderle et al., 2008) exhibited anti-inflammatory and antinoiciceptive effect in a rodent model of inflammation.

CANCER THERAPUTIC EFFECT OF MUSHROOM SUBSTANCES

Cancer is a term used for a heterogeneous disease in which normal cells escape cell cycle regulation, rapidly expand the cell population, and create a mass of tissue called a tumor. Some malignant tumors, having the ability to metastasize, invade their surrounding tissue and establish secondary areas of growth. The main pathways of cancer development include self-sufficiency in growth signals, insensitivity to antigrowth signals and escape from cell cycle regulation. All of these lead to the well-known features of cancer including rapid proliferation of cells, tissue invasion and metastasis, avoidance of apoptosis, and acquisition of sustained angiogenesis, which are all important targets for cancer treatment that have been the focus of much research (Hanahan and Weinberg, 2000). Recently, an increasing number of reports show the ability of mushroom derived substances to interfere with the molecular pathways involved in the different stages of cancer development. In this section we will review some of these reports.

Mushroom-Substances that Interfere with Self-Sufficiency in Growth Signals and Disruption of Cell Cycle Regulation

Normal cells require stimulatory signals in order to shift from quiescence into an active proliferation stage. Self-sufficiency in growth signals is an acquired capacity of tumor cells, in which tumor cells become independent on exogenous growth stimulation and are able to generate many of their own growth signals (Hanahan and Weinberg, 2000). Cellular quiescence and tissue homeostasis are normally maintained by antigrowth signals. In normal cells, cellular division is a well ordered and precisely controlled process which involves multiple checkpoints. This assures that cellular growth, cell size, DNA integrity, and extracellular growth signals do not fall out of regulation. The cell cycle is divided into four distinct phases. The S-phase includes the DNA replication. In the M-phase (mitosis), there is a segregation of the chromosomes into daughter progeny. G1 and G2 are "gap" phases. In G1, the cell prepares for DNA synthesis and once the cell passes the restriction point in late G1 it is obligated to enter the S phase and replicate its DNA. In G2, the cell prepares for mitosis (Figure 13.2). Major regulators in the cell cycle are the cyclins (A, B, D, E), cyclin-dependent kinases (CDK), CDK inhibitors (p21, p27, and p16) and the retinoblastoma (Rb) protein. These proteins control the cell cycle by holding the cells in quiescence or leading them to commit suicide (apoptosis), when the conditions are inappropriate. Tumor cells evade antigrowth signals and accumulate alterations in the controlling proteins, leading to failure of cell cycle arrest and therefore to uncontrolled cellular proliferation (Singhal et al., 2005).

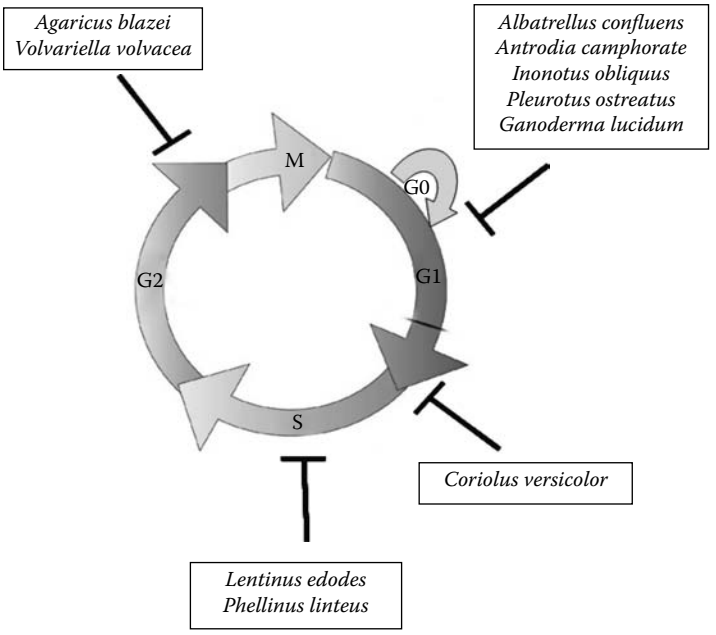

FIGURE 13.2 Mushroom-derived substances induce cell cycle arrest. A schematic of the different phases of the cell cycle is shown. A list of mushrooms that were reported to affect the different phases of the cell cycle is also included. A G0/G1 cell cycle arrest of cancer cells caused by the extracts of the mushrooms *Albatrellus confluens, Antrodia camphorate, Inonotus obliquus, Pleurotus ostreatus*, and *Ganoderma lucidum* which was associated with the downregulation of phospho-Rb, cyclins D1, D2, E, and CDKs 2,4,6 expression levels. It was also associated with increased levels of p21. The level of p27 was downregulated by the mushroom *Inonotus obliquus* and upregulated by the mushroom *Antrodia camphorate.* The mushroom *Coriolus versicolor* caused disruption in G1/S and G2/M Cell cycle phases of Human leukemia cell lines associated with a downregulation of phospho-Rb. Cell cycle arrest at S phase was caused by extracts from the mushrooms *Lentinus edodes* and *Phellinus linteus* and was associated with the suppression of cdk4 and cyclin D1 activity and the upregulation p21 and p27 expression. Extracts from the mushrooms *Agaricus blazei* and *Volvariella volvacea* caused cell cycle arrest at the G2/M phase, which was correlated with a decrease in the levels of cyclin B1, and cdc2 and an increase in the levels of p21, p27, and Rb.

Various mushrooms exerted their antitumor effects by affecting the different stages of the cell cycle. A water extract of *Inonotus obliquus*, for example, was able to inhibit the growth of melanoma B16-F10 cells by causing cell cycle arrest at G0/G1 phase. These effects were associated with the downregulation of phospho-Rb and p27 expression levels (Youn et al., 2009). Furthermore, the extract from the edible mushroom *Pleurotus ostreatus* (oyster) also caused a cell cycle arrest at G0/G1 phase in human breast cancer (MCF-7) and human colon carcinoma (HT-29) cells by inhibiting the phosphorylation of Rb protein and by inducing the expression of p21, a CDK inhibitor (Jedinak and Sliva, 2008).

G0/G1 arrest in the cell cycle, associated with the inhibition of phosphorylated Rb, was also seen in human hepatoma cell lines, HepG2 and Hep3B, after treatment with Chaga mushroom (*Inonotus obliquus*) water extract. The G0/G1 arrest in the cell cycle was also closely associated with the downregulation of cyclins D1, D2, E, cyclin-dependent kinase (Cdk) 2, Cdk4, and Cdk6 expression (Youn et al., 2008). Another study has demonstrated the ability of the water extract, derived from deep-layer cultivated mycelia of the *Coriolus versicolor*, to disrupt the G1/S and G2/M phases of cell cycle progression in treated human leukemia HL-60 cell lines, a disruption which was correlated with a time- and dose-dependent downregulation of Rb protein levels (Hsieh et al., 2006).

Ethyl acetate extract from the Shiitake (*Lentinus edodes*) mushroom have been reported to induce cell cycle arrest in human breast carcinoma cell lines, MDA-MB-453 and MCF-7, and in myeloma cell lines, RPMI-8226 and IM-9, by a significant decrease of S phase, which was associated with the induction of p21 (cdk inhibitor) and with the suppression of cdk4 and cyclin D1 activity (Fang et al., 2006). In addition, treatment with the medicinal mushroom *Phellinus linteus*, caused a growth inhibition of the highly invasive human breast carcinoma MDA-MB-231 cells, mediated by the cell cycle arrest at S phase, and through the upregulation of p27 expression (Sliva et al., 2008). The same mushroom also exhibited cell cycle arrest activity in mouse and human lung cancer cell lines, mediated by the suppression of cdk4 and cyclin D1 activity (Guo et al., 2007). Zaidman et al. (2007) reported the ability of *Ganoderma lucidum* to downregulate cyclin D1 expression, leading to the dephosphorylation of phospho-Rb and to the G1 phase arrest in LNCaP prostate cancer cell lines (Zaidman et al., 2007). Additional report showed the ability of an extract prepared from the mushroom *Agaricus blazei* to inhibit human gastric epithelial (AGS) cell growth in a dose-dependent manner through the arrest of G2/M phase, which was closely correlated to decreased cyclin B1 and cdc2 levels (Jin et al., 2006).

Mushroom extracts and substances were also able to synergize with physiological agents to augment the anticancer activities. The D-fraction, a bioactive extract from the *Grifola frondosa* mushroom, and IFN-α, was shown to cause a G1 cell cycle arrest, indicated by cell cycle analysis. Pyo et al. (2008) demonstrated that following treatment with the two agents, the G1-specific cell cycle regulators, CDK2, CDK4, CDK6, cyclin D1, and cyclin E, have been significantly downregulated in human prostate cancer PC-3 cell lines.

Several chemical structures of mushroom-derived active substances were elucidated, and some of the pure chemicals exhibited anticancer activity by disrupting the cell cycle progression. Compounds such as 4,7-dimethoxy-5-methyl-1,3-benzodioxole (SY-1) (Figure 13.1g), isolated from dried fruiting bodies of *Antrodia camphorate*, profoundly decreased the proliferation of human colon cancer cells (COLO 205) through causing G0/G1 Cell cycle arrest associated with a significant increase in the levels of p21/Cip1 and p27/Kip1 proteins (Lien et al., 2009). Furthermore, Grifolin, a natural product isolated from the mushroom *Albatrellus confluens* (Figure 13.1l), was shown to inhibit the growth of human nasopharyngeal carcinoma cell line (CNE1). Grifolin caused a significant cell cycle arrest at G1 phase in these cells, and led to the inhibition of cyclin D1, cyclin E, and CDK4 expression, and to the subsequent reduction in Rb phosphorylation (Ye et al., 2007).

Also high molecular weight fungal substances such as fungal lectin that were isolated from the mushroom *Volvariella volvacea*, activated the expression of cyclin kinase inhibitors, p21, p27, and Rb, in a dose-dependent manner, resulting in cell cycle arrest at the G2/M phase (Liua et al., 2001).

Mushroom-Substances as Modulators of Cellular Proliferation

Proliferation of cells is one of the processes that are most attributed to cancer. The proliferation mechanism is controlled by many pathways in the cell, and while in the normal cellular mechanisms, proliferation and death programs are tightly linked (Jagani et al., 2008), the ability of the cell to evade death programs and to proliferate is a main cause of tumor development. Cells responding to extra cellular challenges, such as growth factors, depend upon the interaction of external signals with membrane receptors and cell signaling pathways (Godio et al., 2007). The main regulators of these signaling pathways are proto-oncogenes, which have a critical role in the normal cell growth. Mutations by which proto-oncogenes become oncogenes are common reasons for uncontrolled cell growth and tumor initiation.

The Ras proto-oncogenes encode low molecular weight guanine nucleotide-binding proteins that are necessary to cellular growth control. Being associated with many types of cancers, the Ras oncogene has been a main target in cancer therapy (Buday and Downward, 2008). Suppression of Ras oncogene was detected following treatment with extracts from the fruiting bodies and the cultured mycelia of *Ganoderma lucidum* (Hsiao et al., 2004). Another signaling molecule that regulates

proliferation is the extracellular signal-regulated protein kinase (ERK) which is a member of the MAPK family and a part of the Ras-Raf-MEK-ERK signaling cascade controlling cell proliferation (Mebratu and Tesfaigzi, 2009). Suppression of the growth of human leukemia (HL-60) cell lines following treatment with water extracts of *Coriolus versicolor* mushroom, was positively correlated with an increase in signal transducer and activator family of transcription factors (STAT1), and conversely, with a reduction in the expression of the activated form of ERK (Hsieh et al., 2006). Another study has shown the ability of extracts from the *Inontus obliquus* mushroom to cause a significant reduction in the phosphorylation of ERK1/2 and p38 protein kinases in WB-F344 rat liver epithelial cells (Park et al., 2006). Additional signaling molecules that regulate cellular proliferation are the tyrosine kinases in which c-Abl, a nonreceptor tyrosine kinase, exhibited a dominant role in the regulation of the cell growth. Translocation in chromosome 9 and 22 leads to the oncogenic form of the protein, Bcr-Abl, which is the main cause of chronic miyloidic lukemia (CML). Bcr-Abl is constantly activated and simultaneously activates multiple pathways including those involved in cellular proliferation (Jagani et al., 2008). Yassin et al. (2003) showed that treatment with extracts of *Trametes zonata* caused a significant cell growth inhibition and a dramatic reduction in Bcr-Abl expression, in K562 CML cell lines. The antiproliferative effect on K562 cells was also detected following treatment with extracts from *Omphalotus olearius* and *Pleurotus eryngii* mushrooms, an effect that was associated with the inhibition of the MAP Kinase p38. Further investigations revealed that extracts from the mycelium of *Daedalea gibbosa* have selective antiproliferating activities against K562 cells and other laboratory model of CML (Yassin et al., 2008). The active fraction of the extract of the mushroom *Daedalea gibbosa* significantly inhibited the autophosphorylation activity of native and mutated Bcr-Abl, including those carrying the "gatekeeper" T315I mutation, which leads to a complete resistance to Imatinib, the therapy of choice to CML (Yassin et al., 2008).

Mushrooms are also the source of chemicals that exert direct effect on cellular proliferations, such as ganodermic acids (triterpenes) (Figure 13.1k), that were isolated from *Ganoderma lucidum* and inhibit Ras-dependent cell proliferation (Lindequist et al., 2005). Clavaric acid (Figure 13.1h), isolated from the mushroom *Hypholoma sublateritium*, was found to be a potent inhibitor of the Ras farnesyltransferase (Godio et al., 2007). Inhibition of cell growth via the MEK-ERK signaling pathway, has also been observed using grifolin (Figure 13.1l), a natural product isolated from the mushroom *Albatrellus confluens*. In addition, Ye et al. (2007) revealed that MEKK3, MEK1, and MEK5 were strongly downregulated in CNE1 cells following treatment with grifolin. Treatment with grifolin also led to the inhibition of MEK1 expression in human nasopharyngeal carcinoma cell lines, an effect which was associated with a dose-dependent decrease in phospho-ERK1/2, the downstream target of MEK1.

Mushroom-Substances as Modulators of Cellular Apoptosis

Apoptosis is the process of programmed cell death, mediated by a tightly controlled program and by various signaling pathways (Ye et al., 2005). The ability to evade apoptosis is one of the important features of cancer cells and a pathological basis for malignant tumor development (Hanahan and Weinberg, 2000). There are two main pathways in apoptosis: the death receptor pathway and the mitochondrial pathway; both involve many genes which play important roles of regulation, including Bcl-2 family, p53, caspases, and the PI3K/Akt pathway (Singhal et al., 2005). The mitochondrial pathway is mainly regulated by members of the Bcl-2 family of proteins that control cytochroom *c* and other mediators, translocation from the metochondria to the cytoplasms. This family contains pro-apoptotic factors (Bax, Bak) and antiapoptotic factors (Bcl-2, Bcl-xL) and the ratio, Bcl-2/Bax, is one of the main factors regulating apoptosis (Singhal et al., 2005).

Various mushroom substances showed significant antitumor activities with a wide range of mechanisms, some of which have been identified as apoptosis inducers (Ye et al., 2005). For example, the water extracts of I'm-Yunity™ (PSP), derived from mycelia of the *Coriolus versicolor*

mushroom, reduced the expression of the antiapoptotic proteins Bcl-2 and survivin, and simultaneously, increased the expression of Bax protein (Hsieh et al., 2006). Furthermore, treatment with AECM, an aqueous extract of *Cordyceps militaris*, was shown to induce apoptosis through the modulation of pro- and antiapoptotic members of the Bcl-2 family and the activation of caspase-3 in human breast cancer MDA-MB-231 cell lines (Jin et al., 2008). Moreover, *Agaricus blazei* (Ling Jin) was revealed as possessing antitumor activities, and extracts from this mushroom induced apoptosis of gastric cancer cell line, MKN45, mediated by the upregulation of caspase-3 mRNA expression and the inhibition of Bcl-2 expression.

The tumor suppressor, p53, is the key component of a system responsible for maintaining the genetic stability of somatic cells and is one of the most commonly inactivated or mutated genes in human cancers (Almazov et al., 2007). Inactivation of p53 is seen in more than 50% of human cancers, and it is known to have a central role in DNA repair and in apoptosis induction (Oren, 1999). Induction of wild type p53 expression is expected to have a beneficial effect on tumors with unmated p53 gene and has been detected in some cancer cell lines following treatment with mushroom substances. For example, extracts prepared from *Pleurotus ostreatus* induced the expression of p53 in colon cancer HT-29 cell lines and suppressed the proliferation of breast and colon cancer cells via p53-dependent pathway in mice with a significant suppression of mutant p53 mRNA expression (Jedinak and Sliva, 2008).

The PI3K/Akt pathway represents a major downstream target of phosphatidylinositol 3-kinase (PI3K), and has been connected to antiapoptotic functions. Treatment with AECM, an aqueous extract of *Cordyceps militaris*, led to a decrease in Akt activation, which was associated with the induction of apoptosis (Jin et al., 2008). Sliva et al. (2002, 2003), Jiang et al. (2004a,b) demonstrated the ability of *Ganoderma lucidum* extracts to induce apoptosis in androgen independent prostate cancer PC-3 cell lines and breast cancer MDA-MD-231 cell lines by the inhibition of the Akt/NF-κB signaling.

Mushroom-derived chemicals were also effective in inducing apoptosis of tumor cell lines using *in vitro* experimental systems. Examples include acetoxyscirpenediol (Figue 13.1m) which was isolated from *C. sinensis* and induced apoptosis in leukemia cell lines. In addition, triterpenes, isolated from *Ganoderma concinnus*, were effective in inducing apoptosis in HL-60 cell line (Lindequist, et al., 2005). Moreover, Grifolin (Figure 13.1l), isolated from the mushroom *Albatrellus confluens*, significantly suppressed the phosphorylation of Akt and its substrates in U2OS and MG63 osteosarcoma cell lines, causing the induction of apoptosis (Jin et al., 2007). Moreover, the presence of gifolin inducted apoptosis that resulted in the increase of Bcl-2/Bax ratio in human nasopharyngeal carcinoma, cervical cancer, breast cancer, and colon cancer cell lines (Ye et al., 2005).

High molecular weight polysaccharides that were isolated from fruiting bodies of *Antrodia camphorate* (AC) decreased the proliferation of human colon cancer cell lines (COLO 205) by inducing apoptosis and significantly increasing the p53 levels (Lien et al., 2009). Moreover, a novel polysaccharide-peptide isolated from cultured mycelia of *Grifola frondosa*, GFPPS1b, induced apoptosis and was associated with the upregulation of Bax, downregulation of Bcl-2, and activation of caspase-3 (Cui et al., 2007).

Mushroom-Substances as Modulators of Angiogenesis

Tumor angiogenesis, formation of new blood vessels, play a critical role in tumor development. Tumors produce growth factors for the stimulation of angiogenesis or induce surrounding normal cells to synthesize and secrete them (Hanahan and Weinberg, 2000). The main growths factors participating in angiogenesis are vascular endothelial cell growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor α (TGFα), and platelets-derived growth factor (PDGF) (Singhal et al., 2005).

Various mushroom-derived extracts and compounds were reported to interfere with the process of angiogenesis. For example, a water extract of the fruiting body of *Grifola frondosa* (maitake

mushroom) was able to inhibit VEGF-induced proliferation, chemotactic migration, and capillary-like tube formation of human umbilical vein endothelial cells (HUVECs) (Lee et al., 2008). Furthermore, Stanley et al. (2005) demonstrated that *Ganoderma lucidum* extract inhibited the early event in angiogenesis, capillary morphogenesis, of the human aortic endothelial cells. These effects were caused by the inhibition of constitutively active AP-1 in prostate cancer PC-3 cells, resulting in the downregulation of secretion of VEGF and TGF-α1.

Mushroom-derived polysaccharides were also reported to affect tumor angiogenesis seen in the case of a polysaccharide isolated from *Agaricus blazei* and shown to downregulate VEGF mRNA and protein expression in treated BALB/c mice (Niu et al., 2009).

Mushroom-Substances as Modulators of Tissue Invasion and Metastasis

During the development of cancer, tumor cells leave the primary tumor, travel to a distant site via the circulatory system and establish a secondary tumor in a process called metastasis. The ability of tumor cells to invade and metastasize enables them to colonize new area in the body that provides space and nutrients for their growth (Hanahan and Weinberg, 2000). Several proteins are involved in the processes of tissue invasion and metastasis such as integrins, cell adhesion molecules, proteases, and others. Integrins are transmembrane receptors that bind to a variety of extracellular matrix molecules, and play a critical role in the attachment of tumor cells to the extracellular matrix. In addition, cell-cell adhesion molecules (CAMs) are members of the immunoglobulin and calcium-dependent cadherin families which mediate cell-to-cell interactions. Changes in the expression of integrins and CAMs play critical roles in the processes of invasion and metastasis. E-cadherin is a homotypic cell-to-cell interaction molecule that is expressed only in epithelial cells. E-cadherin bridges a couple of adjacent cells enabling transmission of signals. Interference with E-cadherin's functions enhances invasion and metastasis of tumor cells. Furthermore, tumor cells have the ability to degrade the environmental barriers such as the extracellular matrix and basement membrane by various proteolytic enzymes, and often induce the production of cell-surface receptors specific for proteins and polysaccharides found in the basal laminae (e.g., collagens, proteoglycans, and glycosaminoglycans). Type IV collagen is a major constituent of the basement membrane, and the matrix metalloproteinases (MMP)-2 and MMP-9 are enzymes that are highly expressed in cancer cells and have the ability to degrade collagen. Tumor cells also express elevated levels of proteases that promote metastasis by helping tumor cells to digest and penetrate the basal lamina such as the serine protease urokinase-type plasminogen activator (uPA) which is highly expressed in cancer cells.

Weng et al. (2009) reported that *Ganoderma lucidum* extract (GLE) reduced the expression level of MMP-9 in the human hepatoma HepG2 cell line. In addition, using a human tumor xenograft model, a dose—response inhibition was observed in the average size, volume, and weight of tumors upon oral administration of GLE. The number of metastatic tumor-bearing mice, the number of affected organs and the number of tumor foci, as well as the MMP-2 and -9 activities in the serum of mice were also significantly suppressed by oral administration of GLE (Weng et al., 2009). Moreover, Cambodian *Phellinus linteus* (CPL) aqueous extract inhibited platelet aggregation induced by invasive melanoma B16BL6 cell lines and also disrupted the adhesion of the cells to gelatin and the invasion of these cell lines. Similarly, CPL inhibited the pulmonary metastatic colonies in C57BL/6 mice intravenously injected by B16BL6 cells. In addition, CPL also downregulated the expression of urokinase type plasminogen activator (uPA) (Lee et al., 2005).

Chemical entities isolated from the mushroom *Ganoderma lucidum* such as ganoderic acid A, F, and H (GA-A, GA-F, GA-H) (Figure 13.1k), suppressed the growth and invasive behavior of MDA-MB-231, a highly invasive human breast cancer cell lines. The biological effects were mediated through the inhibition of transcription factors AP-1 and NF-κB, resulting in the downregulation of Cdk4 expression and the suppression of uPA secretion (Jiang et al., 2008).

CONCLUSIONS

A variety of polysaccharides, including lentinan, scizophyllan, and PSK, isolated from a number of mushroom species, are in use in a number of countries, such as China and Japan, as cancer therapeutics acting as immune-modulators. In this chapter, we focused on the activity of low molecular weight (LMW) substances with anticancer activity. A large body of scientific reports showed that a variety of mushroom extracts or fractions exhibited anticancer activity through targeting a variety of signaling pathways; however, very few were evaluated in animal models or were advanced to either preclinical or clinical evaluations. In the coming years, one might expect that some of the isolated moieties or derivatives will find their way in the development path. Interestingly, in many cases mushroom substances interfere with multiple signaling pathways which raise the possibility that some of the observed anticancer activity is obtained following synergistic activity of the different substances. Researchers should consider this fact while dealing with the clinical advancement of mushroom-derived substances, and probably favor the route of natureceutical additives of active mushroom extracts or fractions for cancer therapeutics rather than the advancement of selected molecular entity that might be more effective in combating cancer.

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14 Dietary Phytochemicals in Prevention and during Cancer Treatment

Ali-Reza Waladkhani

CONTENTS

INTRODUCTION

Diet as one of the most important health factors can strongly influence the occurrence of cancer. Our diet is composed of plant, and animal foods. Plant foods contain a wide array of compounds that may inhibit cancer, thus conferring a potential chemo-protective effect (Steinmetz and Potter, 1991).

Plant foods are rich in phytochemicals and vitamins. Phytochemicals are being evaluated for the prevention of cancer. They can inhibit carcinogenesis by induction of phase II enzymes, and inhibiting phase I enzymes, scavenge DNA reactive agents, suppress the abnormal proliferation of early, preneoplastic lesions, and inhibit certain properties of the cancer cell (Wattenberg, 1992; Sipes and Gandolfi, 1993) (Figure 14.1). Phytochemicals with potentially anticarcinogenic effects are carotenoids, chlorophyll, indoles, polyphenolic compounds, protease inhibitors, sulfides, and terpens.

CAROTENOIDS

Carotenoids include compounds as diverse as α- and β-carotene, lycopene, lutein, and xanthophylls. Carotenoids are found in almost all coloured vegetables. They are a diverse group of over 600 structurally related compounds synthesized by bacteria and plants. They have protective effect against the development of various cancers (Peto et al., 1981).

Lycopene has multiple cellular effects including functioning as an antioxidant (Bohm et al., 1995), inhibition of cell cycle progression, and inhibition of signalling pathways (Karas et al., 2000). Recent animal studies indicated that dietary lycopene can inhibit nonalcoholic steatohepatitis-promoted hepatocarcinogenesis mainly as a result of reduced oxidative stress (Wang et al., 2009). Clinical studies with lycopene indicated that lycopene supplementation in patients with prostate

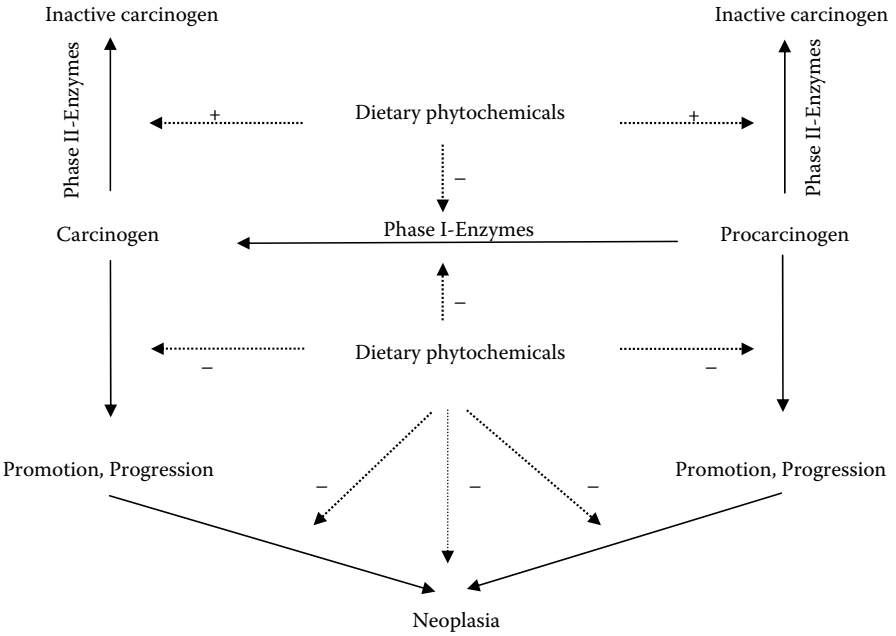

FIGURE 14.1 Relationship between phytochemicals and inactivation of carcinogens. [From Waladkhani A.R. and Clemens M.R. 2008. In *Botanical Medicine in Clinical Practice*. Watson R.R. and V.R. Preedy (eds). CABI Press, 377–387. With permission.]

cancer may decrease the growth of prostate cancer (Kucuk et al., 2001). Several studies have shown that β-carotene prevents DNA damage induced by carcinogenic chemicals (Sarker et al., 1994; Aidoo et al., 1995; Renner, 1995; Uehara et al., 1996). Also, β-carotene has been shown to attenuate hepatic drug metabolising enzymes (Basu et al., 1987), and to inhibit carcinogen activation (Sarker et al., 1994). Recent studies indicate that β-carotene provides protection against ozone-induced skin oxidative stress *in vivo*, which is consistent with a protective role for β-carotene in the skin (Valacchi et al., 2009). A case–control study indicated that consumption of most dietary carotenoids is strongly associated with reduced risk of colorectal cancer. However, smoking significantly attenuated or reversed this observed protective effect on colorectal cancer (Chaiter et al., 2009). In contrast prospective data from the multiethnic cohort study do not support a significant association between carotenoid intake, and colorectal cancer (Park et al., 2009). A recent interventional study in women suggested that a high consumption of carotenoids may reduce the risk of premenopausal but not postmenopausal breast cancer, particularly among smokers (Mignone et al., 2009). In an *in vivo* study with lung epithelial cells, β-carotene increased *in vitro* hydroxyl radical formation in the Fenton reaction, but inhibited the formation of carbon centered radicals (van Helden et al., 2009). Early interventional studies using synthetic β-carotene failed to show the protective effect against cancer (Mayne, 1996).

Processing has the greatest effect on carotenoids bioavailability. Also, human serum lycopene concentrations are greater when heat-processed tomatoes are consumed, as compared with unprocessed tomatoes (Giovannucci et al., 1995). Unprocessed tomatoes contain primarily the *trans* isomer of lycopene, but heat processing converts a substantial amount to the *cis* isomer which may be better absorbed (Schierle et al., 1996). Vine-ripened fruit contains higher concentrations of lycopene than fruit picked green, and ripened in storage, and tomatoes produced in a greenhouse have lower lycopene concentrations than tomatoes produced outside in the summer (Gould, 1992).

CHLOROPHYLL

Chlorophyll is the ubiquitous pigment in green plants. Chlorophyll and their water soluble salts (chlorophyllin) are constituents of the human diet. A number of mechanisms have been proposed for the chemopreventive activity of chlorophyll, and chlorophyllin. Scavenging of reactive oxygen species (Hernaez et al., 1997; Kumar et al., 2001), induction of phase II cytoprotective enzymes (Fahey et al., 2005), and inhibition of xenobiotic transport (Guo and Dashwood, 1994; Mata et al., 2004) are possible mechanisms. Mechanistic studies in mammals, and fish suggest that chlorophyllin acts as a carcinogen-blocking agent, at least in part, by forming tight complexes with aflatoxin B_1 as well as heterocyclic amine, and polycyclic aromatic hydrocarbon carcinogens (Dashwood et al., 1998; Breinholt et al., 1999; Reddy et al., 1999). Animal studies have indicated that administration of natural chlorophyll has substantial protection against aflatoxin B_1 carcinogenesis in the rat liver and colon. The required chlorophyllin to give an overall protection against aflatoxin B_1 induced hepatocarcinogenesis was less than 1500 ppm (Vibeke et al., 1995).

In a clinical chemoprevention trial in eastern China chlorophyllin was tested on a human population with chronic, unavoidably high aflatoxin exposure, and a high incidence of hepatocellular carcinoma (Egner et al., 2001). Administration of 100 mg dietary chlorophyllin three times per day led to a highly significant (50%) reduction in the level of aflatoxin-N7-guanine in the urine of participants (Egner et al., 2001, 2003). Elevated urinary output of this hepatic DNA adduct biomarker in humans is clearly associated with increased risk of liver cancer (Qian et al., 1994), and diminished levels of aflatoxin-N7-guanine have been associated with reduced hepatocellular carcinoma risk in several animal studies (Kensler et al., 1986; Roebuck et al., 1991; Groopman et al., 1992; Yates et al., 2006). Further, chlorophyll inhibits the uptake of aflatoxin B_1 from the rat stomach, and it does so with equal, or greater efficacy than chlorophyllin (Simonich et al., 2007). Formation of direct complexes between the xenobiotic, and chlorophyll or chlorophyllin through interactions between their planar unsaturated cyclic rings would decrease the bioavailability of the xenobiotic agent (Breinholt et al., 1995). In normal human mammary epithelial cell strain, chlorophyllin effectively reduced benzo[*a*]pyrene-DNA adduct formation, and the expression of the benzo[*a*]pyrene-metabolizing genes *CYP1A1*, and *CYP1B1 (*Keshava et al., 2009). In pharmacokinetic compartments outside the gastrointestinal tract such as the liver, serum, and urine, the adduct burden was reduced over a 2- to 13-fold range by chlorophyll coexposure, whereas in the feces of the same animals, roughly 5-fold more aflatoxin B_1 equivalents were eliminated relative to the control animals. Thus, chlorophyll coexposure largely restricted aflatoxin B_1 to the gastrointestinal tract. Moreover, substantial protection by chlorophyll against aberrant crypt focus development in the colon suggests that, in addition to restricting aflatoxin B_1 to the gastrointestinal tract, chlorophyll treatment reduced aflatoxin B_1 metabolism to toxic intermediates in the colon (Simonich et al., 2007). Complex formation

TABLE 14.1
Some Dietary Sources of Phytochemicals

Carotenoide	Apricot, peach, nectarine, orange, broccoli, cabbage, spinach, pea, pumpkin, carrots, tomato, cabbage, apples, olives, red wine, soy products
Flavonoids	Green tea, black tea, citrus fruits, onion, broccoli, cherry, wheat, corn, rice, tomatoes, spinach
Polyphenole	Grapes, strawberries, raspberries, pomegranate, paprika, cabbage, walnut
Protease inhibitors	Soy bean, oats, wheat, peanut, potato, rice, maize
Sulfide	Cabbage, chives, allium, onion, garlic
Terpene	Grape fruits, lemons, limes, oranges, lavander, mint, celery seeds, cherries

Source: From Waladkhani, A.R. and Clemens, M.R. 1998. *International Journal of Molecular Medicine* 1, 747–753. With permission.

appears to block carcinogen absorption, thereby reducing bioavailability to the target tissue resulting in less DNA adduction, and, ultimately, lower tumor incidence (Dashwood et al., 1998). Further, hepatic DNA adduct, and serum albumin adduct burdens were dramatically reduced in chlorophyll-coexposed rats compared with controls (Simonich et al., 2007). In the rat colon, dietary spinach, or an equimolar amount of chlorophyll equally inhibited cytotoxicity, and colonocyte proliferation induced by heme, a red meat, iron-containing pro-oxidant correlated with increased risk of colon cancer (Sesink et al., 2000; de Vogel et al., 2005). Also the chlorophyll-containing diet largely blocked formation of a cytotoxic heme metabolite (de Vogel et al., 2005). Dietary heme injures colonic surface epithelium, which is overcompensated by inhibition of apoptosis, and hyperproliferation of cells in the crypts, resulting in crypt hyperplasia (de Vogel et al., 2008). In contrast, chlorophyllin largely failed to inhibit the damaging effects of heme metabolism (de Vogel et al., 2000).

The reported concentration of chlorophyll in spinach isolates is in the range of 1500–600,000 ppm, depending on agronomic conditions. Also spinach leaves may contain between 2.6% and 5.7% of their dry weight as chlorophylls (Khalyfa et al., 1992). Dietary chlorophyll intake comparable with the 100 mg per meal is obtainable by moderate/high consumption of green vegetables.

INDOLE

In ancient times cruciferous vegetables were cultivated primarily for medicinal purposes (Fenwick et al., 1983). The biologically active compound is glucobrassicin, a secondary plant metabolite that is abundant in cruciferous vegetables (McDanell et al., 1986) (Table 14.2). Glucosinolates are not bioactive in the animal that consumes them until they have been enzymatically hydrolyzed to an associated isothiocyanate (Rouzaud et al., 2003) by the endogenous myrosinase enzyme that is released by the disruption of the plant cells through harvesting, processing, or mastication. The hydrolysis products of common glucosinolates are as follows (Keck and Finley, 2004):

Glucoraphanin: converted to sulforaphane, and sulforaphane nitrile
Sinigrin: converted to allyl isothiocyanate
Glucobrassicin: converted to indole-3-carbinol
Gluconasturtin: converted to phenethyl isothiocyanate

Glucosinolates have been found to act as blocking, and/or suppressing agents against several experimental models of cancers, such as colon (Xu et al., 1997), liver (Oganesian et al., 1999), lung

TABLE 14.2
Glucosinolate Content of Cruciferous Vegetables

Vegetable	Range (μg/g)
Broccoli	450–1480
Brussels sprouts	600–3900
Cauliflower	270–830
Red cabbage	470–1240
White cabbage (kraut)	430–760
White cabbage	260–1060

Source: Adapted from Fenwick, G.R., Heaney, R.K., and Mullin, W.J. 1983. *Critical Reviews in Food Science and Nutrition* 18, 123–201; Flavor and Extract Manufacturers Association. 1991. *D-Limonene Monograph, 1–4*. Flavor and Extract Manufacturers™ Association, Washington, DC.

(Hecht, 1999), and mammary tumors (Wattenberg, 1981). *In vitro*, and *in vivo* studies have shown that isothiocyanates affect many steps of cancer development including modulation of phase I, and II detoxification enzymes (Talalay and Fahey, 2001), functioning as a direct antioxidant (Zhu and Loft, 2003), or as an indirect antioxidant by phase II enzyme induction (Talalay and Fahey, 2001; McWalter et al., 2004), modulating cell signalling (Xu and Thornalley, 2001), induction of apoptosis (Yu et al., 1998; Chiao et al., 2002; Yang et al., 2002), control of the cell cycle (Yu et al., 1998; Wang et al., 2004), and reduction of *Helicobacter pylori* infections (Fahey et al., 2002). There are many glucosinolate breakdown products with chemoprotective activity. Phenethyl isothiocyanate has been shown to inhibit the tumorigenic effects of various carcinogens. *In vivo* pretreatment with phenethyl isothiocyanate inhibits mammary tumors, stomach, pulmonary adenomas (Wattenberg, 1992), and lung tumors (Morse et al., 1990) induced by carcinogens. Further, in rodents, aryl isothiocyanates exert chemopreventive effects against lung (Morse et al., 1990; Jiao et al., 1994; Nishikawa et al., 1996), esophagus (Morse et al., 1993; Stoner et al., 1998), mammary, and stomach (Wattenberg, 1992) carcinogenesis. Phenethyl isothiocyanate significantly inhibits pancreatic carcinogenesis in hamsters (Nishikawa et al., 1996). Feeding animals diets high in cruciferous vegetables and then exposing them to various carcinogens expressed lower tumor yields and increased survival rates (Boy et al., 1982; Wattenberg, 1983). Indole-3-carbinol administration is known to induce cytochrome P450, and glutathione-*S*-transferase activities, resulting in increased metabolic capacity toward chemical carcinogens (Morse et al., 1990). Two interventional studies with glucosinolates in humans showed decreased markers of oxidative damage with consumption of Brussels sprouts (Bogaards et al., 1994; Verhagen et al., 1997). Results from one cross-national study found that those countries with higher cabbage intake had a lower breast cancer mortality rate (Hebert andRosen, 1996). In three small human intervention studies, the daily administration of indole-3-carbinol pills (400 mg/day), or broccoli (500 g/day) significantly increased the urinary 2-hydroxyestrone: 16-hydroxyestrone ratio (Michnovicz and Bradlow, 1991; Bradlow et al., 1994; Kall et al., 1996) consistent with reduced breast cancer risk.

Large quantities of inducers of enzymes that protect against carcinogens can be delivered in the diet by small quantities of young crucifer sprouts (e.g., 3-day-old broccoli sprouts) that have 10–100 times more inducer activity than mature vegetables (Fahey et al., 1997). Glucobrassicin, and glucoraphanin are generally found in high concentrations in broccoli, and constitute as much as 95% of

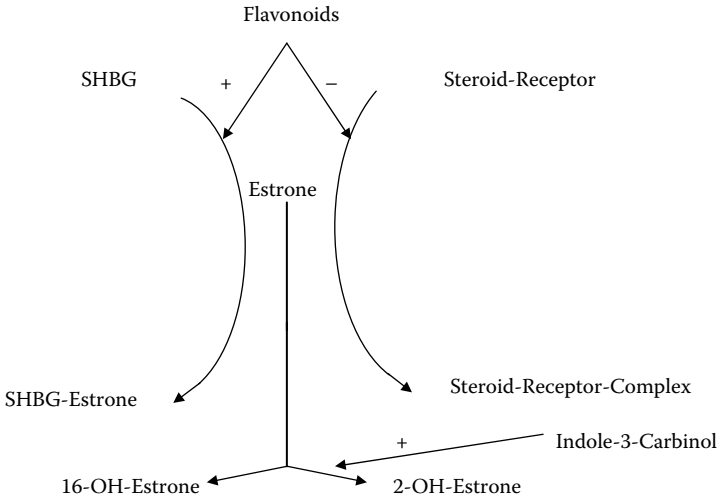

FIGURE 14.2 Possible inhibiting mechanisms of phytochemicals in breast cancer development. (Adapted from Waladkhani A.R. and Clemens, M.R. 2003. In *Functional Foods and Nutraceuticals in Cancer Prevention*. Watson, R.R. (ed.). Iowa State Press, Iowa, pp. 179–197.)

the total amount of glucosinolates (Kushad et al., 1999). Gluconasturtiin is abundant in Chinese cabbage, radishes, and watercress (Fenwick et al., 1983). The average total glucosinolates content of Brussels sprouts was twice that of broccoli, the average glucoraphanin content of broccoli was 7-fold that of Brussels sprouts (Kushad et al., 1999). Glucosinolates, and glucosinolate breakdown products are hydrophilic, and as much as 63% of the glucosinolate content of a vegetable may leach into the cooking water during boiling (Sepkovic et al., 1994). Steaming provides less opportunity for leaching, and stir-fried vegetables retain glucosinolate levels (Betz and Obermeyer, 1993). Light cooking may disrupt plant cell membranes without leaching of the indoles into the cooking medium, providing the opportunity for myrosinase to release these indoles for eventual conversion to indolo[3,2-*b*]carbazole (Kwon et al., 1994; Sepkovic et al., 1994).

POLYPHENOLS

Phenolic acids are abundant in foods. After consumption, the plant flavonoids undergo many metabolic conversions by intestinal bacteria, and both the metabolites, and parent compounds are absorbed into the blood, and then excreted, mainly in the urine (Adlercreutz and Mazur, 1997). The most frequently encountered are caffeic acid, and, to a lesser extent, ferulic acid. Ferulic acid is associated with dietary fiber, and is linked through ester bonds to hemicelluloses. It is commonly found in fruits, and vegetables such as tomatoes, sweet corn, and rice (Srinivasan et al., 2007). The ferulic acid content of wheat grain is approximately 0.8–2 g/kg dry wt, which may represent up to 90% of total polyphenols (Lempereur et al., 1997). Ferulic acid is found chiefly in the outer parts of the grain. The aleurone layer, and the pericarp of wheat grain contain 98% of the total ferulic acid. Rice, and oat flours contain approximately the same quantity of phenolic acids as wheat flour (63 mg/kg), the content in maize flour is about three times higher (Shahidi and Naczk, 1995). Animal studies have shown that ferulic acid exhibits anticarcinogenic effects against azoxymethan-induced colon carcinogenesis (Kawabata et al., 2000). Further, it depresses 12-*O*-tetradecanoylphorbol-13-acetate-promotion of skin tumorigenesis (Asanoma et al., 1994).

Proposed chemopreventive mechanisms of polyphenolic compounds include induction of apoptosis (Gao et al., 2002; Shiono et al., 2002; Hofmann and Sonenshein, 2003; Hsu et al., 2003). Polyphenols may block initiating attacks on DNA (Newmark, 1984), regulate cell signal pathways (Yeh et al., 2003), and may be cytotoxic, for example, resveratrol (Hadi et al., 2000). Resveratrol is synthesized by a wide variety of plant species, including aliments such as grape skins, peanuts, and mulberries, in response to injury, UV irradiation, and fungal attack (Langcake and Pryce, 1976). It is synthesized in the leaf epidermis, and the skin of grape berries, but not in the flesh (Creasy and Coffee, 1988). Resveratrol was shown to induce phase II metabolism, and inhibit cyclo-oxygenase (Jang et al., 1997). Piceatannol a metabolite of resveratrol can protect cultured neurons against A-beta-induced death (Kim et al., 2007). LMP2A, a viral protein-tyrosine kinase implicated in leukaemia, and non-Hodgkin lymphoma which are associated with Epstein–Barr virus, can be blocked by picetannol *in vitro* (Geahlen and McLaughlin, 1989).

Curcuminoids interact with the ATP binding cassette (ABC) drug transporters with very high affinity, and inhibit transporters mediated drug resistance. ABC-B1 (Anuchapreeda et al., 2002; Chearwae et al., 2004), and ABC-C1 (Chearwae et al., 2006b), which are known to play a major role in the development of multiple drug resistance in cancer cells. Curcuminoids also may play an important role in clinical therapy, and the simultaneous administration of recommended therapeutic doses of curcuminoids with anticancer drugs would probably result in an increased bioavailability of the drugs inside the cells (Chearwae et al., 2006a). Curcumin has been considered as a potentially important chemopreventive agent against cancer (Kelloff et al., 1997). Studies demonstrated an inhibitory effect of dietary curcumin when administered continuously during the initiation, and postinitiation phases (Kawamori et al., 1999). Administration of curcumin also may retard growth, and/or development of existing neoplastic lesions in the colon (Kawamori et al., 1999). Further, curcumin also may modulate arachidonic acid metabolism (Rao et al., 1995b). Polyphenols may

affect cell cycle arrest. Also, cell cycle arrest has been induced by curcumin (Hanif et al., 1997), and green tea polyphenol (Jia et al., 2002). Tea polyphenols in concentrations of 100 μM, and 30 μM induce apoptosis, showing apoptosis indices of about 80% and 8–26%, respectively (Yang et al., 1998). Theaflavins from black tea exhibit growth inhibitory activities in cancer cells, although the activities are lower than those with (–)-epigallocatechin (EGC), and (–)-epigallocatechin-3-gallate (EGCG) (Yang et al., 1998). Inhibition of tumorigenesis in mice could be demonstrated by the average EGCG, and EGC concentrations in the range of 0.2 to 0.3 μM (Yang et al., 1996). After ingestion of two, or three cups of tea by human subjects, the average peak plasma values of EGCG, and EGC were also in this range, and the highest individual values observed for EGCG, and EGC were 0.65 μM (Lee et al., 1995).

Quercetin is a glycoside of flavonoids such as rutin, and quercetin found in buckwheat, citrus fruit, and onions. *In vitro* studies quercetin has shown remarkable inhibitory effect on the activities of cytosolic protein kinase C, and trans membrane protein kinase, with the half maximal inhibitory concentration values of about 30.9, and 20.1 μM, respectively, but did not have the effect on membrane protein kinase C or cytosolic protein kinase C activity (Kang and Liang, 1997). Also, treatment with quercetin (5.5 μM) activated both apoptosis, and differentiation programs. After 1 hour exposure to the drug it resulted in apoptosis of the leukemia cells. Differentiation of K562 cells was observed at least after 12 hours of exposure which could be resulted by the early downregulation of c-myc, and Ki-ras oncogenes, and rapid reduction of inositol-1,4,5-triphosphate concentrations (Csokay et al., 1997). Quercetin may act differently on cancer, and normal neuronal tissue. Quercetin decreased the cell viability in glioma cell cultures resulting in necrotic, and apoptotic cell death. It also arrested the glioma cells in the G_2 checkpoint of the cell cycle, and decreased the mitotic index. Furthermore, quercetin protected the hippocampal organotypic cultures from ischemic damage (Braganhol et al., 2006). Recent studies indicated that quercetin has inhibitory effect on MDA-MB-435 cell growth in a time, and dose-dependent manner. The cell cycle analysis of quercetin treated cells showed significant increase in the accumulation of cells at subG1 phase (Choi et al., 2008).

Proanthocyanidins are polyphenolic compounds derived from common dietary foods such as grapes, cranberries and almonds, as well as chocolate, and cacao beans (Bagchi et al., 2000; de Pascual-Teresa et al., 2000; Gu et al., 2004; Seeram et al., 2004). Recent evidence suggests that proanthocyanidins exhibit cytotoxicity against some cancers, including colon, breast, and prostate cancers (Tyagi et al., 2003; Kim et al., 2004; Vayalil et al., 2004; Katayar 2006; Kaur et al., 2006; Matchett et al., 2006). Moreover, studies involving raspberry-, grape-, and grape seed-derived proanthocyanidins have recently demonstrated selective inhibition of oral cancer phenotypes, particularly in oral squamous cell carcinomas (Shirataki et al., 2000; Rodrigo et al., 2006; Chatelain et al., 2008). Grape seed extract, and cranberry extract treatments significantly reduced cell growth, and proliferation of both oral tumor cell lines between 30%, and 50%, compared with the non-treated controls (Chatelain et al., 2008).

The polymethoxyflavones, including tangeretin, sinensetin, and nobiletin has been found in high concentrations in the peel of various citrus species whereas the many hydroxylated flavones clearly predominate in the juice (Lu et al., 2006; Nogata et al., 2006). Nobiletin has previously been reported to induce differentiation of mouse myeloid leukemia cells (Sugiyama et al., 1993), to exhibit antiproliferative activity toward a human squamous cell carcinoma cell line (Kandawaswami et al., 1991), to exert antimutagenic activity (Wall et al., 1998), and to suppress the induction of matrix metalloproteinase-9 (Ishiwa et al., 2000). Nobiletin also suppressed the expression of cyclo-oxygenase-2, and inducible NO synthase proteins, and prostaglandin E2 release (Murakami et al., 2000). Recent studies have shown an antimetastatic effect for nobiletin. Also, nobiletin significantly decreased peritoneal dissemination of stomach cancer nodules, when nobiletin was administered subcutaneously to mice through an osmotic minipump (Minagawa et al., 2001). Tangeretin, and nobiletin given to brain tumor cell cultures have the potential to retard motile, or invasive behaviour (Rooprai et al., 2001).

Soy contains significant amounts of the isoflavones daidezein, and genistein (Price and Fenwick, 1985). Isoflavones, and flavones have been shown to be agonists of estrogen-related receptors

(Suetsugi et al., 2003). Also, there is a modest inverse association between high soy food consumption, and reduced cancer risk (Trock et al., 2006; Wu et al., 2008). Increased levels of estrogens in blood, and urine are markers for high risk for breast cancer (Bernstein et al., 1990; Toniolo et al., 1995). In a large prospective, case–control study there was a significant relationship between serum estrogen levels and the risk for breast cancer in women in New York (Toniolo et al., 1995). Goldin et al. (1986) reported 44% lower blood levels of estrogen, and androgens in oriental women who emigrated to the United States from areas of low breast cancer risk, when compared with Caucasian Americans, who have a higher risk for breast cancer. Women newly diagnosed with breast cancer excreted substantially lower levels of urinary isoflavonoids, and phenols than controls, and high excretions of these compounds were associated with a substantially reduced risk of breast cancer. In particular, high excretions of phenols, and total isoflavonoids were associated with an 85% statistically significant reduced risk of breast cancer (Zheng et al., 1999). Epidemiological studies indicated that genistein/soy exposure during the period preceding puberty reduces later susceptibility to develop breast cancer (Shu et al., 2001; Wu et al., 2002b). In ovarian cancer cells, treatment with the genistein at a pharmacologic dose of 50 μmol/L resulted in apoptosis, and a caspase-independent cell death that exhibited hallmarks of autophagy and was accompanied by inhibition of Akt activation, and glucose uptake (Gossner et al., 2007). Some isoflavonoids have been shown to stimulate the synthesis of sex hormone-binding globulin, and decreasing blood levels of free estrogens that are more available to the target tissues. Further, they may inhibit important steroid biosynthetic enzymes, including aromatase, and 17β-hydroxysteroid dehydrogenase I, thus affecting the level of circulating estrogens (Adlercreutz and Mazur, 1997).

PROTEASE INHIBITORS

Protease inhibitors (PIs) play an important role in the plant defense response to mechanical wounding, which occurs during herbivorous insect feeding (Johnson et al., 1989; Boulter et al., 1990). They are present in seeds, flowers, leaves, and roots and are differentially expressed in tissues over time (Jokufu and Goldberg, 1989; McGurl et al., 1995). Epidemiological, and experimental data suggest that PIs have significant anticarcinogenic activity (Kennedy, 1998). Different types of PIs have been

TABLE 14.3
Some Dietary Sources of Polyphenols

Polyphenol	Source	Range	
Anthocyanins	Cherries (fresh fruit)	4.5	mg/g
	Strawberries (fresh fruit)	0.15	mg/g
	Red wine	26	mg/L
Catechins	Black tea	0.5	g/L
	Green tea	1	g/L
	Red wine	270	mg/L
	Young shoots, dry leaves	200–340	mg/g
Chlorogenic acid	Instant coffee	250–750	mg/L
Ferulic acid	Wheat bran	5	mg/g
Hespeidin	Orange (juice)	125–250	mg/L
Isoflavones (genistein and diadzein)	Soy dry bean	1	mg/g
Quercetin	Onion (fresh)	0.3	mg/g
	Tea	10–25	mg/L
Resveratrol	Red wine	0.3–2	mg/L

Source: From Waladkhani A.R. and Clemens M.R. 2008. In *Botanical Medicine in Clinical Practice.* Watson R.R. and V.R. Preedy (eds). CABI Press, 377–387. With permission.

shown to inhibit the carcinogenic process, and on a molar basis those that inhibit chemotrypsin proteases have been the most effective. A number of clinically important PIs have been found in serum, including α2-protease inhibitor, α2-macroglobulin, α1-antichymotrypsin, α1-acid glycoprotein, and so on-. Many of these PIs are referred to as acute-phase reactants, and are active against serine proteinases (Clawson, 1996). Clinical trials where cancer patients were treated with broad-range protease inhibitors have shown that proteases can act as tumor suppressors (Lopez-Otin and Matrisian, 2007). Potentialy important effects of dietary PIs may occur via hormonal modulation and inactivation of trypsin-, and chymotrypsin in the duodenum. Potato carboxypeptidase inhibitor suppressed the growth of several human pancreatic adenocarcinoma cell lines, both *in vitro*, and in nude mice (Blanco-Aparicio et al., 1998). The Bowman-Birk inhibitor (BBI) which is abundant in soybeans inhibits both trypsin-, and chymotrypsin-like proteases (Birk, 1985), for example, trypsin, and chymotrypsin (Birk, 1985), cathepsin G (Larionova et al., 1993), and elastase (Larionova et al., 1993). BBI is resistant to degradation in the digestive system, and a large proportion is adsorbed into the circulation (Billings et al., 1992). BBI is a potent anticarcinogenic agent that inhibits chemical carcinogen-, and radiation-induced malignant transformation *in vitro*, and suppresses carcinogenesis in several organ systems, and animal species (Kennedy, 1998). Animal studies indicated that addition of BBI, used in the form of BBI concentrate, caused a reduced risk of developing malignant lymphoma in animals exposed to space radiation, and maintained on diets containing the BBI concentrate compared with the irradiated animals maintained on the control diet (Kennedy et al., 2008). BBI, in the form of BBI concentrate, has achieved Investigational New Drug status with the U.S. Food and Drug Administration. In a recently completed phase IIa oral cancer chemoprevention trial in patients with premalignant lesions known as oral leukoplakia, treatment with BBI concentrate at daily doses of 200–1066 chymotrypsin inhibition units for one month led to a dose-dependent decrease in oral leukoplakia lesion size (Meyskens et al., 1999). Recent studies showed synergistic interactions between soybean bioactive compounds BBI, and genistein in inhibiting NO (92.7%), and prostaglandin E2 (95.6%) production (Dia et al., 2008). The relatively rapid urinary excretion, and the lack of BBI accumulation in human subjects after the ingestion of BBI-containing soymilk suggest that daily intake of BBI may be necessary to achieve optimal cancer chemopreventive effects.

Pericellular proteinases are known to play key roles in angiogenesis (van Hinsbergh et al., 2006). Angiogenesis plays a vital role in the development, and progression of various pathological conditions such as rheumatoid arthritis, diabetic retinopathy, and tumor metastasis (Folkman, 1995). The proteinase inhibitors of serine proteinases belonging to the Kunitz, Kazal, α-macroglobulin, and serpin families play critical roles in physiological, and pathophysiological states such as coagulation, intravascular fibrinolysis, wound healing, angiogenesis, and tumor metastasis (Potempa et al., 1994; Rao et al., 1995a). Kunitz-type serine proteinases inhibit the proliferation of basic fibroblast growth factor induced endothelial cells in addition to their inhibitory activity against tissue factor-mediated blood coagulation cascade (Hembrough et al., 2001).

Protease inhibitors are major constituents of seeds of some plants, including soybean. In soybean seeds, there are two major classes of serine protease inhibitors, the BBI, and the Kunitz trypsin inhibitors. These inhibitors may comprise up to 6% of the total protein in the seed. Their content varies considerably with species of soy (Eldridge and Kwolek, 1983), and PI content of several soy protein isolates can vary by as much as 20-fold. The BBI content in soy flour can be as high as 5.5 mg/g (Brandon et al., 1991). Also, 1 g of textured soy protein, tofu, dry cereal, or pancake mix contains 0.48, 0.08, 0.10, or 0.38 mg of BBI, respectively (Di Pietro and Liener, 1989).

SULFIDES

The *Allium* genus includes approximately 500 species, the most widely used of which are onions (*Allium cepa*), garlic (*Allium sativum*), leeks (*Allium porrum*), chives (*Allium schoenoprasum*), and shallots (*Allium ascalonicum*). Organosulfur compounds present in *Allium* vegetables, which are

either lipid or water soluble, are considered responsible for the beneficial effects of these herbs. *In vitro* studies indicated antiproliferative, and apoptotic effects of allyl sulfides on cancer cells (Le Bon and Siess, 2000; Thomson and Ali, 2003). Diallylsulfide (DAS) generally inhibited cancers of the forestomach, colon, esophagus, mammary gland, and lung (Table 14.4) (Ip et al., 1992; Sparmins et al., 1988). Diallyltrisulfide (DATS), and allylmethylsulfide (AMTS) inhibited forestomach cancer. DATS, which contains two allyl groups, has been shown to be more potent than AMTS, the analogue derivative with only one allyl group (Sparmins et al., 1988). Some studies have demonstrated a correlation between their biological potency, and the number of sulphur atoms (Wu et al., 2002a). Also, the number of sulfur atoms on the molecule can influence the degree of protection, with DATS > diallyldisulfide (DADS) > DAS (Tsai et al., 1996; Sakamoto et al., 1997). DADS, and DATS are far more effective in retarding the growth of neoplasms than water soluble allyl sulfur compounds such as *S*-allyl cysteine (Sundaram and Milner, 1993).

In human colon cancer cells, DATS induced oxidative modification of specific cysteine residues in the β-tubulin molecule to form *S*-allylmercaptocysteine, and that this could be the sole cause of cell cycle arrest, and successive apoptosis with activation of caspase-3 (Hosono et al., 2005). The antitumorigenic effects of water-soluble *S*-allylmercaptocysteine, and lipid-soluble DAS, and DADS relate both to a suppression in cell division, and to an induction of apoptosis (Sundaram et al., 1996; Nakagawa et al., 2001; Shirin et al., 2001). The ability of DADS to disrupt cell division corresponds to suppressions in $p34^{cdc2}$ kinase activity, and a block in the progression of cells from the G_2 into the M phase of the cell cycle (Knowles and Milner, 1998). Cytochrome P450 2E1 (CYP2E1) appears to be the one that is particularly vulnerable to the effects of allyl sulfur compounds (Jeong and Lee, 1998; Yang, 2001). An autocatalytic destruction of CYP2E1 has been demonstrated, and may account for the chemo-protective effects of DAS, and possibly other allyl sulfur compounds against some chemical carcinogens (Jin and Baillie, 1997). In mice, the oral application of DAS suppressed the activity of ornithine decarboxylase (Baer and Wargovich, 1989). Depressed carcinogen bioactivation because of reduction in cyclooxygenase, and lipoxygenase activity may also account for some of the lower incidence of tumors after treatment with some carcinogens (Rioux and Castonguay, 1998; Roy and Kulkarni, 1999). *In vitro* studies with human leukaemia HL-60 cells DADS induces apoptosis through the production of reactive oxygen species, and subsequent activation of caspase-3 pathway (Zhang et al., 2008). In studies of the effect of DADS on the growth of transplanted human colon carcinoma cell lines in immunologically compromised nude mice DADS was as effective as 5-FU in inhibiting tumor growth. Combining the DADS, and 5-FU did not increase the effect, but concurrent DADS treatment did significantly reduce the depression of leukocyte counts, and splenic weight associated with chemotherapy administration (Sundaram and Milner, 1996). In Wistar rats, DADS treatment strongly increased all the phase II enzymes activities examined, that is, total

TABLE 14.4
Allyl Sulfides with Antineoplastic Properties

Sulfur Compound	Cell Type
Ajoene	Lymphocytes, colonic, leukemic
Allicin	Lymphoid
Diallyl sulfide	Prostate, leukocytes
Diallyl disulfide	Lung, colonic, skin, prostate, mammary
Diallyl trisulfide	Lung
S-Allyl cysteine	Neuroblastoma, melanoma
S-Allylmercaptocysteine	Prostate, mammary

Source: Adapted from Milner, J.A. 2001. *Journal of Nutrition* 131, 1027S-1031S.

glutathione *S*-transferase activity, quinone reductase activity, and epoxide hydrolase activity (Guyonnet et al., 2001). In an *in vivo* assay intraperitoneal injection of 1, or 2 mg DADS three times a week from the day of tumor cell inoculation until the end of the experiment (after 35 days) caused growth retardation, and 43% reductions in primary tumor weight (Nakagawa et al., 2001).

TERPENS

The terpenoids constitute the largest family of natural products. Over 22,000 individual compounds of this class have been described (Connolly and Hill, 1991). Monoterpenoids are commonly produced by plants, and found in many fruits and vegetables. The principal sources of d-limonene in the diet are the oils of orange, grapefruit, and lemon (Kesterson et al., 1971). D-Limonene has chemopreventive, and chemotherapeutic activities against rodent mammary, liver, and pancreatic tumors (Elegbede et al., 1986; Nakaizumi et al., 1997; Kaji et al., 2001). The chemopreventive efficacy of d-limonene during both the initiation and promotion stages of carcinogenesis has been demonstrated in chemically induced rodent skin (Elegbedge et al., 1986), kidney (Dietrich and Swenberg, 1991), lung, and forestomach (Wattenberg and Coccia, 1991), and mammary (Haag et al., 1992; Gould et al., 1994) tumor model systems. In mammary carcinoma, d-limonene exhibits therapeutic effects against chemically induced mammary tumors in rats, with regression of >80% of carcinomas with little toxicity (Haag et al., 1992). In human studies D-limonene show low toxicity after single and repeated dosing for up to one year (Sun, 2007). D-Limonene and its *in vivo* plasma metabolites have been shown to be the inhibitors of protein isoprenylation of small G proteins, including p21 ras in rats (Bourne et al., 1991). In addition to selectively blocking the isoprenylation of small G proteins, d-limonene has also been shown to have additional cellular effects, including the inhibition of coenzyme Q synthesis, and it is also capable of causing the complete regression of the majority of advanced primary rat mammary carcinomas without significant toxicity (Haag et al., 1992). *In vitro* experiments indicated that nontoxic low doses of d-limonene in the combination treatments with docetaxel enhance significantly apoptotic effect on hormone-refractory DU-145 prostate cancer cells (Rabi and Bishayee, 2009).

Perillyl alcohol is found in small concentrations in the essential oils of lavendin, peppermint, spearmint, sage, cherries, cranberries, perilla *(Perilla frutescens)*, lemongrass, wild bergamot, gingergrass, savin, caraway, and celery seeds (Kelloff et al., 1996). As little as 2000 ppm of perillyl alcohol in the diet can inhibit azoxymethane-induced adenoma, and adenocarcinoma development in rat colons (Kelloff et al., 1996). Transformation of colorectal epithelium to adenomas, and carcinomas has been shown to be associated with a progressive inhibition of apoptosis. The chemopreventive activity of perillyl alcohol during the promotion phase of liver carcinogenesis is associated with a marked increase in tumor cell death by apoptosis, or programmed cell death (Mills et al., 1995). In *in vivo* studies perillyl alcohol showed chemotherapeutic effects on liver and pancreatic cancer (Stark et al., 1995; Burke et al., 1997). In murine models that use human pancreatic cancer cells, perillyl alcohol exhibits significant chemopreventive effects. The tumor growth in perillyl alcohol treated animals was significantly delayed, and survival of animals was prolonged (Lebedeva et al., 2008). The chemopreventive efficacy of perillyl alcohol is probably due to the inhibition of oxidative stress responses, inhibition of the Ras cell proliferation pathway, and induction of apoptosis in murine skin tumor promotion phase (Chaudhary et al., 2009). Perillyl alcohol has been shown to be at least five times more potent than D-limonene in inhibiting rats mammary carcinogenesis (Haag and Gould, 1994). In humans, the three metabolic derivatives detected in plasma after single oral doses of 100 mg/kg limonene are perillic acid, dihydroperillic acid, and limonene-1,2-diol (Crowell et al., 1994).

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15 Effect of Phytochemicals on Stress Management and Mental Health

Ali-Reza Waladkhani

CONTENTS

INTRODUCTION

Diet as one of the most important lifestyle factors can strongly influence the stress susceptibility, neurodegenerative disorders, and mental health. Our diet is composed of plant, and animal foods. Plant foods which are rich in phytochemicals, and vitamins show a variety of positive effects on health. Due to their positive influences on stress, mood, and cognitive function plant foods might be preferred (Waladkhani and Hellhammer, 2008). Human studies indicate that persons who eat green or yellow vegetables every day show a lower incidence of the stress syndrome (irritation, sleeplessness) than those who do not eat them daily (Hirayama, 1992). Fruit, vegetables, and common beverages as well as herbs have been shown to be rich sources of the micro chemicals with different healthy effects. Due to ignorance, and indifference they are not identified as nutrients and essential to health.

The food chemists, and natural product scientists have identified hundreds of the phytochemicals, for example, carotenoids, chlorophyll, polyphenols, and sulfides. They have the potential to modulate stress. Also, these compounds may be responsible for the multitude of beneficial effects that have been reported for fruits, and vegetables, with an array of health-related bioactivities. Phytochemicals can selectively regulate multiple signal pathways at the level of transcription, especially these involving mitogen-activated protein kinase (Frigo et al., 2002). *In vitro* studies suggested that flavonoids such as delphinidin inhibit endothelial cell proliferation, and cell cycle progression through extracellular signal-regulated kinase activation (Martin et al., 2003).

Extracts of various fruits, and vegetables exhibit neuroprotective properties in cell culture, and animal models that are relevant to the pathogenesis of many different neurodegenerative conditions including stroke, Alzheimer's disease (AD), and Parkinson's disease (Joseph et al., 2005; Mattson and Cheng, 2006). Animal studies indicated that long-term feeding (from 6 to 15 months of age) of rats with a diet supplemented with strawberry, or spinach extract (1–2% of the diet) retard

age-related decrements in cognitive, and neuronal function (Joseph et al., 1998). Feeding studies with tea (Unno et al., 2004; Chan et al., 2006), grape juice (Shukitt-Hale et al., 2006), or flavonols such as quercetin (Patil et al., 2003; Singh et al., 2003) were beneficial in reversing the course of neuronal, and behavioral aging. Such effects can be mediated by stimulation of hippocampal neurogenesis (Casadesus et al., 2004), modulation of neurotransmitter release (Joseph et al., 1998, 1999), and changes in neuronal signalling (Goyarzu et al., 2004; Joseph et al., 2005). Feeding studies with rats from 19 to 21 months of age indicated that aqueous strawberry, spinach, and particularly blueberry extracts were able to reverse several parameters of the brain aging (e.g., deficits in cell communication) such as dopamine release (Joseph et al., 1999; Youdim et al., 2000), and age-related motor and cognitive deficits (Joseph et al., 1999; Bickford et al., 2000; Youdim et al., 2000). Reversals in age-related declines might be accomplished by increasing the dietary intake of the fruits, and vegetables (Cao et al., 1997; Wang et al., 1996). Also, consumption of green tea (Haque et al., 2006), blueberries (Joseph et al., 1999), pomegranates (Hartman et al., 2006), and strawberries enhance memory, and synaptic plasticity (Maher and Akaishi, 2006).

Carotenoids

Carotenoids are found in almost all colored vegetables. They are a diverse group of over 600 structurally related compounds synthesized by the bacteria, and plants. Human studies indicated that β-carotene suppresses the secretion of CRH in a dose-dependent manner (Hasegawa, 1993). It has been suggested that CRH is a neurotransmitter as well as a neurohormone (Tellam et al., 2000).

It has been suggested that the effective site of β-carotene is the hypothalamus, where β-carotene suppresses the secretion of CRH induced by the exercise stress, and consequently the secretion of ACTH in the pituitary. While CRH stimulates the sympathetic neurons (Kurosawa et al., 1986), β-carotene inhibits the stimulation of noradrenaline, and adrenaline secretion through the suppression of CRH secretion (Hasegawa, 1993). Increases of CRH, ACTH, and cortisol levels in anticipation of or during stressful stimulation are interpreted as allostatic (Munck, 1971).

Chlorophyll

Chlorophyll is the ubiquitous pigment in green plants. Chlorophyll, and their water soluble salts (chlorophyllin) are constituents of the human diet. The reported concentration of chlorophyll in spinach isolates is in the range of 1500–600,000 ppm depending on agronomic conditions. Also spinach leaves may contain between 2.6% and 5.7% of their dry weight as chlorophylls (Khalyfa et al., 1992). Dietary intervention studies indicated that individual pretreatments with chlorophyll prevent ischaemia-reperfusion induced cerebral injury (Rehni et al., 2007).

Polyphenols

Polyphenolic compounds have been found to possess neuroprotective properties under conditions like hypoxia, ischemia, and Parkinson's disease (Hidgon and Frei, 2003). The most abundant polyphenols in our diets are flavonoids. Dietary intervention studies in humans, and animals with flavonoid-rich plant, or food extracts have highlighted their potential to influence cognition, and learning (Youdim and Joseph, 2001; Galli et al., 2002; Haque et al., 2006; Kuriyama et al., 2006; Wang et al., 2006). Also, they are capable of improving both memory, and learning (Youdim and Joseph, 2001; Galli et al., 2002; Youdim et al., 2002; Unno et al., 2004; Haque et al., 2006; Kuriyama et al., 2006; Wang et al., 2006). Further, they have protective effects against neuronal death in both oxidative stress-induced (Inanami et al., 1998), and A-beta-induced neuronal-death models (Luo et al., 2002). Also, they enhance existing neuronal function, and stimulate neuronal regeneration. Imaging studies in humans drinking a flavanol-rich cocoa beverage showed enhanced cortical blood flow (Fisher et al., 2006; Francis et al., 2006). Increased cerebrovascular function, especially in the

hippocampus, a brain region important for memory, may facilitate adult neurogenesis (Gage, 2000; Pereira et al., 2007). Further, interactions of flavonoids with the benzodiazepine binding sites of GABA-A receptors, and with adenosine receptors (Medina et al., 1997; Dekermendjian et al., 1999) have been reported. Animal studies indicated that rats fed diets containing extracts high in the both falvanoid as well as total antioxidant activity for 6 weeks before being subjected to 48 hours of exposure to 100% normobaric O_2 showed no loss in the striatal muscarinic, or cerebellar GABAergic receptor sensitivity (Chadman et al., 1997). These oxygen-induced decreases in the neuronal function have been shown to be sensitive to aging, and have been associated with the behavioral deficits (Joseph et al., 1998). Flavonoids have been shown to be effective in protecting the neurons against the oxidative insults, possibly by acting selectively within the protein, and lipid kinase signaling cascades, and not through their potential to act as the antioxidants (Schroeter et al., 2001; Williams et al., 2004).

Human studies with a total of 1640 subjects (aged ≥65 years) free from dementia indicated that flavonoid intake was associated with significantly better cognitive performance at baseline, and with a better evolution of the performance over time. After 10 years of follow-up, subjects with the lowest flavonoid intake were found to have lost on average 2.1 points on the mini-mental state examination, whereas subjects with the highest quartile had lost only 1.2 points (Letenneur et al., 2007). Flavonoids may have a multiplicity of the direct, and indirect effects that can profoundly affect the different neuronal parameters that lead to alterations in the motor, and cognitive behaviors (Youdim et al., 2004). In support to the concept of multiple, and complementary flavonoid effects beyond the simple antioxidant function, a prospective study of dementia in the subjects older than 65 years reported an inverse relation between the baseline intake of dietary flavonoids, and development of dementia (Commenges et al., 2000).

Multiple studies have shown that the protein kinase activity is important in the memory formation in the particular spatial memory (Micheau and Riedel, 1999), and that extracellular signal-regulated kinase is involved in the striatal-dependent learning, and memory (Mazzucchelli and Brambilla, 2000), and the hippocampal-dependent spatial memory (Selcher et al., 1999). Changes in the hippocampal plasticity parameters such as the hippocampal neurogenesis, extracellular receptor kinase activation, insulin-like growth factor-1, and insulin-like growth factor-1 receptor levels were increased in the blueberry-supplemented aged animals (Casadesus et al., 2004). Therefore, the cognitive improvements afforded by the polyphenolic-rich fruits such as blueberries appear in part to be mediated by their effects on the hippocampal plasticity (Shukitt-Halle et al., 2006).

Polyphenols such as resveratrol were initially identified as the plant's defensive response against stress from ultraviolet radiation, pathogens, and physical damage (Ferguson, 2001). Recent studies have shown that resveratrol stimulates sirtuins, and can exert antiaging effects at the cellular level (Lamming et al., 2004). Further, resveratrol activated the extracellular signal-regulated kinase-1, and extracellular signal-regulated kinase-2 in a neuronal cell model (Tredici et al., 1999). Resveratrol metabolite piceatannol can protect cultured neurons against A-beta-induced death (Kim et al., 2007). Studies with cell culture models of Parkinson's disease also demonstrated neuroprotective effects of resveratrol in alleviating oxidative damage induced by neurotoxins (Gelinas and Martinoli, 2002; Alvira et al., 2007). The multiple roles of resveratrol as an antioxidant, and as a life-promoting agent make it an attractive candidate for treatment of neurodegenerative diseases (Anekonda, 2006; Baur and Sinclair, 2006; Mancuso et al., 2007). Resveratrol combined with other polyphenolic compounds, such as catechin from green tea, can produce synergism in the protective effects (Conte et al., 2003a,b). In a rat model of sporadic AD, chronic administration of resveratrol ameliorated the cognitive impairment, and oxidative damage induced by intracerebroventricular injection of streptozotocin (Sharma and Gupta, 2002). Resveratrol protected cultured PC12 neural cells against A-beta-toxicity (Jang and Surh, 2003), and dopaminergic neurons in midbrain slice cultures against several different insults (Okawara et al., 2007). Resveratrol also protected hippocampal neurons against nitric oxide-mediated death (Bastianetto et al., 2000), prevented axon degeneration (Araki et al., 2004), and protected nematode, and mammalian neurons against mutant polyglutamine toxicity

(Parker et al., 2005). Grape polyphenols also prevented chronic ethanol-induced increase in COX-2 mRNA expression in the rat brain (Simonyi et al., 2002). Dietary supplement of polyphenols extracted from grape skin, and seeds could ameliorate oxidative damage in synaptic membranes in the brain induced by chronic alcohol consumption (Sun et al., 1999a,b). A number of studies using cell models have provided information for the underlying mechanisms for neuroprotective effects of resveratrol (Gao et al., 2006; Lu et al., 2006; Raval et al., 2006; Tsai et al., 2007; Cho et al., 2008).

Anthocyanins are highly pigmented polyphenolic compounds found in many red, purple, and blue fruits, and vegetables. Studies with anthocyanins have suggested that these brightly colored compounds are associated with a wide variety of health benefits, including decreased risk of cardiovascular disease (Kaplan et al., 2001; Rechner and Kroner, 2005; Bell and Gochenaur, 2006; Dai et al., 2007), improved glucoregulation (Jayaprakasam et al., 2005, 2006), protection of brain tissue from hypoxia (Loren et al., 2005; West et al., 2007), reduced risk of cancer (Kang et al., 2003; Katsube et al., 2003; Yi et al., 2005; Ding et al., 2006; Lala et al., 2006; Hecht et al., 2006; Dai et al., 2007), and reversal of age-related neurodegenerative declines (Galli et al., 2002; Goyarzu et al., 2004; Andres-Lacueva et al., 2005; Joseph et al., 2005; Hartman et al., 2006). Grape seed proanthocyanidin can reduce ischemia/reperfusion-induced activation of JNK-1, and c-Jun, and reduce cardiomyocyte apoptosis (Sato et al., 2001). Strawberries, and radishes are the most abundant sources of pelargonidin-based anthocyanins in the diet (Wu et al., 2006a), and these provide ~3% of total anthocyanin intake (Wu et al., 2006b).

Chronic administration of tee polyphenols in scopolamine-induced amnesic mice improved cognitive performance, and inhibited acetylcholine esterase activity (Kim et al., 2004). Loss of cholinergic innervation, demonstrated by reduced choline acetyltransferase, and elevated acetylcholine-esterase activity is correlated with the degree of dementia, and the severity of the neuropathological hallmarks of AD (Zubenko et al., 1989; Law et al., 2001). Green tea extract (–)-epigallocatechin-3-gallate (EGCG) was shown to inhibit 6-OHDA-induced NF-κB-mediated expression of cell death, and cell cycle genes (Levites et al., 2002a,b). EGCG may also exert protection through controlling calcium homeostasis, activation of MAPK, PKC, antioxidant enzymes, survival genes, and modulating enzymes for processing of the amyloid precursor protein (Mandel et al., 2004; Weinreb et al., 2008). After ingestion of two, or three cups of tea by human subjects, the average peak plasma values of EGCG, and (–)-epigallocatechin (EGC) were also in this range, and the highest individual values observed for EGCG, and EGC were 0.65 μM (Lee et al., 1995). Green tea contains chemicals capable of activating adaptive cellular stress responses, and protectecting neurons against a range of oxidative, and metabolic insults. Examples of neuroprotective effects of chemicals in green tea include:

Protection of dopaminergic neurons from damage induced by 6-hydroxydopamine in a rat model of Parkinson's disease (Guo et al., 2007)
Protection of retinal neurons against ischemia–reperfusion injury (Zhang et al., 2007)
Reduction of mutant Huntington misfolding, and neurotoxicity in Huntington's disease models (Ehrnhoefer et al., 2006)
Direct protection of neurons against A-beta toxicity (Bastianetto et al., 2006)
Protection against A-beta-induced cognitive impairment in a rat model relevant to Alzheimer's disease (Haque et al., 2008)

The neuroprotective phytochemicals in green tea include catechins, and epicatechins which can induce the production of cytoprotective proteins (Mandel et al., 2005). Feeding studies with (–)epicatechin-containing diet showed enhancing effects on memory, hippocampal vascularization, and neuronal spine density (van Praag et al., 2007). Further, (–)epicatechin treatment in combination with exercise is even more beneficial for cognition (van Praag et al., 2007). Early animal feeding studies in dogs have showed that environmental enrichment combined with a diet containing phytochemicals, and vitamin E had a greater effect on cognition than either condition alone

(Milgram et al., 2005). Further, the tea flavanol epigallocatechin gallate accesses the brain after oral administration to mice (Suganuma et al., 1998).

Curcumin (diferuloylmethane) a plant polyphenol has been considered as a potentially important chemopreventive agent against cancer (Kelloff et al., 1997). It is derived from turmeric, the powdered rhizome of the medicinal plant *Curcuma longa* Linn. Animal studies indicated that dietary curcumin prevents A-beta-induced spatial memory deficits in the Morris water maze, and postsynaptic density loss, and reduced A-beta deposits (Frautschy et al., 2001). Recent studies indicated antiamyloidogenic effects of curcumin (Ono et al., 2004). Curcumin can bind amyloid directly, and inhibit A-beta aggregation as well as prevent fibril, and oligomer formation (Yang et al., 2005). Also, curcumin supplementation has been considered as an alternative, nutritional approach to reduce oxidative, inflammatory damage, and amyloid pathology associated with AD (Wu et al., 2006a).

In vitro studies with curcumin either through i.p. injection (30 mg/kg), or through a dietary supplementation (2.0 g/kg diet) for 2 months indicated significantly attenuated ischemia-induced DND as well as glial cell activation in the gerbil model (Wang et al., 2005). This administration ameliorated the increase in loco-motor activity observed at 24 hours after ischemic insult, thus correlating behavioral deficits with the extent of neuronal damage (Wang et al., 2005). Further, curcumin has been shown to reverse chronic stress-induced impairment of hippocampal neurogenesis, and increase expression of brain-derived neurotrophic factor in an animal model of depression (Xu et al., 2007).

Ferulic acid is the other commonly found polyphenols in fruits, and vegetables such as tomatoes, sweet corn, and rice (Srinivasan et al., 2007). It has been shown that ferulic acid can significantly protect against amyloid beta-peptide toxicity by modulating oxidative stress, and by inducing the expression of protecting proteins in hippocampal cultures (Sultana et al., 2005).

Fisetin has been found in fruits, and vegetables, such as strawberry, apple, cucumber, grape, onion, and persimmon (Arai et al., 2000). It has the ability to promote the differentiation of nerve cells (Sagara et al., 2004). The induction of differentiation by fisetin depends on the activation of the Ras–extracellular signal-regulated kinase (ERK) cascade, and in particular on the activation of the ultimate kinase in this cascade. Animal studies indicated that fisetin can activate signalling pathways in hippocampal slices that are implicated in the development of long-term memory (Maher and Akaishi, 2006). Fisetin is also a potent inhibitor of β-amyloid fibril formation *in vitro* (Kim et al., 2005), and can decrease myelin phagocytosis by macrophages (Hendricks et al., 2003).

Epidemiological investigations have provided evidence that postmenopausal women who undertake oestrogen-replacement therapy have a significantly lower risk for the onset of Alzheimer's disease than women who do not (Henderson, 2006). The sudden decline in estrogen levels after menopause coincides with acceleration of several aging processes (Yaffe et al., 1998). Several studies demonstrated that flavones are effective inhibitors of aromatase (estrogen synthetase) (Kao et al., 1998). Dietary isoflavones, and flavones have also been shown to be agonists of estrogen-related receptors (Suetsugi et al., 2003). They have been shown to stimulate the synthesis of sex hormone-binding globulin (SHBG), decreasing blood levels of free estrogens that are more available to the target tissues (Adlercreutz and Mazur, 1997). Significant amounts of the isoflavones daidezein, and genistein have been found in soy (Price et al., 1985). They may inhibit important steroid biosynthetic enzymes, including aromatase, and 17β-hydroxysteroid dehydrogenase I, thus affecting the level of circulating estrogens (Adlercreutz and Mazur, 1997). Animal studies using a rat model showed a clear effect of an isoflavone-rich diet on cognitive function (Pan et al., 2000; Lund et al., 2001). Human studies indicated that intake of isoflavones improve cognitive function in both college students, and postmenopausal women (File et al., 2001; Duffy et al., 2003; Kritz-Silverstein et al., 2003). Recent studies indicated a statistically significant better cognitive performance, notably in processing capacity, and speed, and in executive function in participants who have a higher lignan intake, but not isoflavones (Sanne et al., 2007). The mechanism of action for lignans might be their antioxidant properties (Wang, 2002).

Indole

The biologically active compound of cruciferous vegetables is glucobrassicin, a secondary plant metabolite that is abundant in cruciferous vegetables (McDanell et al., 1988). After consuming cruciferous vegetables the enzyme myrosinase transforms glucoraphanin which is a glucosinolate to sulforaphane. The young sprouts of broccoli, and cauliflower are particularly rich in glucoraphanin. Sulforaphane protected cultured neurons against oxidative stress (Kraft et al., 2004), and dopaminergic neurons against mitochondrial toxins (Han et al., 2007). Through induction of phase II enzymes expression, sulforaphane protected neurons against death in a Drosophila model of Parkinson's disease (Trinh et al., 2008).

Sulfides

The Allium genus includes approximately 500 species, the most widely used of which are onions (*Allium cepa*), garlic (*Allium sativum*), leeks (*Allium porrum*), chives (*Allium schoenoprasum*), and shallots (*Allium ascalonicum*). The organosulfur compounds present in the Allium vegetables, which are either lipid or water soluble, are considered responsible for the beneficial effects of these herbs. Recent animal studies indicated preventive effects of the garlic extracts for brain atrophy (Moriguchi et al., 1997) as well as learning, and memory impairments (Nishiyama et al., 1997).

CONCLUSION

Diet as one of the most important lifestyle factors can strongly influence the stress susceptibility, neurodegenerative disorders, and mental health. Plant foods which are rich in phytochemicals, and vitamins show a variety of positive health effects. Extracts of various fruits, and vegetables exhibit neuroprotective properties in cell culture, and animal models that are relevant to the pathogenesis of many different neurodegenerative conditions including stroke, Alzheimer's, and Parkinson's diseases. Also, intake of foods containing phytochemicals is beneficial in reversing the course of neuronal, and behavioral aging. Due to their positive influences on stress, mood, and cognitive function plant foods might be preferred.

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16 Fruits, Vegetables, and Their Extracts in Health of the Upper Gastrointestinal Tract

Simin Bolourchi-Vaghefi

CONTENTS

INTRODUCTION

The use of botanicals, herbs, fruits, and vegetables have increased in the past decades. Consumption of vegetables and fruits are being encouraged by health professionals because of recent findings of their beneficial properties. So are the uses of edible nonfood natural plants that have been in use for as long as history can reveal. They have been used in both folk medicine and traditional treating of diseases for centuries in the Far East and the Middle East. They have become popular and are used now in combination, as alternatives or complementary to prescribed medications. The practice of alternative or complimentary medicine also has increased. Therefore, this subject has been the focus of many scientific investigations. Popular use of these edible plants has been on the rise and naturalists are encouraging people to use them in lieu of pharmacological drugs. There are numerous literature reports on the effectiveness of these foods and herbs in preventing and treating diseases. However, the safety and compatibility and interaction with medicinal drugs, as well as the metabolism and their mechanism of action have not been completely elucidated.

Promoters claim that these items are safe, natural, and contain no chemicals, the fact is that plants are made of chemicals and have their own metabolism, some compatible with the metabolic processes of humans and some not. There are many benefits of including plants and herbs in the diet but on the other hand some plants can induce toxicity, inhibit some metabolic processes or can interfere with drug metabolism.

For the above reasons this specific field of nutrition research is very active. Numerous scientists are engaged in research to provide information as to the safety and compatibility of the botanicals and their role in providing health and protection against disease. In recent years there have been many reports of interaction of botanicals with pharmacological drugs (Boullata, 2000; Emst, 2002; Greenblatt and von Moltke, 2005). "Mechanism investigated to date include the possibility that botanical medicines may induce or inhibit the activity of human cytochrome P450 enzymes or the

activity of transport proteins such as P-glycoprotein" (Zhou et al., 2003). The objective of this chapter is to discuss foods with bioactive components such as varieties of botanicals or the extracts of bioactive components therein as possible protective agents against upper gastrointestinal (GI) cancers.

FRUITS, VEGETABLES, AND GRAINS AND HEALTH

In addition to being extremely rich sources of nutrients, fruits, vegetables, nuts, seeds, and grain contain nonnutrient compounds that are bioactive and protective against chronic and degenerative diseases. The food consumed by individuals contain beneficial as well as carcinogenic and mutagenic compounds. The genetic makeup of the individuals determine how these compounds interact in the body and how protective the bioactive components of fruits, vegetables, and botanicals as a whole can be in different individuals. The scientific and nutritional interest in the role fruits, vegetables, and grains play in protecting the health of individuals against degenerative diseases have resulted in multitudes of research reports on the effects of these foods. Many epidemiological studies have proven that fruits and vegetables protect against cancer. Communities who consume fewest versus most servings of fruits and vegetables have double the risk of developing lung, oral cavity, larynx, esophagus, and colorectal cancer.

Interest in the medicinal effects of foods in treating disease may well date back to the time when the first humans appeared on the planet. Many ancient beliefs regarding medicinal effects of foods are handed down from generation to generation. The first historic evidence comes from Hippocrates about 2500 years ago. Eastern philosophies of healthy living are intertwined with eating natural food to protect health. Most agricultural societies up to the advent of industrialization relied predominantly on natural agricultural foods instead of the high fat, high meat, and highly processed foods.

Today, introduction of highly refined, processed, and genetically engineered foods has replaced natural foods; this has come at a high price with the disadvantage that consumers do not receive all the needed nutrients which the whole, unadulterated agricultural produce can provide for good health. This change in diet pattern has resulted in increased incidence of obesity and the resulting diseases as well as deficiency of some essential nutrients in populations around the world.

The recent renewed interest in natural and botanical foods could be a reaction to the processed and altered foods and responsible for the advances in research in these areas. Some of the findings from research resulted in reports indicating that those who consume fruits, vegetables, grains, and nuts as major part of their daily diet are healthier and live longer than those who do not benefit from these whole foods in adequate quantities.

FACTORS CAUSING CANCER: MECHANISMS OF ACTION

Mitochondria that are the sites of production of energy in the cells become less efficient as a result of aging. The aged mitochondria produce more mutagenic oxygen radicals that can oxidize and damage DNA. The production of cardiolipin, a lipid in mitochondrial membrane responsible for mitochondrial electrical potential is decreased by aging resulting in reduced oxygen utilization and increased in production of oxygen free radicals causing oxidative lesions in DNA.

Studies have shown that by feeding acetyl carnitine to old rats they show less mitochondrial and DNA damage. DNA lesions can translate into production of wrong types of proteins that may cause uncontrolled cell proliferation causing cancers (Ames, 2004).

Many carcinogens find their way into the diet and depending on the genetics and the immune system of individual, cancer can be initiated. Diet has been the focus of many studies in its relation to cancer. The main dietary problem in the United States is believed to be the deficiency of vitamins, minerals, and presence of obesity. Deficiency of micronutrients can affect the general health by causing the inability of the immune system to respond to foreign bodies, rendering individuals vulnerable to many diseases including cancer.

Obesity has been shown to be the underlying cause of several types of cancer. A diet dominated by meat, refined carbohydrate foods, and fat combined with a lifestyle of inactivity is a recipe for obesity in young and old. High fat, high meat meals can also be carcinogenic due to preparation methods, types of fat content, additives, and lifestyle of people. Production of hydrogenated vegetable oil has introduced *trans* fatty acids in the diet of people (Emken, 1984). In an extensive review about the evolution of Western diet from the beginning, Cordian et al. (2005) explain that the degenerative diseases do not arise from one or few dietary factors but "from a complex interaction of multiple nutritional factors directly linked to the excessive consumption of novel Neolithic and industrial era foods (diary products, cereals, refined cereals, refined sugars, refined vegetable oils, fatty meats, salt, and combinations of these foods). These foods, in turn, adversely influence proximate nutritional factors, which universally underlie or exacerbate virtually all chronic diseases of civilization." They further indicate that "the collision of our ancient genome with the new conditions of life in affluent nations including the nutritional qualities of recently introduced foods" is the ultimate underlying factor responsible for degenerative diseases in our civilization.

The only recourse, therefore, is to restrict quality and quantity of meals, be conscious of selection of healthy, natural foods in the diet and to prevent obesity. The latter can be accomplished by balancing intake and output of energy, which means more physical activity, eating less caloric foods, and more vegetables and fruits.

BOTANICAL DIVERSITY AND OXIDATIVE BIOMARKERS IN HUMAN METABOLISM

Diets dominated by fruits, vegetables, grains, nuts, and spices, and so on, provide a variety of diverse edible foods for us to enjoy. There is a wealth of information about the health status of vegetarians and their long, active, and healthful living. Fruits and vegetable varieties can exceed thousands and they add variety and taste to the menu. People with interest in culinary creativity can prepare hundreds of delicious meals using different varieties of vegetables and fruits in their creations to please the palate of the most discriminating food critics. These nature's edible cornucopias provide, in addition to taste and variety, valuable nutrients and nonnutrient compounds that preserve health and prevent diseases. Phytochemicals in plant foods contain many bioactive compounds that are antioxidants, antimutagenic, anticarcinogenic, and have vitamin A, C, D, and E activity.

"Phytochemicals are defined as agents in plants that are biologically active and maybe used for both preventive and therapeutic purposes. Phytochemicals affect oxidation potential, cellular differentiation, inflammation, lipid and drug metabolism and other metabolic processes. These agents are at the frontier of research on cancer prevention" (Rivin, 2006). It has been shown that the antioxidant activity of green leafy vegetables is the highest followed by wheat and rice. Clove's eugenol content has a protective effect against induced hepatotoxicity in rats. Turmeric and curcumin have proven both to be antimutagen *in vivo* and capable of reducing adducted levels of DNA in rat liver challenged with benzo[*a*]pyrene, B[*a*]P. Turmeric and curcumin both given to rats after initiation phase of carcinogenesis were proven to have better antitumor activity. Antimutagenic actions of popular vegetables onion and garlic have been recognized (Krishnswamy and Raghuramulu, 1998).

Lampe (1999) conducted studies involving human subjects and demonstrated that bioactive compounds in plants may be able to modulate "the activity of many biological systems in mammalian species." The plant phytochemicals are protective against plant diseases and can be protective against the risk of some diseases of animals and humans including cancer, cardiovascular and other degenerative diseases depending on the process of digestion, hydrolysis, absorption, and metabolism of the phytochemical components. Most of the investigations into these topics have emphasized the role played by dietary phytochemical bioactive compounds in the protection of cellular oxidation in biological systems. It is now well established that the bioactive compounds of the plants modulate many biological system activities in humans. Plant phytochemical components have been

the focus of many investigators, and the roles they play in preventing cellular oxidation have been well studied.

Thompson et al. (2005, 2006) reported that intakes of high (more than 10 servings) quantities of fruits and vegetables per day modestly reduces the levels of both DNA oxidation in peripheral lymphocytes of human subjects and their urinary concentration of a marker of lipid peroxidation. The antioxidant effects were more pronounced in participants who had elevated levels of oxidation biomarkers initially at the start of the study (Thompson et al., 2005a,b, 2006).

Kudo et al. (2008a,b) compared the incidences of Barrett's esophagus in men and women in a case–control study of "health conscious" subjects who habitually consumed a diet of fruit, vegetables, and non-fried fish. The results showed a significant inverse relation between Barrett's esophagus and amounts of fruits and vegetables consumed by each group. Another report of that study indicated that significant inverse association existed between Barrett's esophagus and intake of fruits, vegetables, vitamin C, vitamin E, beta carotene, and selenium. However, taking supplements of the above vitamins and selenium long term did not influence the risk of Barrett's esophagus.

In a community-based study Thompson et al. (2009) measured servings of fruits and vegetable intakes of patients newly diagnosed with Barrett's esophagus (by biopsy of tubular esophagus) and compared them with the intake of controls chosen from general population. The number of servings of fruits and vegetables were measured by using a self-administered validated 131-items Food Frequency Questionnaire. The study showed that reduction in the risk of Barrett's disease was greatest among those who had about 2.5 servings of vegetables per 2000 kcal daily. A combined vegetable and fruit intake of about 4.6 serving per 2000 kcal per day showed greatest reduction in the risk of the disease among men and women studied.

The author of this chapter was involved in a survey of food intake by population in a remote area of northeast Iran with a semi desert, very arid climate environment where no farms, farm products, or fruit orchards existed. The incidences of cancer of esophagus were the highest there as compared with other areas studied then. Fruits and vegetables consumed by the population were extremely low, but cases of esophageal cancer were very high among the inhabitants of that region, especially among young adult females (Bolourchi-Vaghefi, 1975).

Riboli and Norat (2003) used meta-analysis approach and reported that case–control trials show the risk of cancers of esophagus, lung, and stomach were significantly reduced in subjects consuming higher amounts of fruit and vegetables. They reported in the case–control studies that met the criteria for analysis that fruit intake reduced the risk of oral and pharyngeal cancer consistently. This protective effect was consistently significant for fruits but not for vegetables. They reported that the risk of laryngeal cancer was consistently reduced by consumption of fruits, but one study found that vegetable consumption reduced the risk but not fruit intake.

For esophageal cancer they found significant protective effects of fruits and vegetables in reducing the incidences, but the effect was more important for fruit intake than for vegetables. These investigators found significant protective effect for fruit in the case–control studies for gastric cancer, but the cohort studies did not show any effect for either fruits or vegetables in reducing the risk of gastric cancer. The overall results of the meta-analysis studies were heterogeneous for the case–controls and for geographic subgroups. They generally found that fruits had a higher protective effect than vegetables in Asian studies but not in European or North American studies.

Gastric cancer is associated with cigarette smoking and is one of the leading causes of cancer deaths in the world (Kelly and Duggan, 2003). The risk of gastric cancer is higher in cigarette smokers than nonsmokers. Epidemiological studies have shown that risk of gastric cancer is reduced in people who consume high levels of vegetables and fruits containing carotenoid lycopene (tomatoes, watermelon, pomegranate, red beets, etc.) and have a high plasma levels of lycopene (Geovannucci, 1999; Tsubono et al., 1999; Yuan et al., 1999). These studies suggest lycopene in fruits and vegetables has a protective role against development of gastric cancer. Supplementation of lycopene in the study with ferrets increased level of lycopene in the gastric mucosa in a dose-related response. Exposure to cigarette smoke reduced lycopene in the gastric mucosa of ferrets significantly.

Supplementation with either low or high dose of lycopene significantly reduced increased levels of p53 indicating that lycopene protects against stress caused by cigarette smoke through production of reactive oxygen species (ROS) causing cellular DNA damage; thus excess increase in both total and phosphorylated p53 in the tissues of ferrets (Liu et al., 2006).

Seifried et al. (2004) believe that intake of adequate amounts of fruits and vegetables cut the risk of lung cancer by half, even in smokers. Smoking produces a great number of oxidants in the body which lowers the body's levels of vitamin C. *Helicobacter pylori* infection that causes gastric cancer also reduces levels of vitamin C in the body. Consuming fruits and vegetables provide needed vitamin C and other antioxidants that reduce the levels of oxygen radicals produced by both smoking and infection. Phytochemical components of fruits and vegetables have influences in cells beyond antioxidant activity. Carcinogens from cigarette smoke when metabolically activated can affect the p53 tumor suppressor gene. This gene is responsible for maintaining balance between proliferation and apoptosis in cellular response stressors (Levine, 1997). p53 is overexpressed in gastric cancer in response to damage to the cell DNA by the carcinogens through ROS released by cigarette smoke. Increased consumption of fruits and vegetables are encouraged by any dietary guidelines or advise from nutritionists, to provide antioxidants to combat the damage of ROS to DNA.

Supplements of antioxidants, plant phytochemicals, and certain vegetables, especially Allium family, is often recommended for cancer prevention and treatment. Supplements of extracts and oils of some of these vegetables and some fruits are available in the market. Caution should be practiced because the generation of excess levels of ROS is important in initiating the internal cell programs for cell apoptosis. Cells are programmed for suicide or apoptosis that is essential in destroying the cancer cells, including preneoplastic and neoplastic cells through invoking a cascade of cellular enzymatic activities that kill the affected cells. Overconsumption of supplements may interfere with normal process of cell apoptosis.

Zeisel (2004) cautions that before cancer patients include antioxidants supplements in their diet they consult with their treating physician regarding suppression of apoptosis by excess antioxidants.

ROLE OF FRUIT AND VEGETABLES IN COUNTERING CANCER CAUSING ELEMENTS

Factors that influence the effect of plant-based diets on the risk or prevention of cancer include: genetics, food tolerance, differences in taste preference, and metabolism. Biotransformation enzymes in human systems metabolize diets containing a mixture of protective compounds as well as carcinogens and mutagens. The risk of carcinogens and mutagens are modified by genetic polymorphism altering enzyme function or protein expression according to the situation. Genotypes that are more favorable toward handling carcinogens may not be able to metabolize phytochemicals the same favorable way. "Genetic polymorphism in enzymes that metabolize phytochemicals may account in part for variation in disease risk and also have to be considered in the context of other aspects of human genetics, gut bacterial genetics and environmental exposure." One of the factors that modify response to constituents of high plant diet in population-based studies is human genetic variations (Lampe, 2009).

Walle et al. (2005) reported that dietary flavonoid glycosides are hydrolyzed and activated in oral cavity due to the activity of bacteria and epithelial cells to create biologically active aglycones at the surface of oral epithelial cells. These aglycones, quercetin and genistein are known to inhibit proliferation of oral cancer cells. In one experiment grape seed extract and methanol extract of grape seed was more able to successfully kill two human oral cancer cell lines than did grape peels alone and human gingival fibroblast (Shirataki et al., 2000).

Garlic and onion of the *Allium* genus are popular vegetables with potent flavors that are used as flavoring spices in culinary creations. They both are used extensively in Eastern and Mediterranean cuisines. Garlic has been used for medicinal purposes since ancient times as preventive and curative

agents for many diseases. The early civilizations known in history, or even before written history was available, used garlic for preservation of health, and prevention and treatment of diseases. Garlic was used in Asia, Middle East, and Mediterranean countries for health purposes and also as condiments in food preparations. Garlic is a vegetable containing high amount of organosulfur compounds, flavonoids and other bioactive compounds including nonsulfur compounds. These compounds work synergistically in providing health benefits and preventing degenerating diseases.

The most active components of garlic with pharmacologic effects are the sulfur-containing compounds known as diallyl. These are water soluble such as *S*-allyl methylcysteine and fat soluble such as diallyldisulfide. Due to the fact that chemistry of garlic is so complex, preparations and processing of garlic yields different varieties of compounds. This diversity of bioactive compounds gives the garlic components varieties of bioactivities. Both water soluble and fat soluble allyl sulfides can effectively change outcomes of molecular events that can involve preventing cancer. These changes "include inhibiting mutagenesis, blocking carcinogen DNA adduct formation, scavenging free radicals as well as blocking cell proliferation, differentiation, and angiogenesis" (Milner, 2006). There are a good number of investigations that confirm the above facts; however, still more research and evidence are necessary to elucidate the exact mechanism of action of each fraction for each of the above-mentioned actions.

Garlic is a popular culinary herb throughout the world; long-term consumption of garlic is believed to reduce risk of some cancers. Most notable cancers that, according to popular belief, can be prevented or cured by garlic are stomach and colon cancers. Wargovich (2006) explains the mechanism of carcinogen inhibition through suppression of cytochrome 4502E1 (CYP2E1) by some garlic-derived agents. CYP protein enzymes are a group of enzymes that metabolize zenobiatics (chemicals, drugs, carcinogens, and other environmental toxins). CYP2E1, although not very important in human metabolism, can activate carcinogens in animals. It has been shown that a single allyl side chain attached to a sulfur atom from either diallylsulfide, allylmethyl, allylpropyl, and dipropylsulfide may dramatically suppress action of CYP2E1-inhibiting carcinogenesis.

Garlic compounds have also been shown to involve activities of phase II enzymes which are known to inhibit carcinogenesis. A comparison of naturally occurring water soluble sulfur compound of garlic with synthetic analogs of garlic compounds may suggest that premalignant lesions caused by azoxymethane are suppressed dramatically by the allyl groups. Wargovich (2006) also reports his findings that, in particular, azoxymethane inhibits CYP2E1, and this inhibition may extend to other carcinogens such as nitrosamines, liver carcinogens, colon carcinogens, and small intestine carcinogens.

Other investigators also have found that volatile and aromatic diallyl sulfide components of garlic can modulate cytochrome P450 isozyme activities, blocking carcinogen activities, detoxifying carcinogens by induction of glutathione peroxidase, increasing the activities of glutathione-*S*-transferase, epoxide hydrolase, and UDP-glucuronosyl transferase. It is believed that cancer chemoprevention by zenobiotics in plants can be due to diallyl sulfide component of garlic and other plants.

El-Bayoumy et al. (2006) studying the effect of sulfur and selenium compounds in garlic on chemoprevention in rat mammary glands conclude that diallyl sulfide and diallyl selenide exert similar effects in chemoprevention of cancer. They indicate that investigation in other laboratories have confirmed that diets high in selenium such as broccoli, garlic, and wheat are more effective in prevention of cancer than other nutritious foods.

Onion is another popular vegetable of the Allium family that is consumed regularly and used in culinary practices for almost every meal of the day. In addition to adding flavor to every meal, it is one of the folk remedies that is believed to restore youth, provide stamina, and prevent diseases. Analysis has shown that the active molecule in onion oil is propyl sulfide, not unlike allyl sulfide in garlic. Wu et al. (2006) demonstrated in a cell culture experiment that onion oil added to the human lung cancer A549 cells at 12.5 mg/L, induced apoptosis significantly (13% increase in apoptotic cells) shown by sub-G1 DNA content and caused arrested cell cycle at G2/M phase. When onion oil was doubled to 25 mg/L the percentage of G2/M cells was increased almost sixfold as compared

with dimethyl sulfoxide control. Addition of antioxidants *N*-acetylcysteine, exogenous glutathione to the medium blocked the cell cycle arrest and apoptosis. Thus it was suggested that the action of onion oil may have been due to ROS-dependent pathway. In that experiment, a marked collapse of mitochondrial membrane potential occurred pointing to the dysfunction of mitochondria due to oxidative burst and apoptosis caused by onion oil. Also phospho-cdc2 and phospho-cycling B1 expression were downregulated by onion oil. These investigators concluded that "onion oil may exert chemopreventive action by inducing cell cycle arrest and apoptosis in tumor cells."

Health benefits of garlic (Allium family) include hypolipidemic effect, antiplatelet activity, and pro-circulatory effect. It enhances the immune system preventing cold and flu symptoms and has chemopreventive and anticancer activities. Studies and experiments with experimental animals have shown that consumption of garlic preparation including aged garlic extract (AGE) show a wide variety of biological activities including hepatoprotective and neuroprotective in addition to antioxidant properties.

There are reports in the literature that show no or little effect of garlic in reducing or alleviating the risk of cancer. However, the number of studies relating the reduction of risk of cancer to consumption of garlic and onion, belonging to the same botanical family, is much greater than the ones that do not show a relation. The relation of garlic intake and reduction risk of cancer was evaluated by Kim and Kwon (2009) using the U.S. Food and Drug Administration's evidence-based review system for scientific evaluation of health claims. These reviewers did not find credible evidence that supported a relation between consumption of garlic and reduced risk of gastric, breast, lung, or endometrial cancer. However, limited evidence showed a weak inverse relation between intake of garlic and its preparations and esophagus, larynx, and oral cancers.

Gail and You (2006), in a factorial double-blind placebo controlled trial, treated 4326 subjects with *H. pylori* in a region of China where a high percentage of people die of gastric cancer. They used one time administration of amoxicillin and omeprazole as well as long-term supplementation with garlic preparation consisting of AGE and steam distilled garlic oil. The results of the study indicated that after five and nine years of follow-up, 95.9% and 91.5%, respectively of the participants were alive, and histopathological data indicated that precancerous gastric lesions were not present in the subjects who remained alive by the ninth year of follow-up.

Patients with colorectal, liver, or pancreatic cancer who were judged inoperable by their attending physicians were entered in a randomized double blind study. The study group received AGE and the control group received a placebo for six months to evaluate the effect of AGE on the quality of life of the patients. Although at the end of the study there were no changes in the quality of life of the patients, significant increase in the natural killer (NK) cells activity was observed in peripheral blood three months after the administration of AGE group, but specific activity (activities in 100 cells) did not change. The investigators concluded that the increase in the number of NK cells meant increased activity of these cells. These results are in agreement with similar investigations reported (Ishikawa et al., 2006).

CONCLUSION

Consumption of more vegetables, fruits, and plant foods in the daily diet have a possible prevention and reduction in risk of chronic, degenerative diseases such as cancer, cardiovascular, and diabetes. The reviews of research in cancers of upper GI tract prove that populations consuming equal or more servings of vegetables and fruits to USDA recommendations had lower risk of oral, pharyngeal, esophageal, gastric, lung, and other cancers. Reduction of the risk or possible prevention of these cancers is related to both the antioxidants content and other nonnutrient phytochemicals in plants that prevent damage to the DNA of the cells (environment), genetics, aging, or other causes. Although the risk of cancer was inversely related to the intake of vegetables and fruits, the risk decreased as amount/servings increased per day, and intake of fruits seemed to reduce the risk more than of vegetables.

On the basis of the results of studies, it is imperative to recommend increased intakes of fruits, vegetables, and plant foods in daily diet while reducing high energy, high fat and highly refined carbohydrates, and increasing the physical activity to prevent a rise in BMI.

The administration of supplements or any concentrated form of antioxidants, phytochemicals, and other nonnutrients in botanicals did not reduce the risk. They can interfere with the chemistry of the cell metabolism or the clinical treatments for cancer. Caution should be exercised when prescribing these forms of chemicals to patients or general public.

SUMMARY

Scientific research has proven that fruits, vegetables, grains, nut, and seeds are rich sources of both nutrients and nonnutrient bioactive compounds that can preserve health and prevent chronic and degenerative diseases. Foods that we eat contain both beneficial compounds and harmful compounds; the latter may be carcinogenic, mutagenic, or toxic, their synthesis being part of the natural defense mechanism of the plants. They enter food chain naturally, in processing or preparation, intentionally such as additives, preservatives, and so on, or nonintentionally from environment. Health, genetics, and state of nutrition of the individual determine how these compounds and risk factors interact and how protective the bioactive components of vegetables, fruits, and botanicals can be in different individuals.

In numerous studies it has been shown that increased consumption of fruits, vegetables, and other natural foods can protect against chronic diseases such as cancer.

There are more pros and less cons in the literature of the beneficial and protective effects of consumption of more fruits and vegetables and less of meat, fat, and highly refined carbohydrates by human subjects in prevention of cancers of upper GI tract.

Consumption of a plant-based diet with a high intake of fruits, vegetables, botanicals, and less processed foods are recommended. On the other hand, intake of highly processed food, fortified and loaded with additives and preservatives are not encouraged. It is believed that plant antioxidants and phytochemicals protect against chronic diseases such as cancer depending on the genetics of individuals, digestion, absorption, and metabolism by humans and interaction of the phytochemicals and antioxidants at the cellular level.

Consumption of natural fruits and vegetables is of primary importance as the balance of metabolism and energy production at the mitochondria is best maintained with natural foods.

Supplements, extracts, oils, and any concentrated form of the fruits and vegetable antioxidants and phytochemicals have shown to have adverse effects on the cellular functions and may interfere with treatment of disease by interaction with pharmacological medicines. Any concentrated nutrient, nonnutrient bioactive component of foods (supplements) is treated by liver enzymes as drugs and are detoxified by these enzymes. Thus regular or excess consumption of supplements can interfere with normal function of these enzymes and cause problems.

Therefore, it is recommended that in order to reduce the risk of cancer of upper GI tract a few simple guidelines are followed:

An active lifestyle should be pursued.
Natural and unadulterated foods should be eaten.
More servings of fruits and vegetables should be eaten each day.
Less meat, less fat, no *trans* fat and no processed, refined foods should be consumed.
Restriction should be exercised in consumption of excess calories.
Prevention of obesity should be the goal, at any expense.

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17 Significance of Processing for the Chemopreventive Potential of Tomato-Based Products

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CONTENTS

INTRODUCTION

Food processing is a fundamental activity of modern humans which was, along with tool making and communication skills, one of the prerequisites for the unprecedented geographical spread and adaptation to almost every environmental niche on earth by the human race. Common food processing techniques include mechanical (peeling, chopping, mixing), thermal treatment (steaming, boiling, roasting), addition of preservatives (pickling, fermentation), as well as dehydration. Historically, the major beneficial outcomes of food processing were preservation, decontamination, and increase in nutritional value. Current views on the significance of changes in human diets that were determined by the invention of food processing for evolution of modern man include population health issues, among others (Wrangham and Conklin-Brittain, 2003; Cordain et al., 2005).

In concordance, epidemiological studies suggest that most cancers are induced by environmental factors and that more than two-thirds of human cancers could be prevented by lifestyle changes, including dietary modification (Go et al., 2001; Anand et al., 2008). The past two decades have seen a massive number of published reports suggesting an inverse relationship between fruit, vegetable, and whole grain consumption and cancer risk (Steinmetz and Potter, 1996; Gingras and Beliveau, 2007), with particularly strong protective effects being documented for raw vegetables. Although there were no studies directly comparing the effects of raw vs. cooked vegetables, a recent meta-analysis (Link and Potter, 2004) of publications, which distinguished between raw and cooked

vegetables, has concluded that the inverse relationship between consumption of raw vegetables and epithelial cancers was more significant than that established for cooked or total vegetables. However, one notable exception to this trend, and the topic of this chapter, is the tomato vs. prostate cancer relationship.

Food processing, as a rule, brings about a myriad of chemical reactions that may dramatically change the molecular composition of the ingested products. For example, introduction of plant foods to thermal treatment dramatically increased their nutritive value, to a large extent due to the chemical modification of complex carbohydrates that constitute the main part of plant biomass. While digestible sugars (glucose, sucrose, lactose) and starches provide the ingested energy value of foods, there is also increasing evidence that nondigestible carbohydrates such as pectins, gums, hemicellulose, inulin, etc., are essential for normal functioning of symbiotic microflora in the gut and are responsible for a host of other beneficial effects on health (Meyer and Tungland, 2001) that include protection from cancer (Pelucchi et al., 2004). In general, light food processing could provide some protection against cancer risk for such reasons as: decontamination from potentially tumorigenic liver-invading parasites, *Helicobacter pylori* (Plummer et al., 2004), algal and fungal toxins (Kabak et al., 2006); activation or increase in bioavailability of natural chemopreventive agents, such as phenolics (Son, 2008), carotenoids (Bohn, 2008) or isothiocyanates (Hayes et al., 2008); increase in antioxidant capacity (Manzocco et al., 2000), and generation of novel potentially chemopreventive entities. On the other hand, a significant proportion of vitamins and other temperature- and oxygen-sensitive phytochemicals are lost to autoxidation and degradation reactions, while pickling or excessive thermal treatment of foods may result in the production of potential mutagens and carcinogens (Weisburger, 1996). Therefore, optimized food processing technologies may be an additional resource for improving the nutritional value of foods in regard to lowering cancer risk in populations.

Due to the steadily increasing lifespan in modern man, there has been a significant increase of public awareness and interest in diet and nutrition as efficient ways to improve health in general and reduce the risk of cancer in particular. Numerous publications for professionals and general public have assessed the beneficial effects of dietary tomato supplementation on the reduction of prostate cancer risk. With a main focus on lycopene, there has been relatively little attention paid to other potentially protective agents, especially those present in processed tomato products. However, the most recent studies on this subject matter call for search of other agent(s) that may complement or synergize with lycopene against the tumorigenesis (Basu and Imrhan, 2007; Grainger et al., 2008a). Here we discuss several aspects of the chemistry behind tomato processing and its impact on the cancer preventive potential of tomato products.

EPIDEMIOLOGICAL AND CLINICAL EVIDENCE

The tomato is the second-most produced vegetable in the Western world. Over 80% of all tomato fruit is processed and consumed in the form of products such as juice, soup, purée, paste, ketchup, sautés, sauces, or seasonings. Lycopene, a carotenoid which is present in high concentrations in tomatoes, is responsible for the red color of tomato fruits and whose specific physiological functions include protection from photosensitization and aid in photosynthesis. It has also been suggested as a potential cancer protective agent over three decades ago (Petyaev et al., 1977). One of the earlier case–control studies established that high consumers of lycopene, which was available predominantly (over 80%) from dietary tomato products, had a 30% lower risk of prostate cancer than those with a low tomato intake (over 14 vs. less than 3 meals per month) (Giovannucci, 2002). However, no association between lycopene and prostate cancer was found in the Hawaiian case–control study when only tomatoes, rather than tomato products, were considered (Le Marchand et al., 1991). In 1995, a large prospective cohort study, the Health Professionals Follow-Up Study, suggested an inverse relationship between the intake of tomato products/lycopene and the risk of prostate cancer (Giovannucci et al., 1995). The initial survey of food intake in 1986 and the follow-up surveys (Chan et al., 2006) were thoroughly analyzed by Giovannucci et al. (2002) for trends in eating habits and

various diseases. Only consumption of lycopene from processed tomato-based foods such as tomato sauce and pizza was associated with prostate cancer risk reduction [relative risk (RR) = 0.79, confidence interval (CI) = 0.64–0.99], while the risk reduction for raw tomatoes was not significant. Tomato juice did not show the association, possibly because of its low consumption rate. This study inspired a considerable interest among health professionals and the public, resulting in a number of laboratory, clinical, and epidemiologic studies which initially corroborated the original findings. For example, the British case–control study (Key et al., 1997) did not establish a statistically significant association between the intake of raw or cooked tomato, but RR was lower for cooked tomato. In the Greek case–control study (Tzonou et al., 1999), raw tomatoes had only a weak, inverse association with prostate cancer risk (two-sided $p = 0.12$), whereas cooked tomatoes had a much stronger inverse association ($p = 0.005$). A compilation of these and other available publications which allowed for comparative analysis of raw versus processed tomato is given in Table 17.1. Although statistically significant results were obtained only for a smaller fraction of studies, a common trend is that the inverse relation between the prostate cancer risk and tomato consumption is always stronger for tomato products, as compared with raw tomato. A meta-analysis of case–control and cohort studies carried through early 2003 (Etminan et al., 2004) has also established that serum lycopene (RR = 0.71, CI = 0.59–0.92), lycopene intake (RR = 0.89, CI = 0.81–0.98), and cooked tomato intake (RR = 0.81, CI = 0.71–0.92) were associated with a significant decrease of the prostate cancer risk, while intake of raw tomatoes (RR = 0.89, CI = 0.80–1.00) was not. However, more recent epidemiological studies (Wu et al., 2004a; Jian et al., 2005; Key et al., 2007; Zhang et al., 2007) showed inconsistent support for the protective effect of dietary lycopene/tomato on prostate cancer risk, which prompted the U.S. Food and Drug Administration to assume a rather unsupportive stance to claims of cancer preventive potential of dietary tomato/lycopene (Kavanaugh et al., 2007).

A number of clinical trials addressed the potential impact of dietary tomato on prostate cancer risk and progression. Since the subjects of such studies were largely patients with either established tumors, benign prostate intraepithelial neoplasia or high risk of developing prostate cancer, a chief outcome was the response of prostate-specific antigen (PSA) to the lycopene or tomato consumption. Tomato sauce (Chen et al., 2001; Bowen et al., 2002; Kim et al., 2003), paste (Edinger and Koff, 2006), and tomato products (Grainger et al., 2008b) consumption led to statistically significant improvement in this biomarker parameters in all of the studies. On the other hand, similar clinical studies with pure lycopene supplementation produced only mixed results (Kucuk et al., 2001; Ansari and Gupta, 2004; Bunker et al., 2007; Jatoi et al., 2007). Moreover, dietary tomato powder significantly increased survival from prostate cancer of NMU/testosterone-induced Wistar-Unilever rats (Boileau et al., 2003; Mossine et al., 2008a), while lycopene alone did not produce any protection from the induced tumorigenesis. In the syngeneic Dunning rat prostate cancer models, lycopene alone was not effective against the transplanted tumor growth, while tomato powder or a combination of lycopene with antioxidant FruHis from tomato powder significantly inhibited tumor growth (Siler et al., 2004; Canene-Adams et al., 2007; Mossine et al., 2008a).

Taken together, these and other epidemiological, clinical, and laboratory studies suggest that processing of tomatoes may be essential for their protective efficiency against prostate cancer risk and that ingested lycopene may require the presence of other components in order to decrease the initiation and/or progression of prostate cancer. For years, the main emphasis in both epidemiological and intervention studies remained on lycopene alone. This was due, in part, because laboratory studies suggested that lycopene may possess unique antioxidant properties and accumulate in the prostate tissue, thus altering carcinogenesis, and because it is an agent readily assessable by modern analytical techniques (Grainger et al., 2008a). Therefore, the reported inconsistencies in the above-mentioned epidemiological studies might stem from a one-sided, lycopene-based mechanistic approach which lacked consideration of content, availabilities, and chemopreventive potential of other tomato nutrients and phytochemicals that include carotenoids, phenolics, salicylates, vitamins C and E, or other, yet unidentified chemical entities, which may synergize with lycopene or contribute to the cancer prevention on their own. Despite these shortcomings, tomato holds a unique place

TABLE 17.1
Comparative Prostate Cancer Risk for Tomato Products and Raw Tomatoes

Reference, Place	Type of Study	No. of Subjects	Tomato	Intake	RR, HR, or OR @ 95% CI	PC Risk Lowering Effect
(Key et al., 1997) UK	Case–control	328 cases	Raw	≥5/week vs. <3/month	1.06 (0.55–1.62) $p = 0.88$	No effect
		328 controls	Cooked	≥2/week vs. <1/month	0.92 (0.57–1.42) $p = 0.64$	No effect
			Lycopene	≥718 vs. <402 µg/day	0.99 (0.68–1.45) $p = 0.88$	No effect
(Tzonou et al., 1999) Greece	Case–control	320 cases	Raw	>30 vs. <20/month	0.65 (0.40–1.0) $p = 0.12$	Suggestive positive
		246 controls	Cooked	>28 vs. <13/month	0.52 (0.33–0.83) $p = 0.005$	Significantly positive
(Cohen et al., 2000) Seattle	Case–control	628 cases	Raw	≥3 vs. <1/week	0.93 (0.67–1.30) $p = 0.76$	No effect
		602 controls	Cooked	≥3 vs. <1/week	0.73 (0.48–1.10) $p = 0.13$	Suggestive positive
(Norrish et al., 2000) New Zealand	Case–control	317 cases	Raw	>35 vs. <13 g/day	1.01 (0.66–1.53) $p = 0.93$	No effect
		480 controls	Raw + Cooked	>64.2 vs. <18.7 g/day	0.82 (0.53–1.26) $p = 0.30$	Suggestive positive
			Lycopene	≥1994 vs. <662 µg/day	0.76 (0.50–1.17) $p = 0.30$	Suggestive positive
(Kolonel et al., 2000) USA–Canada	Case–control	1619 cases	Raw + Cooked	>108.1 vs. ≤20 g/day	1.07 (0.83–1.38) $p = 0.85$	No effect
		1618 controls	Cooked	>92.7 vs. ≤18.3 g/day	0.94 (0.58–1.52) $p = 0.56$	No effect
(Giovannucci et al., 1995) USA	Prospective cohort	773 cases	Sauce	2–4 vs. 0 servings/week	0.66 (0.49–0.90) $p = 0.001$	Significantly positive
		47893 total	Juice	2–4 vs. 0 servings/week	1.15 (0.90–1.49) $p = 0.67$	No effect
			Combined products	>10 vs. <1.5 servings/week	0.65 (0.44–0.95) $p = 0.01$	Significantly positive
(Giovannucci et al., 2002) USA, follow-up	Prospective cohort	2481 cases 47365 total	Sauce	≥2/week vs. <1/month	0.77 (0.66–0.90) $p < 0.001$	Significantly positive
(Chan et al., 2006) USA, follow-up	Prospective cohort	392 cases[a]	Raw	Quartile 4 vs. 1	1.58 (1.10–2.25)	Negative
		1202 total[a]	Sauce	Quartile 4 vs. 1	0.56 (0.38–0.82)	Positive
(Kirsh et al., 2006) USA	Prospective cohort	1338 cases 29361 total	Raw	>3/week vs. <2.5/month	1.04 (0.86–1.27) $p = 0.84$	No effect
			Pizza	≥1/week vs. <0.5/month	0.83 (0.67–1.03) $p = 0.06$	Suggestive positive
			Ketchup	>2/week vs. <1/month	0.99 (0.82–1.19) $p = 0.68$	No effect
(Ambrosini et al., 2008) Australia	Prospective cohort	97 cases	Raw	>4.1 vs. ≤1.7/week	1.04 (0.60–1.80) $p = 0.89$	No effect
		1985 total	Cooked	>2.2 vs. ≤0.6/week	0.67 (0.38–1.16) $p = 0.13$	Suggestive positive

[a] Cases of the cancer progression among total diagnosed with prostate cancer.

as the main source of dietary lycopene and the most widely and consistently recognized dietary means to decrease risk of prostate cancer, and which likely needs processing to enhance its health benefits.

CHEMICAL TRANSFORMATIONS IN THERMALLY PROCESSED TOMATOES

Studies of chemical composition of fresh tomato fruits and tomato products have long been and remain an issue of significant importance to the food industry, while more recent discoveries of health beneficial effects of tomato-based phytochemicals, such as lycopene, dramatically expanded an interest to chemical and biochemical transformations of these constituents as well. In their comprehensive review, Davies and Hobson (1981), admitted a great deal of variation in analytical data for ripe tomato fruit and gave the following averaged relative amounts of the tomato components per dry matter: 25% fructose; 22% glucose; 1% sucrose; 8% protein; 7% pectic substances; 4% hemicelluloses; 6% cellulose; 8% minerals; 2% dicarboxylic amino acids; 2% lipids; 4% malic acid; 9% citric acid; 0.5% ascorbic acid; 0.4% carotenoids; 0.1% volatiles; 1% other amino acids, vitamins and polyphenols. The total dry matter amounts for 5–7.5% of ripe tomato fruit tissue. Most of these components undergo chemical transformations to some extent during industrial processing, and some new chemical entities appear in tomato products as a result (Table 17.2).

Industrial Processing of Tomatoes

Typical technological steps of tomato processing are shown in Figure 17.1 (Gould, 1992). At the initial step, freshly scalded and chopped tomato fruits are either: (a) immediately mashed and kept briefly at 90–105°C or, (b) mashed at about 66°C and maintained at that temperature for 2–25 min. The processes are commonly referred to as "Hot Break" (HB) and "Cold Break" (CB), respectively. Breaking is necessary to deactivate most of pectolytic enzymes, which affect the texture quality. Thus, HB tomato products retain polymeric structure of pectic polysaccharides to a large extent and, consequently, possess relatively high viscosity that is preferred for tomato-based sauces or ketchup, while less viscous, more flavorful CB tomato is better suited for production of soups or juice. Finishing of the breaker mash is normally done by sieving off skins and seeds, and some 20% of the liquid serum may be squeezed off as well. The resulting tomato juice (about 5% solids) is deaerated by applying vacuum briefly. Deaeration prevents excessive loss of vitamin C and other antioxidants. The juice may then be seasoned, pasteurized, and canned for consumption or processed further. Tomato paste is made from tomato juice by evaporation to a predetermined percentage of soluble solids (which is also commonly referred to as Brix), usually in a range of 20–37%. During this processing step, tomato pulp is additionally exposed to elevated temperatures of 50–95°C, and a number of chemical reactions, largely of nonenzymatic hydrolytic and autoxidative origin, continue to affect its original composition. These reactions continue on, although at much slower rates, during storage of seasoned and sterilized tomato paste at ambient temperatures, although frozen storage is gaining more popularity.

A significant proportion of tomato is made into dehydrated products (Sabarez, 2008). Dehydrated tomato products have many advantages over tomato paste, including ease of packing, storage, transportation and mixing. Tomatoes can be sun-dried, dehydrated, or spray-dried. Sun-drying of ripe tomatoes is the oldest dehydration technology in which freshly halved fruits are soaked in a preservative solution containing sulfur dioxide before being exposed to the sun for 7–10 days, resulting in a product that contains 12–24% moisture. Notably, exposure to sunlight may significantly decrease antioxidant content and promote darkening, even in the presence of preservatives. A modern industrial modification, convective dehydration, uses warm air to dehydrate tomatoes and produce a final product with 5–7% moisture, having higher antioxidant content and possessing a milder taste. Currently, the mainstream technology of tomato dehydration is spray-drying in which droplets of tomato paste are briefly exposed to hot drying air at >110°C. However, due to water evaporation,

TABLE 17.2
Changes in the Content of Some Tomato Constituents in Raw and Processed Tomato (mg/100 g)

	Raw	Processed	Dehydrated
Glucose	1790[a]	25,800 → 20,700[b]	10,100[b]
Fructose	1860[a]	27,800 → 24,800[b]	20,400[b]
Pectic substances insoluble	320[c]	220[c]	
Pectic substances soluble	65[c]	160[c]	
Hemicelluloses	274[d]	265[d]	
Cellulose	576[d]	386[d]	
Amino acids	45.1[e]	337.6[e]; 6500 → 6700[b]	2,400[b]
Pyroglutamic acid	n.d.[b]	1630 → 1650[b]	1,690[b]
Fructose-amino acids	n.d.[b]	640[b]	9,500[b]
Phenolics, total antioxidant activity	16.2[f]; 14.2[g]; 22.3[h]	19.4 → 22.9[f]; 14.6[g]; 31.3[h]	
Flavonoids	0.9[g]	1.0[g]	
Vitamin C	13.4[g]; 12.8[h]	9.5[g]; 7.4[h]	
Lycopene, all-*trans*	19.6[i]	30.8 → 14.5[j]	99 → 99 → 84[i]
Lycopene, *cis* isomers		1.7 → 12.0[j]	
Lycopene, total	10.7[a]	32.5 → 26.5[j]	
Other carotenoids	2.1[a] 2.2[i]		11.2 → 11.0 → 9.9[i]
Tocopherols	41.9[k]	42.7[k]	
Total water soluble antioxidant activity, relative to controls	10.9[i]; 45[h]	25.2[i]; 83[h]	
Total methanol soluble antioxidant activity, relative to controls	5.2[i]	64.8[i]	
Total lipid soluble antioxidant activity, relative to controls	18.2[i]	28.4[i]	

[a] Ripe cherry tomato (Raffo et al., 2002).
[b] Per dry weight, tomato paste, processed at: 65°C → 90°C (Schräder and Eichner, 1996).
[c] Per fresh weight, tomato paste, processed at 90°C (Anthon et al., 2008).
[d] Per fresh weight, tomato purée, processed at 90°C (Reinders and Thier, 1999).
[e] Tomato juice (Gould, 1992).
[f] Per dry weight, tomato juice, processed at: 65°C → 90°C (Re et al., 2002).
[g] Cooked at 88°C (Dewanto et al., 2002).
[h] Baked at 200°C (Gahler et al., 2003).
[i] Corrected to tomato paste conc: raw → HB juice → paste (Takeoka et al., 2001).
[j] Tomato sauce, processed at: 75°C → 127°C (Unlu et al., 2007).
[k] Per dry weight, tomato paste, processed at: 93°C (Capanoglu et al., 2008).

their actual temperature does not exceed 90°C, and the final product contains only 3–4% moisture and preserves most of its vitamin C, other antioxidants, flavor, and color. That being said, tomato dehydration does bring about other changes in its composition, primarily related to (poly)condensation and degradation of dominant sugars, glucose and fructose.

Tomato powder is mainly rehydrated and reconstituted at food plants, restaurants, pizzerias, etc., to prepare the same range of tomato products (sauces, soups, juice, etc.) that are made from tomato paste or juice. As a result, one can expect then that chemical composition of tomato soup made from paste will differ from the one obtained with use of rehydrated tomato powder. This fact is rarely reflected in literature and may serve as a complicating factor in studies involving human consumption of tomato products.

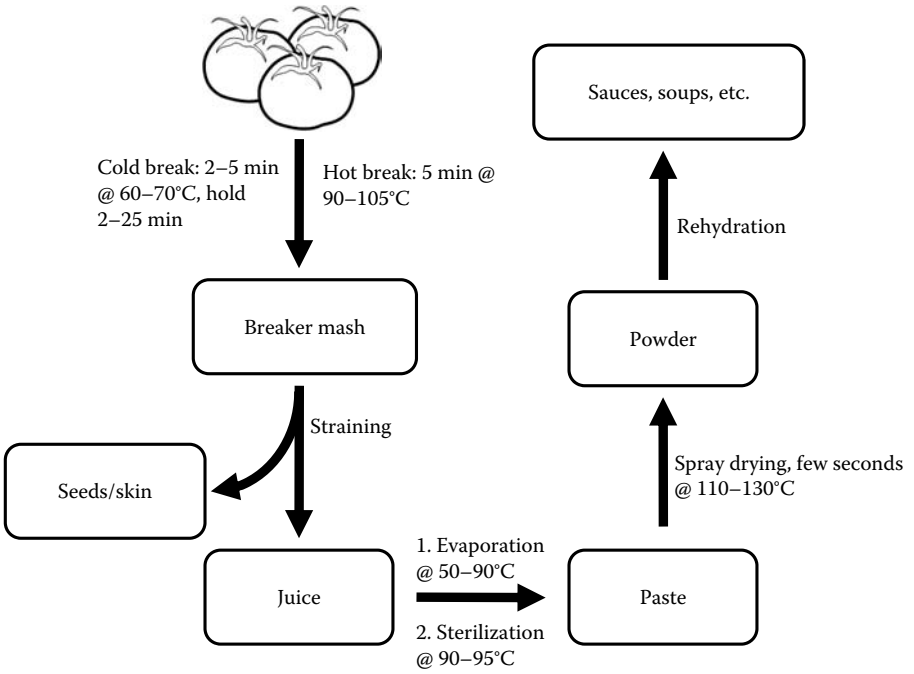

FIGURE 17.1 A simplified industrial tomato processing scheme.

Pectin and Other Cell Wall Polysaccharides

The principal structural elements of plant cell wall are rigid cellulose microfibrils held together by a network of intertwined matrix glycans (also referred to as hemicelluloses), pectins and structural glycoproteins (Kalamaki et al., 2006). The most abundant matrix glycan in tomato is (galacto)xyloglucan, a linear polymer of β-D-glucose, with α-D-xylose side substituents (Figure 17.2). Other common hemicellulose structures are represented by (galacto)glucomannan, a β-D-glucose and β-D-mannose block copolymer decorated with α-D-galactose, and glucuronoarabinoxylan, a β-D-xylose polymer with attached side chains of α-L-arabinose and α-D-glucuronic acid. While hemicellulose molecules cover cellulose microfibrils, the spaces in the cellulose/matrix glucan network are filled by pectins (Brummell & Harpster, 2001). Pectins are perhaps the most complex molecules found in nature and can include over 20 different monosaccharide units (Voragen et al., 1996). Their backbone consists of alternating regions of poly(α-D-galacturonic acid, mostly in methyl ester form) and poly(α-L-rhamnose-α-D-galacturonic acid) repeats. The side chains are versatile in size and composition and include, among others, oligomers of β-D-galactose, α-L-arabinose, or their branched combination known as arabinogalactan (Figure 17.2).

The cell wall rigidity gradually decreases upon tomato fruit ripening. This is achieved chiefly by hydrolysis and depolymerization of pectin molecules with the aid of enzymes such as polygalacturonase, pectin methylesterase, β-galactanase, and others (Kalamaki et al., 2006). Cellulose and hemicelluloses are also affected with help of glycan-modifying enzymes such as endo-β-glucanase, xyloglucan endotransglycosylase, etc. (Ali et al., 2004) Upon crushing tomato fruit at tomato processing plants, acceleration of pectin and (hemi)cellulose hydrolysis is halted by thermal inactivation of the hydrolytic enzymes in the HB or CB processes. The nonenzymatic degradation and solubilization of pectins and other matrix polysaccharides continues, however, at elevated temperatures and results in tenderizing the flesh, better homogenization, and increased bioavailability of encapsulated nutrients (phenolics, carotenoids, etc.).

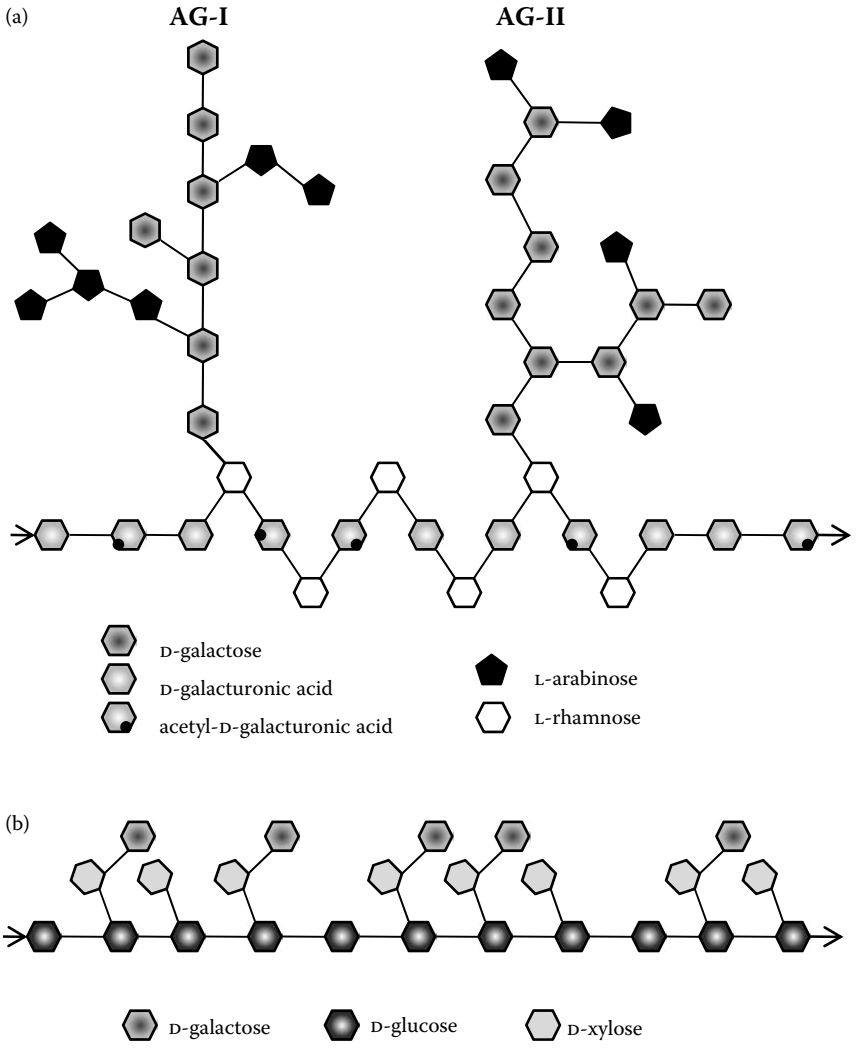

FIGURE 17.2 (a) Primary structure of a pectin fragment, rhamnogalacturonan I (RG-I). The backbone of RG-I is formed by alternating residues of β-D-galactopyranuronic acid (partially acetylated) and α-L-rhamnopyranose. The side chains of type I and type II arabinogalactans (AG-I and AG-II), as well as arabinan (not shown), are attached to the backbone of RG-I exclusively through L-rhamnose residues. The main chains in AG-I are formed by β-D-galactopyranose units, connected through (1 → 4) links. The side chains in AG-I consist of either β-D-galactopyranose or short α-L-arabinofuranose oligomers, both connected to the main chains through (1 → 3) links. In AG-II, the main galactan chains are built with (1 → 3) links, while the side chains consist of (1 → 6) linked β-D-galactopyranose oligomers, decorated with (1 → 3) attached α-L-arabinofuranose. (b) A fragment of galactoxyloglucan (a hemicellulose). The backbone is similar to cellulose and consists of (1 → 4) linked β-D-glucopyranose polymer. The side chains are short and are represented by (1 → 6) linked α-D-xylopyranose monosaccharides or β-D-galactopyranosyl(1 → 2)α-D-xylopyranose disaccharides. In both structures, availability of terminal β-D-galactopyranose residues is essential for potential blocking of tumor-associated galectins.

Polyphenols, Carotenoids, and Vitamins

Compared with other foods, the content of polyphenolic compounds in tomato is relatively small, though given the scale of tomato consumption, it may serve as an important dietary source of these antioxidants. Chlorogenic, caffeic, and *p*-coumaric acids represent the most abundant group of phenolic compounds in ripe tomato fruit, which is followed by flavonoids (Slimestad and

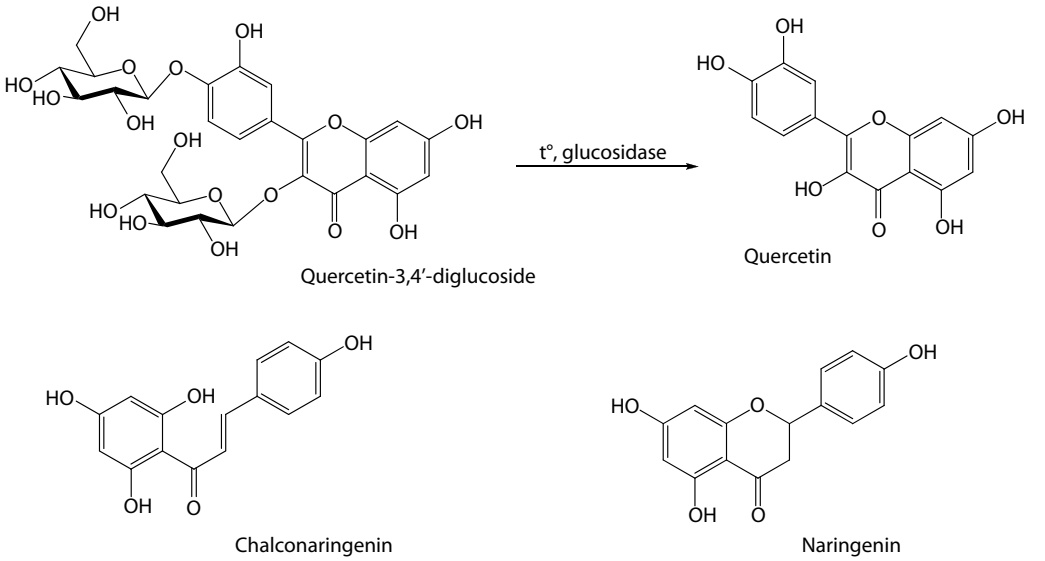

FIGURE 17.3 Typical structures of tomato flavonoids. The glycosylated structures are the chief form present in raw tomato. Thermal processing of tomato causes both enzymatic and nonenzymatic partial hydrolysis of the glycosidic bonds and release of the aglycons, which are believed to possess better bioavailability, antioxidant, and cancer cell proliferation inhibiting potential.

Verheul, 2009). Fresh tomato fruit contains flavonoids chiefly in the form of glycosides such as rutin (RhaGlc-quercetin), phloretin diglucoside, Api-RhaGlc-quercetin, RhaGlc-kaempherol, and others, but aglycons naringenin and chalconaringenin are also present in comparable concentrations (Figure 17.3). Most of the major phenolic structures are localized in the skins (Slimestad et al., 2008). Nevertheless, significantly higher bioaccessibility of polyphenols, such as naringenin or chlorogenic acid, has been proven to be present in cooked tomatoes (Bugianesi et al., 2004) as compared with the fresh fruit. This phenomenon was explained as loosening of the cell wall matrix which takes place during tomato processing and is reflected in an increase of total phenolic content in processed tomatoes (Re et al., 2002; Gahler et al., 2003). In addition, increase in bioavailability of flavonoids may be assisted by means of enzymatic and nonenzymatic hydrolysis of glycosidic bonds with a concomitant release of free phenolic groups in the aglycons (Nemeth et al., 2003).

There are several carotenoids found in ripe tomato fruit (Figure 17.4), with the most abundant being, in decreasing order, lycopene, carotene, phytoene, and phytofluene (Raffo et al., 2002). Due to its highest concentration in raw tomato (5–10 mg/100 g) as compared with other food sources, tomatoes serve as the main source of dietary lycopene, providing about 80–90% of this carotenoid to the Western population. In spite of its nutritional value, the fate of lycopene during industrial tomato processing has been a subject of numerous studies. Lycopene and other carotenoids are distributed unequally throughout tomato tissues and are mostly concentrated in skin and pericarp (Shi and Le Maguer, 2000). Not surprisingly, as skins are mechanically removed at the tomato pulp finishing step, there is significant lycopene loss. Another potential source of lycopene loss is its autoxidation, which may be promoted by elevated temperatures or sunlight during drying (Shi and Xue, 2008). Although the amount of lycopene in tomato decreases during processing by 5–30%, its bioavailability from tomato products or supplements may increase up to 50%, as compared with only 5% available from raw tomato (Shi and Le Maguer, 2000). It was hypothesized that fragmentation of the tissue matrix, presumably hydrophilic cell wall polysaccharides during tomato processing, makes hydrophobic, water insoluble carotenoids available to fatty substances, such as oils from food, which can solubilize and make carotenoids bioaccessible. In fresh tomato, the majority of

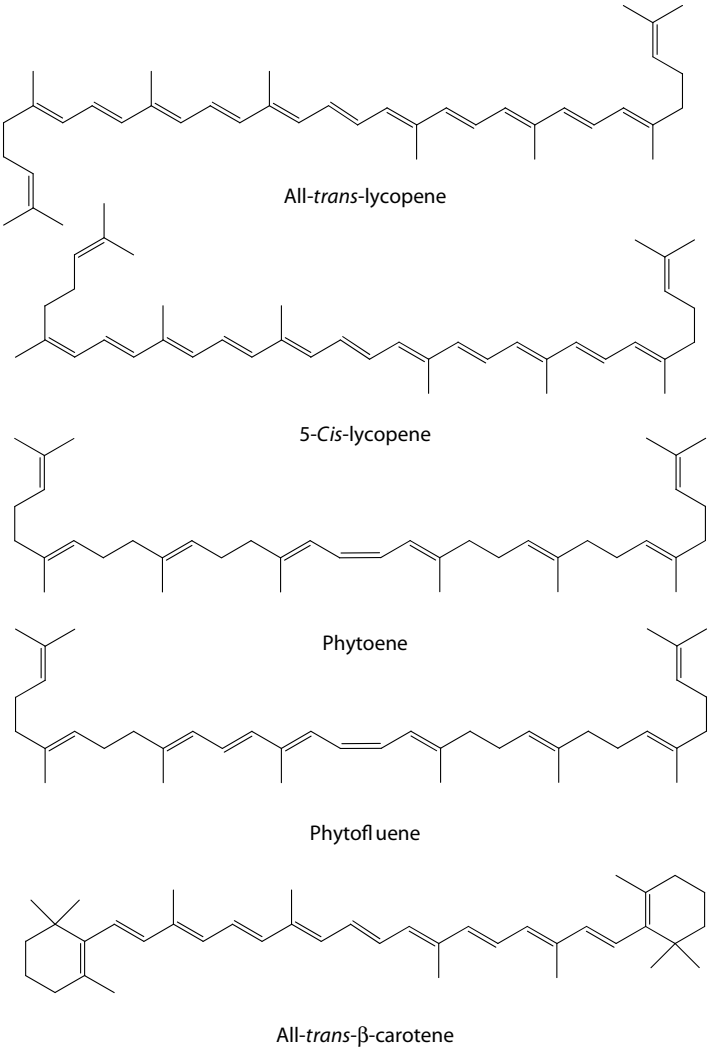

FIGURE 17.4 Structures of the most abundant carotenoids from tomato.

lycopene is present in the all-*trans*-isomeric form (Figure 17.4), while several *cis*-isomers constitute about 5%. During processing, the *cis*-isomers content increases to 6–18% (Seybold et al., 2004). The *cis*-isomers of lycopene are better incorporated into chylomicrons thus additionally improving lycopene bioavailability (Unlu et al., 2007). Interestingly, the isomeric composition of circulating lycopene is about 50/50, which may be a consequence of a further isomerization of lycopene in gastric juice and *in vivo*, better ingestibility of the *cis*-isomers into circulation, or both. In practice, however, total lycopene concentration in blood serum reaches saturation at 1–1.5 μM and lycopene absorption from food or supplements does not exceed 5 mg per day in healthy men (Diwadkar-Navsariwala et al., 2003).

The vitamin content in thermally processed tomatoes decreases as a consequence of thermal treatment-induced autoxidation. Ascorbic acid is particularly sensitive and its concentration drops more dramatically in tomato products, as compared with tocopherol (Table 17.2). The main product of ascorbate oxidation, dehydroascorbic acid, is relatively unstable and can degrade further (Serpen and Gökmen, 2007).

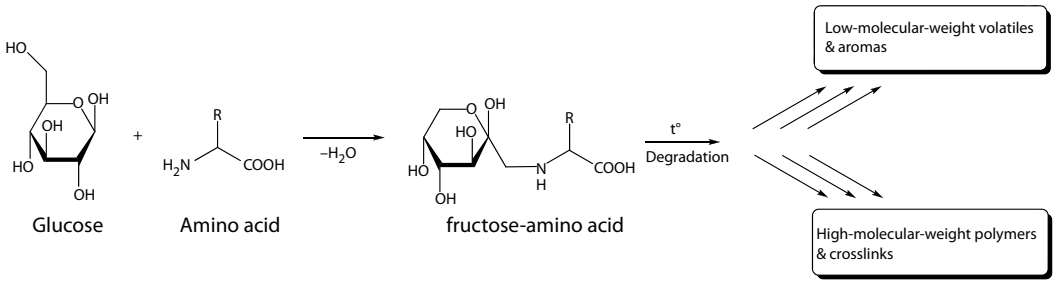

FIGURE 17.5 Initial steps of the Maillard reaction in dehydrated foods.

Maillard Reaction

The Maillard reaction is a term used for a large array of reactions which normally start between free glucose, fructose, or other reducing sugars with the amino groups of amino acids or proteins (Nursten, 2005). In the presence of organic acids, such as citric acid, D-glucose and amino acid molecules conjugate and rearrange into relatively stable D-fructose-amines (Figure 17.5). Equally abundant in ripe tomato, free D-fructose may conjugate with amino acids and rearrange to form D-glucosamine derivatives (Mossine et al., 1996). Subsequent steps of the Maillard reaction in thermally processed foods give rise to a broad variety of products, from dicarbonyl intermediates (Feather et al., 1996) to volatile aromas, to brown-colored polymers (Nursten, 2005). Normally, the formation of glucose-amino acid conjugates is accelerated under conditions of elevated temperatures and, especially, dehydration. Therefore, the extent of the Maillard reaction is the most prominent in dehydrated tomato flakes or powder where the total content of fructose-amino acids, not detectable in raw tomato, may reach 5–9% per total dry weight (Schräder and Eichner, 1996).

Total Antioxidant Content

Most studies indicate increase of total antioxidant capacity of tomato, of both hydrophilic and lypophilic origin, as a result of processing (Table 17.2). The likely factors which may positively affect total antioxidant content in tomatoes during industrial processing include: (a) release of incapsulated antioxidants, such as carotenoids, from the matrix; (b) deglycosylation of glycosylated polyphenols, both nonenzymatic and β-glucosidase catalyzed; (c) de-esterification of pectic uronic acids which may bind redox-reactive metal ions; (d) formation of novel antioxidants such as fructose-amino acids and other Maillard reaction products. These and other transformations may compensate for those that negatively affect the antioxidant capacity of processed tomato, such as autoxidation and degradation of vitamins C and E, polyphenols, and carotenoids upon heating, removal of polyphenol-rich tomato peels, etc.

POSSIBLE MECHANISMS AND TARGETS FOR THE PROCESSING-INDUCED TOMATO INGREDIENTS

Although there is a considerable epidemiological, clinical, and laboratory evidence in favor of fruit and vegetable consumption as a source of biologically active fibers, antioxidants or other phytochemicals that may reduce cancer risk, the identification of these molecules and their targets *in vivo* remains a major challenge and a subject of extensive research. The protective properties of plant fibers and phytochemicals have been implicated in all generally recognized chemoprevention mechanisms such as stimulation of the host's defense against DNA damage (Conney, 2003), cell growth inhibition and induction of apoptosis in tumor cells, inhibition of angiogenesis (Tosetti et al., 2002), tumor cell migration, and others.

Lycopene

Lycopene is widely implicated as the chief chemopreventive component in tomato products. Multiple epidemiological studies reported an inverse association between circulating lycopene and prostate cancer (Etminan et al., 2004; Wu et al., 2004a). A few clinical trials have supported a therapeutic potential of lycopene as well. Thus, in patients with newly diagnosed, clinically localized prostate cancer, supplementation with 15 mg of lycopene, in the form of "Lyc-O-Mato" capsules twice daily for 3 weeks before surgery, significantly decreased tumor growth, plasma PSA and IGF-1 levels in the intervention group vs. control (Kucuk et al., 2001). Although an exact *in vivo* mechanism of its protective activity against prostate cancer is not known, a number of important potential molecular targets for lycopene have been identified over the years. The best *in vitro* characterized property of lycopene is its antioxidant potential. Several research groups reported that lycopene protected cultured cells from ROS- or radiation-induced DNA damage, lipid peroxidation, and other deleterious effects of oxidative stress (Matos et al., 2000; Srinivasan et al., 2009). *In vivo*, lycopene was effective in protection of rat prostate against induced oxidative stress (Matos et al., 2006). In patients with localized prostate adenocarcinoma, consumption of lycopene-rich, tomato sauce-based diet led to a decrease in DNA oxidative damage (Chen et al., 2001). It has therefore been initially suggested as an inhibitor of oxidative stress in the prostate tissue, due to its propensity to accumulate in this organ. However, lack of a consistent evidence for improved antioxidant status in patients ingesting purified lycopene supplements rather than tomato products, put into a question the significance of direct antioxidant action provided by lycopene (Erdman et al., 2009). In experimental rats, a short-term increase in tissue and serum lycopene or phytofluene resulted in a 40–50% drop in circulating testosterone (Campbell et al., 2006a). The authors considered negative regulation of steroidogenic enzymes mRNA as a possible mechanism of the carotenoid-altered androgen status, which, in turn, may contribute to the protective effect of tomato. Other molecular targets for lycopene have been identified and include growth factor receptor cyclin D1, cell signaling molecules Akt and NF-κB, while cellular effects of lycopene include inhibition of cancer cell proliferation, regulation of transcription, gap junctional communication, and others [Table 17.3; see also Preedy and Watson (2008)].

Phenolics and Other Antioxidants

The hydrophilic antioxidant fraction accounts for about 90% of total antioxidant capacity in tomato and is thought to be provided mainly by phenolics (Lavelli et al., 2000; Wu et al., 2004b). Like in the case of lycopene, direct scavenging of reactive oxygen species is no longer considered as the chief *in vivo* bioactivity mechanism for this class of compounds. Many phenolics make good inhibitors for tyrosine kinases and phosphatases, and in such quality, can participate in modulation of cellular signaling pathways, including those implicated in cancer (Ramos, 2008).

The most abundant tomato flavonoid naringenin (Figure 17.3) inhibits the activity of aromatase (CYP19), thus decreasing estrogen biosynthesis and producing antiestrogenic effects, important in breast and prostate cancers (Moon et al., 2006). It also inhibited AKR1C3, a redox regulator of hydroxysteroid/ketosteroid transformations and a potential target in prostate cancer (Škarydová et al., 2009), but stimulated DNA repair from oxidative damage in LNCaP cells (Gao et al., 2006). Naringenin has been proposed as a potential suppressor of metastasis to lungs (Du et al., 2009) that may act by improving the immunosuppressive environment through downregulating TGF-β1 and reducing regulatory T cells.

Quercetin has often been implicated as an active ingredient in plant foods with a cancer preventive potential, although, to date, no evidence has been generated for its relation to prostate cancer risk. In chemically induced rodent carcinogenesis models, quercetin aglycone consistently modulated development of aberrant crypt foci in the colon, or adenomas in other sites. In contrast, its glycoside derivatives generally failed to demonstrate any protective effects (Murakami et al., 2008).

TABLE 17.3
Some Cancer-Related Molecular Targets and Biological Effects Affected by Agents that are More Abundant or Bioaccessible in Processed vs. Raw Tomato

Compound	*In Vitro* Molecular Targets	*In Vitro* Cellular Effects	*In Vivo* Effects	Literature
Lycopene	↓ROS; ↓IGF-I; ↓NF-κB; ↓PI3K/Akt; ↓cyclin D1; ↑phase II detoxicant enzymes (GST, UDP-GT, MnSOD, etc.)	Antioxidant; increased apoptosis; decreased cell cycle progression; transcription regulation; increased gap junctional communication	Decreased tumor growth, plasma PSA and IGF-I levels in PC patients; antioxidant in rat prostate	Ben-Dor et al. (2005); El-Agamey et al. (2004); Karas et al. (2000); Kucuk et al. (2001); Matos et al. (2000); Matos et al. (2006); Nahum et al. (2006); Heber & Lu (2002)
Oxidized lycopene		Increased apoptosis; transcription regulation		Ben-Dor et al. (2005); Nara et al. (2001)
Phytoene, phytofluene	↓ROS	Antioxidant; increased apoptosis		Ben-Dor et al. (2005); Nara et al. (2001); Shaish et al. (2008)
Naringenin	↓ROS; ↓aromatase; ↓steroid oxidoreductase	Antioxidant	Immunostimulation by downregulation of TGF-β1	Du et al. (2009); Moon et al. (2006); Gao et al. (2006); Škarydová et al. (2009)
Quercetin	↓ROS; ↑NRF-2; ↓fatty acid synthase; ↓transcription factors; ↓PI3K; ↓EGFR; ↓NF-κB; ↓cyclin D1; ↓Bcl-2; ↑Bax; ↑PARP; ↑Gadd 45; ↑caspases; ↑p53; ↓Erb2R; ↓VEGF; ↓VCAM; ↓MMP-9	Increased apoptosis; cell cycle arrest; transcription regulation; antitubular	Carcinogenesis modulation	Brusselmans et al. (2005); Murakami et al. (2008); Ramos (2008); Wang et al. (2003).
Arabinogalactan, solubilized pectin	↓Galectin-3	Antiproliferative, antiadhesive	Antimetastatic; source of short fatty acids in gut; heavy metal sequestrant	Mossine et al. (2008b) Pienta et al. (1995); Eliaz et al. (2006)
Fructose-amino acids	↓ROS	Antioxidant; antiproliferative	antitumorigenic (with lycopene/ tomato); antimetastatic	Glinsky et al. (1996); Ide et al. (1999); Mossine et al. (2008a)

Based on its chemical properties, quercetin was primarily recognized as an efficient antioxidant, capable of scavenging free radicals such as superoxide and peroxyl radicals. The ROS-scavenging activity in quercetin depends on availability of the catechol hydroxyl groups. For this reason, glycosylated or glucuronylated quercetin derivatives are less potent antioxidants, as compared with the aglycon molecule. A current consensus view, however, is that the protective effects of quercetin must be related to targets other than ROS. Over the last decade, a number of molecular targets

relevant to the chemopreventive potential of quercetin was suggested (Murakami et al., 2008; Ramos, 2008) and included such cellular receptors as cell surface laminin receptor 67LR, insulin-like growth factor-I receptor (IGF-IR), aryl hydrocarbon receptor (AhR), estrogen and androgen receptors, epidermal growth factor receptor (EGFR) and death receptor DR5. Multiple potential targets that are involved in cellular signal transduction pathways associated with tumorigenesis and are affected by quercetin and other flavonoids, include apoptosis-related kinases JNK, RAF, MEK, Akt, ERK, and, most likely, a number of others. Modulation of the pathways involved in the production of pro-inflammatory mediators by quercetin is thought to involve targeting of ERK or p38 MAPK, and the same target kinases that were implicated in a quercetin-assisted cellular detoxification. Treatment of human prostate cancer cell lines such as LNCaP, PC-3, or DU-145 with quercetin resulted in the cell death through apoptosis (Aalinkeel et al., 2008; Lee et al., 2008). One proposed mechanism to explain this finding includes inhibition of a survival protecting kinase Akt in the neoplastic, but not normal human prostate epithelial cells, and a subsequent translocation of proapoptotic Bax to the mitochondrial membrane and the activation of caspases. Another identified target for quercetin in the proapoptotic cascade is heat shock protein 90. Its downregulation has been induced by quercetin in the cultured cancer cells, while no normal prostate epithelial cells were affected. Quercetin and kaempherol inhibited IGF-I-stimulated proliferation of a rat prostate adenocarcinoma cell line by reducing the insulin receptor substrate-1 (IRS-1) content in cells and thereby modulated IGF-I signal transduction cascades (Wang et al., 2003). Interestingly, quercetin, along with kaempherol, also showed a potential for activation of immune response to prostate tumors, by enticing secretion of GM-CSF in human prostate PC-3 line *in vitro* (Bandyopadhyay et al., 2008).

Soluble pectin and galactoxyloglucan fragments (Figure 17.2) may also provide a boost to the protective potential of processed tomato. The structural complexity and variability of pectic oligosaccharides define a variety of physico-chemical properties of these substances, which may provide for a multitude of nutritional and physiological effects reported for this class of dietary agents. Thus, multiple health-benefitting effects were attributed to dietary pectic substances, including glycemic control in diabetics, lowering blood cholesterol and LDL (Chandalia et al., 2000), immunostimulation and immunoprotection (Yamada, 1996), heavy metal detoxification, and related antioxidant activities (Eliaz et al., 2006). Significant epidemiological and experimental evidence suggests that diets rich in soluble fiber, including pectic substances, reduce the risk of colorectal and breast cancers (Moore, 2002). While the possible anticarcinogenic mechanisms are not clear, the suppression of pro-carcinogenic bile acids by the polysaccharides and the production of the protective short-chain fatty acids (SCFA) during pectin fermentation in the gut may play an important role. More definite data have been obtained in mechanistic studies which established galectins as a potential target for soluble pectin in several breast and prostate cancer models (Mossine et al., 2008b). Galectins are a group of mammalian β-galactoside-binding lectins that are abnormally expressed in many cancers. Galectins have been implicated in the tumor proliferation and dissemination, and suspected in cancer immune protection and identified as novel targets for cancer therapies (Liu and Rabinovich, 2005). These galectin functions can be affected through its blockage by galactoside-rich pectic oligomers such as modified citrus pectin (MCP) or arabinogalactan. MCP displays high affinity toward galectins, due to the multivalent mode of binding to these proteins (Mossine et al., 2008b). *In vitro*, MCP prevented adhesion of human prostate, colon, and breast cancer cells to vascular endothelial cells, inhibited aggregation and clonogenic growth of the tumor cells. MCP ingested by animals in advanced prostate, colon, and breast cancer models significantly decreased number and incidence of lung metastases (Nangia-Makker et al., 2002; Mossine et al., 2008b). Another potentially important target for β-galactoside-bearing pectic substances is cancer-associated Thomsen-Friedenreich antigen (Khaldoyanidi et al., 2003), which is commonly expressed in prostate and colon adenocarcinomas and is also involved in β-galactoside-mediated interactions in cancer proliferation and dissemination. Despite the growing evidence in favor of cancer protective potential of soluble pectic substances, the field is still in an embryonic state. Many critical issues,

such as bioavailability and pharmacokinetics of these oligosaccharide molecules, and their possible interaction with other potential targets in cancer, remain essentially unexplored.

Research investigating the potential contribution of the main Maillard reaction products in dehydrated tomatoes, that is, fructose-amino acids, to cancer prevention is also new. Scattered available data suggest that fructose-amino acids possess antioxidant activities. Thus, D-fructose-L-arginine has been identified as a contributor to antioxidant potential of dried plants known to reduce cancer risk, such as garlic powder (Ryu and Rosen, 2003) or ginseng root (Joo et al., 2008). In tomato powder, even more potent antioxidant, D-fructose-L-histidine (FruHis), has been discovered (Mossine and Mawhinney, 2007), and its ability to prevent both chemically induced carcinogenesis in the prostate and to inhibit implanted prostate tumor growth was demonstrated in two rodent models (Mossine et al., 2008a). Synthetic fructose-amino acids have also shown inhibition of primary and secondary breast and melanoma tumor growth in mice (Denisevitch et al., 1995; Glinsky et al., 1996), but the mechanism of their action has not been established yet.

Interactions between Components from Processed Tomato

Boileau et al., (2003) demonstrated that supplementation of pure lycopene to diets of *N*-methyl-*N*-nitrosourea/testosterone-treated rats did not decrease spontaneous carcinogenesis rate in the prostate of the experimental animals, while supplementation of tomato powder proved to be protective. Plasma lycopene concentration in rats receiving lycopene beadlets was about 35% higher than in rats on the tomato powder diets. The authors concluded that, to exert its protective potential, lycopene may need to interact with other component(s) from tomato products. Similar conclusions were drawn from the analysis of multiple intervention studies (Basu and Imrhan, 2007). In support of this hypothesis, a number of investigations established synergistic interaction between lycopene and other antioxidants *in vitro* and *in vivo*. For example, antioxidant synergism of lycopene and vitamins C, E, other phenolics has been documented in a number of studies which employed lipid peroxidation or free radical trapping assays for testing *in vitro* (Shixian et al., 2005; Milde et al., 2007; Liu et al., 2008).

A possibility of interaction between lycopene and other dietary plasma antioxidants was tested in a small case–control study (Goodman et al., 2006), which established a significant inverse association between prostate cancer risk and an exposure to a combination of lycopene with α-tocopherol and β-carotene (OR = 0.57, CI = 0.20–1.58), and that was even stronger in men with the Arg/Arg genotype. Treatment of transgenic *Lady* mice with a combination of dietary lycopene, vitamin E, and selenium resulted in a fourfold reduction in the incidence of prostate cancer, as compared with controls (Venkateswaran et al., 2004). An explanation to a possible *in vivo* mechanism of interaction between lycopene and vitamin E can be drawn from an observation of their additive effect to repress androgen target genes, such as steroid 5α-reductase, in the Dunning (MAT-LyLu) advanced rat prostate carcinoma model (Siler et al., 2004). One of the Maillard reaction products from dehydrated tomato, antioxidant FruHis, strongly synergized with lycopene against proliferation of the same Dunning MAT-LyLu cell line *in vitro* and *in vivo* (Mossine et al., 2008a). When FruHis-supplemented tomato paste was included into the diets of NMU/testosterone treated Wistar-Unilever rats, the animals had a significantly better survival from cancer, while incidence of carcinogenesis in the prostate dropped sixfold, as compared with the controls. It is presently not clear whether the protective effect of FruHis can be credited to its antioxidant properties, cytotoxic interaction with lycopene, or both.

Interaction of lycopene from tomato with active components from other foods against tumorigenesis has also been suggested in intervention studies, such as testing tomato/broccoli combination versus implanted rat prostate carcinoma in the Dunning model (Canene-Adams et al., 2007), tomato/garlic vs. aberrant crypt foci in colons of carcinogen-treated rats (Sengupta et al., 2004), or the same dietary combination versus chemically induced carcinogenesis in the buccal pouches of hamsters (Bhuvaneswari et al., 2005).

Other than lycopene, tomato phytochemicals demonstrated synergistic interactions as well. Flavonoids in the aglycon form, such as quercetin, kaempherol or naringenin, interacted with each other against Hepa-1c1c7 and LNCaP cancer cell lines proliferation *in vitro* (Campbell et al., 2006b). When compared with the individual antioxidants, combinations of β-carotene with these flavonoids have also better protected against ROS-induced cellular DNA damage (Yeh et al., 2006) and suppressed release of pro-inflammatory cytokines by leukocytes (Yeh et al., 2009). In simulated gastric juice, dietary plant oligosaccharide preparations enhanced antioxidant capacity of ascorbic acid (Sun-Waterhouse et al., 2007).

CONCLUSIONS AND PERSPECTIVES

Tomatoes and tomato products are an important dietary source of health beneficiary nutrients and phytochemicals. Epidemiological and experimental data suggest an inverse relation between ingested tomato and prostate cancer risk. The protective effect of tomato is likely determined by lycopene and may be largely dependent on synergistic interaction of lycopene with other bioactive components from tomato or other foods. Tomato processing significantly enhances bioavailability of lycopene, other phytochemicals and nutrients, and gives rise to novel species which may be involved in the protective mechanisms of lycopene. Nevertheless, there is no universal agreement on whether dietary tomato/lycopene protects humans from prostate cancer, due to a significant proportion of contradictory conclusions coming from intervention and other studies. Given the scale of tomato consumption and its rather exclusive place as a promising natural means against prostate cancer risk, a more comprehensive approach to the evaluation of its protective potential will need to address a few important issues.

Choice of relevant clinical endpoints in the intervention studies is one of these issues. For example, since prostate cancer develops slowly in the later stages of life, reducing mortality from prostate cancer through lifestyle and diet management may generally be more feasible than preventing its occurrence (Giovannucci et al., 2007), and monitoring PSA levels may not be the most optimal clinical endpoint.

Precise chemical control of tomato products in the intervention studies remains another important issue. Since the chemical composition of tomato products may differ dramatically, the reliance on carotenoid analysis alone could have led to the frustrating data inconsistencies and confusions noted above. This, in turn, has diminished any initial enthusiasm regarding the protective potential of tomato/lycopene against prostate cancer. Indeed, routine analytical procedures for major bioactive components of processed tomato, such as soluble fibers or fructose-amino acids, have not yet been developed, although the emergence of powerful analytical techniques based on novel mass-spectrometric instrumentation will help to establish the missing methods.

An important related issue to be addressed in the future studies is to differentiate protective effects of food products based on dehydrated tomato, or other vegetables/fruits. So far, only a few studies included evaluation of protective effects of dried fruits on cancer risk. In a prospective cohort study surveying about 14,000 Adventist men (Mills et al., 1989), intake of ≥5 vs. <1 servings of raisins, dates, other dried fruit per week, when related to prostate cancer risk, produced RR = 0.62 (0.36–1.06) and $p = 0.06$. In a more recent cohort study (Schuurman et al., 1998), the protective effect of dietary dried fruits has been documented as well, with RR = 0.49 (0.18–1.32). More such studies will be needed, as improving dehydration technologies may allow dried fruits to acquire a larger part in the human diet and thus become an important source of bioactive carbohydrate derivatives, such as those that are specifically related to the early Maillard reaction.

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18 Glycemic Index, Glycemic Load, and Cancer Risk

Helen G. Mulholland, Liam J. Murray, and Marie M. Cantwell

CONTENTS

INTRODUCTION

Dietary carbohydrate is currently a hot topic in human nutrition and health in the wake of the "Atkins" era, which has generated a poor reputation for carbohydrate in the role of weight management, in spite of little supportive scientific evidence. Current dietary guidelines advise individuals to obtain at least 50% of total energy intake from carbohydrate; therefore, it is important the public are aware that carbohydrate-containing foods should not be avoided. The glycemic index (GI) and glycemic load (GL) are relatively new concepts of carbohydrate classification that seek to guide the public to make choices that may be beneficial to health.

What is the Glycemic Index?

The dietary glycemic index (GI) concept was conceived in 1981 by Jenkins et al. at the University of Toronto as a way of classifying foods that contain carbohydrate according to the physiological

response their consumption induced (Jenkins et al., 1981). This innovative classification scheme differed from previous carbohydrate classifications that focused mainly on the size and structure of carbohydrate.

In order to measure the GI of a particular food, study volunteers consume, on separate occasions, the test food and a standard food containing 50 g of available carbohydrate. Their blood glucose concentrations are then measured periodically over the following 2-hour postprandial period (Jenkins et al., 1981). The GI value of the test food is then calculated by plotting a blood glucose response curve, and subsequently expressing the incremental area under the curve of the test food as a percentage of the area under the curve of a standard food (Jenkins et al., 1981).

The standard reference food can be glucose, whereby glucose is assigned a GI value of 100, or white bread, whereby white bread is assigned a value of 100. Glucose is the preferred control food due to its more stable composition, however, white bread is more palatable and is still utilized widely (Foster-Powell et al., 2002). The suggested cut-off points for ranking GI values, according to the glucose reference scale, are 55 or lower for low GI foods, above 55 and less than 70 for medium GI foods and 70 or higher for high GI foods (Foster-Powell et al., 2002).

What is the Glycemic Load?

Food GI calculations are usually based on portions containing 50 g of carbohydrate; however, in reality an individual's blood glucose, and hence insulin, response varies after consuming differing amounts of carbohydrate. An additional measure, the glycemic load (GL) concept, was introduced in 1997 by researchers at Harvard University as an extension of GI to better reflect the overall glucose demand of a food by incorporating both the GI value and the total carbohydrate content of usual portion sizes of foods (Salmeron et al., 1997).

GI and GL are therefore considered to be measures of carbohydrate quality, and quality and quantity, respectively. Notably though, the GL concept has yet to be officially endorsed, and experts recommend that data on GL is used with caution, given the likely geographical, intra- and inter-individual variations in usual portion sizes (Foster-Powell et al., 2002).

It should be noted that the relationship between the GI and GL of a food is not straightforward. A high GI food can be a low GL food if it is low in carbohydrate content, for example watermelon, or if it is normally eaten in small quantities. Alternatively, a low GI food can have a high GL if it is eaten as a large portion size. It is more desirable to achieve a low GL diet by consuming moderate-large quantities of low GI carbohydrates rather than consuming smaller amounts of high GI carbohydrates.

Examples of Low and High Glycemic Index/Glycemic Load Foods

Examples of low and high GI foods are displayed in Table 18.1. Most fruits and vegetables, legumes, barley, pasta, all-bran and wholegrain breads are considered to be low GI foods (Foster-Powell and Miller, 1995; Foster-Powell et al., 2002; Atkinson et al., 2008). There are some exceptions; for example watermelon is classified as a high GI food, but is low in carbohydrate and is therefore a low GL food and is also a good source of other beneficial nutrients such as lycopene. Limiting consumption of this fruit is therefore not recommended.

Potatoes, white and wholemeal breads, and refined breakfast cereals such as cornflakes fall into the high GI category (Atkinson et al., 2008). The fibre content of foods appears to have little predictive value for GI values, as wholemeal and enriched high-fiber white breads have a very similar GI to white breads and baked potatoes have a slightly higher GI value than boiled or mashed potatoes (Atkinson et al., 2008). When examining sugars, fructose and lactose are considered to have low GI values, thus it is not surprising that most fruits and milk are also low GI foods, while sucrose has a medium GI value (Atkinson et al., 2008).

TABLE 18.1
Examples of Low and High Glycemic Index (GI) Foods

Low GI	High GI
Legumes	White rice
Wholegrain bread	White or wholemeal breads
Pasta	Potatoes
All-bran	Cornflakes

The authors of the International tables of GI and GL values have strongly advised against using the GI value in isolation as an indicator of a "good" or "bad" food and suggest that the GL, amount, and type of fat, fiber, and salt content of each food should also be taken into consideration (Foster-Powell and Miller, 1995; Foster-Powell et al., 2002; Atkinson et al., 2008).

GLYCEMIC INDEX, GLYCEMIC LOAD, AND CANCER RISK

Recently, Jenkins et al. (2002)—the founders of the GI concept—called for more studies to assess the relationship between GI and chronic disease risk, including cancer. High GI and GL intakes are associated with many risk factors for cancer common in Western societies, including hyperglycemia, hyperinsulinemia, diabetes, sedentary lifestyles, and obesity (Brand-Miller, 2003).

Proposed Mechanisms Linking Glycemic Index, Glycemic Load, and Cancer Risk

There are several biologically plausible mechanisms linking GI and GL with cancer risk, which are interrelated, as presented in Figure 18.1. Although the proposed mechanisms may differ slightly for different cancer types, the suggested pathways are largely generic to carcinogenesis.

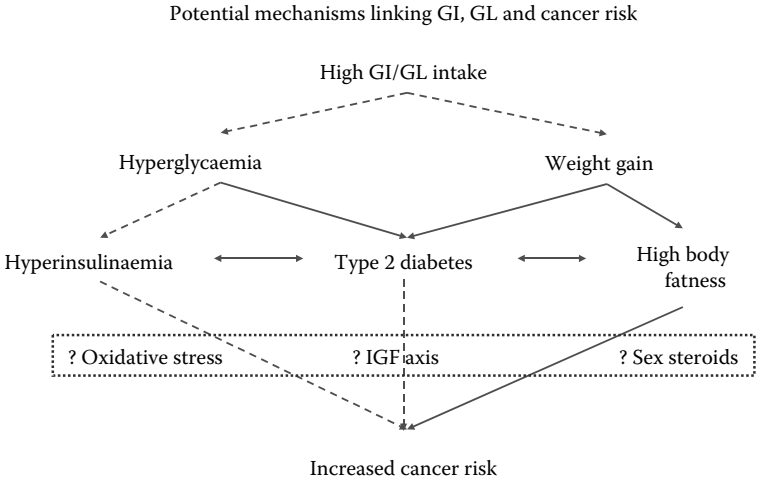

FIGURE 18.1 Potential mechanisms linking GI/GL and cancer risk. GI: glycemic index, GL: glycemic load, IGF: Insulin-like growth factors. Dashed line indicates suggested evidence of a link. Solid line indicates conclusive evidence of a link.

Hyperglycemia

Consuming a low GI or GL food results in a slower rate of food digestion and glucose absorption, and in turn a smaller, more gradual rise in postprandial blood glucose concentrations than a comparable high GI or GL food. Over time, habitual consumption of a high GI or GL diet may lead to a prolonged state of elevated postprandial glycaemia (Jenkins et al., 2002). Hyperglycemia may induce chronic inflammation and tissue damage via excess oxidative stress from the generation of reactive oxygen species and the liberation of pro-inflammatory cytokines, all of which may be implicated in carcinogenesis (Jenkins et al., 2002; Giugliano et al., 2008). Australian researchers have sought to explore the relationship between nuclear factor-κβ (NF-κβ), a transcription factor which is an important mediator of both pro-inflammatory gene transcription and also other inflammatory markers, such as cytokines (Dickinson et al., 2008). In their small study, which incorporated 10 young healthy subjects, NF-κβ activation in mononuclear cells was significantly higher after consuming a high GI meal compared with a low GI equivalent. The authors concluded that the relationship between GI and chronic disease, including cancer, may be explained by aggravated inflammatory responses induced by elevated blood glucose concentrations (Dickinson et al., 2008).

Chronic hyperglycemia is also associated with the development of Type 2 diabetes mellitus (Barclay et al., 2008), which in turn is linked to an elevated risk of many malignancies including kidney, liver, pancreas, colorectal, prostate, breast, and endometrial cancers (Mori et al., 2000). Therefore, it could be speculated that habitual consumption of a high GI or GL diet may indirectly increase cancer risk via hyperglycemic and/or Type 2 diabetes mellitus development.

The Insulin Hypothesis and Related Growth Factors

Long-term consumption of a high GI or GL diet is proposed to induce hyperglycemia, may in turn lead to chronic hyperinsulinemia. There has been much speculation that elevated blood insulin levels are contributing to a heightened risk of cancer (Augustin et al., 2002; Pisani, 2008). Meta-analyses have illustrated that colorectal and pancreatic cancer risks are positively associated with high levels of circulating insulin and C-peptide, a marker of insulin secretion (Pisani, 2008). Insulin itself conveys mitogenic properties *in vitro* promoting tumor cell growth but also acting indirectly through the actions of insulin-like growth factors (IGFs) and associated binding proteins (IGFBPs) (Giovannucci, 2001).

The majority of circulating IGF-1 is bound to IGFBP-3, which controls the availability of free IGF-1 by modulating its access to the IGF-1 receptor (Augustin et al., 2002). Elevated blood insulin concentrations suppress IGFBP concentrations, which consequently increases circulating concentrations of free IGF-1 (Augustin et al., 2002). IGF-1 has been shown to promote cell growth as it is required for cell cycle progression, and has angiogenic and antiapoptotic properties that may stimulate tumor development (Giovannucci, 2001; Augustin et al., 2002; Kaaks et al., 2002). A comprehensive systematic review was conducted by Renehan et al., investigating IGF-1 and IGFBP-3 in relation to cancer risk (Renehan et al., 2004). Direct associations were observed between IGF-1 concentrations and risk of prostate, colorectal, and premenopausal breast cancer risk but had no effect on lung or postmenopausal breast cancer risk (Renehan et al., 2004). No significant inverse associations were identified between total IGFBP-3 concentrations and prostate, lung, colorectal, or breast cancer risk (Renehan et al., 2004).

Nutrition is a key regulator of IGF-1 concentrations and the IGF axis may be a potential link between GI and cancer risk, even independently of insulin (Biddinger and Ludwig, 2005). A validation study of the effect of GI on the insulin response reported an overall 70% reduced insulin response after consumption of a low GI food compared with a high GI food (Brand-Miller et al., 2005). However, alterations to IGF-1 concentrations were minimal following the low compared with the high GI food. Notably, this study was conducted in lean young subjects and so the application of these findings to obese people is currently unknown. Obese subjects are known to have

elevated circulating IGF-1 as a result of overnutrition (Augustin et al., 2002) and it is therefore plausible that a high GL diet in people with a higher BMI may have a more profound effect on IGF-1 levels.

High Body Fatness

High body fatness, as measured by body mass index (BMI), contributes to an increased risk of most malignancies including esophageal adenocarcinoma, thyroid, colorectal, kidney, gallbladder, and postmenopausal breast cancer, yet contrastingly, probably protects against premenopausal breast cancer (Renehan et al., 2008). Excess body weight, and abdominal obesity in particular, is usually accompanied by a state of insulin resistance, which may be implicated in the etiology of many cancers (Renehan et al., 2006).

Consuming a low GI diet may be beneficial in avoiding weight gain and obesity by altering appetite and substrate oxidation in the body (Brand-Miller et al., 2002). It is proposed that recurrent postprandial hyperglycemia and hyperinsulinemia induced by a habitually high GI/GL diet leads to increased carbohydrate oxidation and reduced fat oxidation. The resulting increased dependence on carbohydrate and protein fuel sources, which are limited, may stimulate appetite and create a consequential positive energy imbalance resulting in a gain in body fat (Brand-Miller et al., 2002).

In reality, low GI diet interventions have been shown to produce modest but significant reductions in body weight in randomized controlled trials according to the results of a Cochrane systematic review (Thomas et al., 2007). However, the findings from a well-designed randomized crossover food provision intervention trial published since the Cochrane review do not support claims that a low GI diet can affect body weight (Aston et al., 2008). In their study of overweight and obese English women, free-living diets differed by 8.4 units of GI between groups and no differences in energy intake, body weight, or composition or subjective ratings of satiety were observed. The researchers highlighted that previous studies often did not match low and high GI diet interventions in fiber content, energy density or macronutrient composition, which may have accounted for any associations previously seen with body fatness (Aston et al., 2008). The current available evidence is inconclusive and robust judgements on GI and GL intakes and the prevention, or reversal, of body weight gain and any subsequent indirect effects this may have on cancer risk is difficult.

Sex Steroids

Excess body weight and hyperinsulinemia can induce alterations in sex steroid synthesis and bioavailability that may be conducive to tumor development (Renehan et al., 2006). Hyperinsulinemia in obesity is thought to suppress sex-hormone binding globulin activity, resulting in higher levels of circulating estradiol which can promote carcinogenesis (Augustin et al., 2002; Renehan et al., 2006). Increased IGF-1 bioactivity inhibits apoptosis and sex-hormone binding globulin synthesis, while stimulating cell proliferation and sex steroid synthesis, all of which could be implicated in the development of hormonal cancers, in particular, breast, endometrial, and ovarian cancer (Kaaks and Lukanova, 2001).

There is only limited evidence currently available investigating the effects of dietary GI and GL on sex steroid metabolism. In the DIANA randomized trial, 49 postmenopausal women were assigned to an intervention group which followed a regime low in total fat and refined carbohydrate intake, high in omega 3 fatty acids, dietary fiber, and phytoestrogens (Kaaks et al., 2003). The intervention was also reported to be plentiful in low GI foods, although the actual GI and GL values of the intervention and control diets were not detailed. After five months of following the intervention, women had increased circulating sex-hormone binding globulin concentrations and reduced testosterone and free estradiol concentrations, compared with the control group; changes which may eventually contribute to a reduction in breast cancer risk (Kaaks et al., 2003). More evidence for a role of GL in sex steroid metabolism has emerged from small studies of young male acne sufferers (Smith et al., 2008). In controlled feeding trials, a high GL diet was associated with significantly reduced sex-hormone binding globulin concentrations, and elevated circulating free androgen, and so may augment sex

hormone bio-activity (Smith et al., 2007, 2008). However, the high GL diets were also lower in protein, and therefore these results are only speculative, although this is a promising research area that may have noteworthy consequences for unravelling the association between GI, GL, and cancer risk.

EVIDENCE FROM EPIDEMIOLOGICAL STUDIES OF GLYCEMIC INDEX, GLYCEMIC LOAD, AND CANCER RISK

The publication of the international tables of GI and GL values in 1995 and subsequent updates in 2002 and 2008 has fuelled a surge in GI and GL research and they have been applied to numerous large-scale epidemiological studies to answer many research questions. Over the last decade, reviews have suggested that high GI and GL intakes are directly related with cancer risk, and could be implicated in breast and colorectal cancer etiology in particular (Augustin et al., 2002; Brand-Miller, 2003).

Endometrial Cancer

A systematic review of GI, GL, and endometrial cancer risk has been published incorporating four prospective cohorts, one of which originates from the renowned European Prospective Investigation into Cancer and Nutrition, and one further hospital-based case–control study (Mulholland et al., 2008a). All studies were conducted in Europe or North America, and principally employed either self-reported or interviewer-administered food frequency questionnaires (FFQs) to assess diet.

In that review, the combined adjusted relative risk (RR) from prospective cohort studies of endometrial cancer in the highest compared with the lowest reported category of GI intake was 1.06; [95% confidence intervals (CI): 0.92–1.21], indicating no evidence of an association (Mulholland et al., 2008a). The lack of any association remained when GI and endometrial cancer risk was investigated across normal, overweight or obese BMI categories (Mulholland et al., 2008a). Notably though, the hospital-based case–control study, based in Italy, did report a significant increased endometrial cancer risk in overweight women consuming high GI intakes (Augustin et al., 2003).

The pooled association between endometrial cancer risk and GL intake for the four cohort studies from the systematic review is shown in Figure 18.2. The combined adjusted RR was statistically significant, showing a 20% increased risk of endometrial cancer in women consuming a high GL diet (RR 1.20; 95% CI: 1.06–1.37).

Furthermore, findings from the review also indicated that a high GL diet increases the risk of endometrial cancer as BMI increases, suggesting that BMI may be an effect modifier of the association between GL and endometrial cancer. It is possible therefore that high GL diets exaggerate risk in women who are more likely to be insulin resistant. This is illustrated in Figure 18.3, which shows a dose-response increment in endometrial cancer risk as BMI increases, peaking at a significant 54% increased risk for obese women consuming the highest category of GL intakes.

In addition, one further study has been published investigating GI, GL, and endometrial cancer risk after the systematic review inclusion date of July 2008 (George et al., 2009). In that study by George et al., the association between GI and GL intake and endometrial cancer risk was explored in 87,678 women participating in the National Institutes of Health–AARP Diet and Health Study in eight locations across the United States. Over eight years of follow-up, 1041 cases of endometrial cancer were identified via linkage with state cancer registries. Results from that study also confirmed that GI was unrelated to endometrial cancer risk, and also showed that women consuming the highest GL intakes were at a 25% increased risk of endometrial cancer risk, although this was not statistically significant (George et al., 2009). This is not surprising, since none of the cohort studies in the systematic review reported statistically significant results although all studies showed associations in a positive direction, demonstrating the advantage and increased statistical power of a meta-analysis.

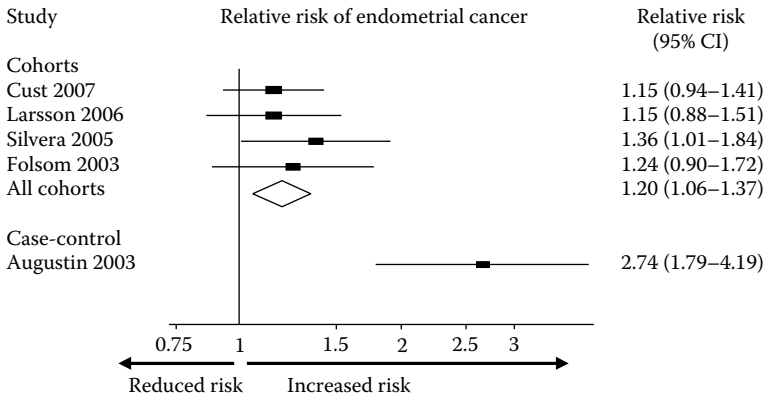

FIGURE 18.2 Forest plot of GL and endometrial cancer risk. GL: glycemic load. Pooled estimates of study results of endometrial cancer risk in women consuming the highest versus the lowest category of GL intake. (Adapted from Mulholland, H. G. et al. 2008. *British Journal of Cancer* 99, (3): 434–41.)

The lack of an association between dietary GI and endometrial cancer risk would suggest that endometrial cancer risk is related to the actual blood glucose and insulin demand induced by the consumption of usual portion sizes of carbohydrate-containing foods, as represented by GL. Long-term consumption of a high GL diet may lead to hyperglycemia, and consequently hyperinsulinemia (Augustin et al., 2002). Hyperinsulinemia has been shown to lower IGFBP concentrations thereby increasing IGF-1 levels, which may stimulate tumor development (Kaaks et al., 2002). Increased IGF-1 bioactivity inhibits apoptosis, stimulates cell proliferation and sex steroid synthesis and inhibits sex-hormone binding globulin synthesis, all of which could be implicated in the development of endometrial cancer (Kaaks and Lukanova, 2001).

To summarize, there is convincing evidence in the literature that an association exists between a high GL diet and an increased risk of endometrial cancer, which appears to be pronounced in obese women. Dietary GI does not appear to be related to endometrial cancer risk.

Breast Cancer

Low GI diet interventions have been shown to significantly reduce body weight in randomized controlled trials (Thomas et al., 2007). High body fatness contributes to an increased risk of postmenopausal breast cancer risk, yet contrastingly, probably protects against premenopausal breast cancer (Renehan et al., 2008). In addition to menopausal status, the link between body weight and breast cancer risk is also highly dependent on the hormonal receptor status of the tumor. The positive association between BMI and postmenopausal breast cancer risk, and the contrasting inverse association between BMI and premenopausal breast cancer risk, appear to be evident only for estrogen and progesterone-positive tumors (Suzuki et al., 2009). Conversely, no associations have been demonstrated between BMI and breast cancer risk for estrogen and progesterone-negative tumors (Suzuki et al., 2009). Thus, any potential association between dietary GI, GL, and breast cancer risk may also differ by menopausal or hormone receptor status.

Premenopausal Breast Cancer

A meta-analysis has examined GI intake and premenopausal breast cancer risk in nine epidemiological studies (Mulholland et al., 2008b), all of which used FFQs to assess habitual dietary intake. Most studies sourced GI and GL values from international tables (Foster-Powell and Miller, 1995; Foster-Powell et al., 2002) with the notable exception of one prospective Italian study (Sieri et al., 2007) that primarily used GI and GL values calculated from their local foods.

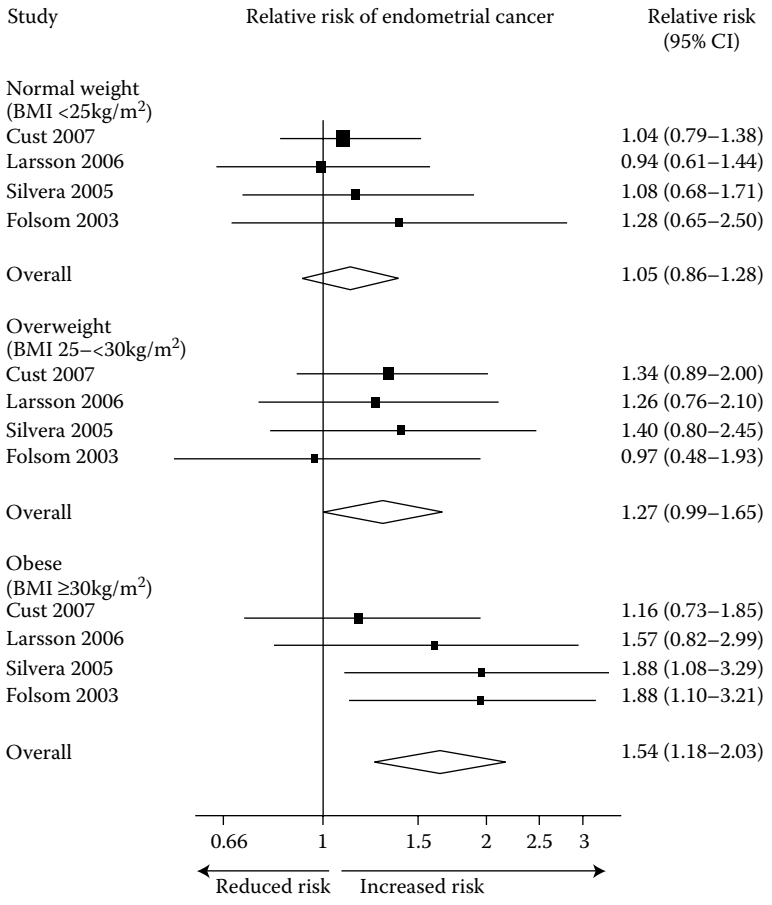

FIGURE 18.3 Forest plot of GL and endometrial cancer risk by BMI categories. BMI: body mass index. GL: glycemic load. Pooled estimates of study results of endometrial cancer risk in women consuming the highest versus the lowest category of GL intake by BMI categories. (Adapted from Mulholland, H. G. et al. 2008. *British Journal of Cancer* 99, (3): 434–41.)

When risk estimates from these studies were pooled, there was a modest 11% elevated risk of premenopausal breast cancer risk in women consuming the highest GI intakes, although these results did not achieve statistical significance (Mulholland et al., 2008b). Adolescent GI intake has also been assessed as part of the Nurses' Health Study cohort in relation to premenopausal breast cancer risk. In that investigation, a high GI intake in teenage years significantly increased the risk of developing premenopausal breast cancer later in life, although this was a retrospective study design which may have incorporated some recall bias (Frazier et al., 2004).

Moreover, most studies have failed to demonstrate any evidence of an association between GL intake and premenopausal breast cancer risk (Mulholland et al., 2008b). Despite this, atypical positive associations between GL and premenopausal breast cancer risk were observed in the Italian cohort that sourced GI and GL values estimated from local foods (Sieri et al., 2007). The majority of GI and GL values in international tables are derived from North American and Australian studies and therefore their reproducibility and application to different population groups is questionable. It would be preferable if future studies utilized country-specific GI and GL values, due to possible international variation in the amylose content, and physical and chemical characteristics of otherwise similar foods that may have an impact on their GI value (Foster-Powell et al., 2002).

The majority of participants in included studies were of Caucasian origin and therefore the application of these findings to other ethnic groups is unknown. Since the cut-off date for the systematic review, one study has been published on GI and GL intake and breast cancer risk in Chinese women participating in the Shanghai Women's Health Study (Wen et al., 2009). In that study, high GL intakes were associated with an increased premenopausal breast cancer risk, while no significant associations were found with postmenopausal breast cancer risk. No significant findings were demonstrated between dietary GI and breast cancer risk in either premenopausal or postmenopausal women.

Postmenopausal Breast Cancer

Overall, findings from a comprehensive systematic review did not ascertain any strong associations between GI or GL intake in relation to postmenopausal breast cancer risk (Mulholland et al., 2008b), an outcome that has been confirmed in a subsequent report (George et al., 2009).

It may be important to report risk estimates for diet and breast cancer separately by different hormonal receptor profiles and three studies of GI, GL, and postmenopausal breast cancer risk have done so to date (Nielsen et al., 2005; Giles et al., 2006; Lajous et al., 2008). Of these, the two European cohorts suggested that high GI or GL intakes elevated risk for estrogen receptor negative tumors only; however these findings were not replicated in an Australian cohort (Giles et al., 2006). There are relatively few known risk factors for hormone receptor-negative versus hormone receptor-positive tumors, and so these findings are certainly worth further exploration. Current knowledge suggests that hormone receptor-negative tumors are unrelated to body weight (Suzuki et al., 2009) and so plausible mechanisms for a direct association between GI, GL and estrogen receptor-negative breast cancer risk are unclear. Progesterone receptor status does not appear to modify the null associations between GI nor GL intake and postmenopausal breast cancer risk (Giles et al., 2006; Lajous et al., 2008).

There are a few studies that have investigated the association between GI, GL, and breast cancer risk while stratifying by BMI; however, most have failed to identify any significant associations. It should be noted though that none of these studies were sufficiently powered to examine the association in obese women as a separate group; the association for overweight and obese women have been combined. Since the association between GL and endometrial cancer was exacerbated in obese women, further research in this population subgroup is called for.

In summary, there is little evidence of an association between GI, GL and breast cancer risk, although further research is required to confirm if an association may exist for particular hormone-receptor-defined cancers or for obese women.

Colorectal Cancer

In a systematic review that examined the association between GI, GL, and colorectal cancer risk, disparate results were observed from studies of case–control design, compared with findings from prospective cohort studies. No significant associations were observed between the highest versus the lowest category of GI intake and colorectal cancer risk when results from cohort studies were combined (RR 1.04; 95% CI: 0.92–1.16) (Mulholland et al., 2009a). Similarly, there was no evidence of an association between the highest versus the lowest category of GL intake and colorectal cancer risk from the pooled cohort study results (RR 1.06; 95% CI: 0.95–1.17) (Mulholland et al., 2009a).

In contrast, strong direct associations between GI, GL, and colorectal cancer risk were observed in the European hospital-based case–control studies included in the review (Franceschi et al., 2001; Levi et al., 2002). This may be explained by the high range of GI and GL intakes observed among the European study participants (Franceschi et al., 2001; Levi et al., 2002); however, another cohort study observed similar intakes among their population in Sweden and found no significant association with colorectal cancer risk (Larsson et al., 2007). Therefore, it is likely that the results in the case–

control studies may have been positively skewed by dietary recall bias and the use of hospital-based controls in these studies (Franceschi et al., 2001; Levi et al., 2002). Additionally, these two European case–control studies were unable to adjust for BMI in their analysis, which is an important risk factor for colorectal cancer development (Renehan et al., 2008) and may have confounded the associations shown.

Several published studies have indicated that they performed subgroup analyses based on strata of BMI categories, although results from these have been extremely inconsistent. Some studies observed positive associations between GI or GL intake and colorectal cancer risk among participants with an above-normal BMI (Franceschi et al., 2001; McCarl et al., 2006), while others observed no associations in any BMI or physical activity strata (Michaud et al., 2005; Kabat et al., 2008; Weijenberg et al., 2008) and indeed a further study detected an inverse association between GL intake and colorectal cancer risk among sedentary overweight individuals (Strayer et al., 2007).

Findings from two studies included in the systematic review also gave little indication of a positive association between dietary GI, GL, and the risk of colorectal adenoma development, suggesting that GI and GL are not of etiological importance in the initiation stages of colorectal tumor development (Mulholland et al., 2009a).

The lack of a strong association between GI, GL intake, and colorectal cancer is particularly surprising, since one of the main hypothesized mechanisms for this association is via hyperinsulinemia, markers of which have been strongly linked with elevated colon cancer risk (Giovannucci, 2001; Pisani, 2008). However, current evidence from prospective epidemiological studies give little support to the hypothesis that dietary GI and GL influence colorectal cancer risk, which casts doubt on the suggested influence of dietary GI and GL on blood insulin concentrations. A validation study of the effect of GI on the insulin response did report an overall 70% reduced insulin response after consumption of a low GI food compared with a high GI food (Brand-Miller et al., 2005). In the same small trial though, IGF responses were also measured and minimal differences in IGF-1 concentrations were observed, although serum concentrations of IGFBP-3 were markedly elevated after consuming the low GI food (Brand-Miller et al., 2005). However, a thorough meta-regression analysis has previously concluded that only IGF-1, and not IGFBP-3, appears to play a role in colorectal cancer development (Renehan et al., 2004). Thus, even if GI and GL are impacting upon components of the IGF axis, these particular components may not be of importance in colorectal cancer etiology.

Overall, evidence from well-designed prospective cohort studies demonstrates that GI and GL are not related to the risk of colorectal cancer.

Pancreatic Cancer

The majority of cohort studies that have investigated GI, GL, and pancreas cancer risk have originated from North America, and all have utilized FFQs to assess habitual GI and GL intake. In a recent systematic review, combining the results from well-designed prospective cohort studies did not reveal any indications of an association between GI (RR 0.99; 95% CI: 0.83–1.19) or GL (RR 1.01; 95% CI 0.86, 1.19) and pancreatic cancer risk (Mulholland et al., 2009a).

Even when restricting analysis to inactive or overweight individuals, the majority of studies to date have failed to identify a link between GI, GL, and pancreas cancer development. Only data from the Nurses' Health Study cohort identified elevated risks among sedentary overweight individuals consuming high GI and GL intakes (Michaud et al., 2002); however, their subgroup analysis was based on a very small number of cases, and no significant associations were observed by separate strata of BMI or physical activity levels. The results of a further prospective cohort study, published after the systematic review findings, and including 1151 pancreas cancer cases, have since replicated the lack of an observed association between GI, GL, and the risk of this malignancy (Jiao et al., 2009).

To summarize, there are consistent findings from prospective studies that pancreas cancer risk is not related to dietary GI and GL.

Other Cancers

Several other cancer sites have been explored in relation to dietary GI and GL. Evidence appears to be accumulating for a role for GI and GL in esophageal and stomach carcinogenesis in particular. The large National Institutes of Health–AARP Diet and Health Study conducted throughout the United States illustrated a direct link between high GI intakes and stomach cancer risk in males (George et al., 2009). Additionally, an Irish population-based case–control study has demonstrated a significantly increased risk of esophageal adenocarcinoma in individuals consuming in the highest category of GI intake (Mulholland et al., 2009b).

Studies of ovarian cancer also appear to show direct associations between GI, GL, and risk and deserve further investigation, since the underlying mechanisms may be similar to those for GI, GL, and endometrial cancer (Mulholland et al., 2008a). Other malignancies that have been investigated with regard to dietary GI and GL include prostate, head and neck, thyroid, melanoma, non-Hodgkin's lymphoma, kidney, bladder, and lung tumors (George et al., 2009); however, too few studies have been done to make even suggestive judgments about potential associations. More research is required into GI, GL, and the risk of these cancers.

DISCUSSION

Overall, research findings suggest that high GI and/or GL intakes are directly related to endometrial cancer risk. Why this cancer appears to be more highly related to GI and GL intakes compared with colorectal, breast, or pancreas cancer remains to be clarified.

One of the main proposed mechanisms linking a high GI and GL diet with cancer risk is via hyperinsulinemia. Hyperinsulinemia has been linked with an elevated risk of endometrial cancer risk (Pisani, 2008); however, by far the strongest evidence to date originates from studies of hyperinsulinemia and colon carcinogenesis (Giovannucci, 2001), and yet there was little evidence to support a role of GI/GL in colorectal cancer from a robust systematic review (Mulholland et al., 2009a).

One possible explanation is that endometrial cancer is the malignancy most highly related to body fatness in women, according to a comprehensive systematic review of BMI and cancer risk (Renehan et al., 2008). Body fatness is also directly related to colorectal, pancreas, and postmenopausal breast cancer development, but to a lesser extent than endometrial cancer risk (Renehan et al., 2008). Therefore, if the carcinogenic effect of a high GI/GL diet is mediated via the promotion of body fatness, then it may have been easier to detect an association with endometrial cancer. However, the evidence that a high GI or GL diet increases body fatness is certainly not definitive and more research is required to assess if high GI/GL diets encourage weight gain and to evaluate plausible biochemical influences of high GI and GL intakes on glucose and insulin metabolism in overweight and obese individuals.

It is also possible that dietary measurement error associated with FFQs may have caused misclassification of individuals' actual GI and GL intakes. Self-administered FFQs were used in all of the prospective cohorts, none of which were specifically designed or validated for assessing GI or GL intake. FFQs are known to incorporate some dietary measurement error, especially among overweight or obese individuals but are the most convenient assessment tool available for large-scale studies (Black et al., 1993). The FFQs used were quite variable in length, ranging from 61 items to 192 items and most studies assessed dietary intake in the 1–2-year period prior to completing the FFQs. This time period may not be the appropriate exposure period to capture an association between GI, GL, and cancer risk if one truly exists. Future studies utilizing superior dietary assessment methods would be extremely useful in confirming or rebutting the current available evidence on GI, GL and cancer risk studies.

Future studies should ideally collect information on BMI, body fat distribution, and menopausal status at multiple time-points during follow-up periods. The inclusion of appropriate biomarkers, for

example fasting serum insulin, IGFs and associated binding proteins, C-peptide, and estrogens, in addition to dietary assessment of GI and GL will be essential in elucidating any potential associations with cancer risk.

To conclude, diabetic patients are currently encouraged to consume low GI and GL diets in order to achieve optimal glycemic control. In light of the evidence that high GI and GL intakes appear to increase endometrial cancer risk, particularly among overweight and obese women, it may also be appropriate to advise people in the general population to consume low GI/GL carbohydrates where possible. However, the GI and GL concepts are quite complex, and therefore, widespread promotion of consuming a low GI/GL diet may not be appropriate unless accompanied by dietary counselling from a health professional, to ensure individuals achieve a low GL diet that is comprised of low GI/moderately high total carbohydrate content rather than high GI/low total carbohydrate content.

SUMMARY POINTS

- GI and GL are measures of carbohydrate quality, and quality and quantity, respectively.
- The mechanisms underlying the association between GI, GL, and cancer risk remain elusive, although risk may be mediated via high body fatness, insulin, or sex steroids.
- High GL diets are associated with an increased risk of endometrial cancer and the risk appears to be elevated in obese women.
- GI and GL do not appear to be of etiological importance for breast cancer, although verification from further studies on hormone-receptor defined cancers is needed.
- Evidence that GI and GL are positively associated with colorectal cancer risk is limited to case–control studies and is not supported by more robust cohort study findings.
- GI and GL intakes appear to be unrelated to pancreas cancer risk.
- More studies are needed to explore the association between GI, GL, and risk of other cancer sites, particularly stomach and esophageal cancers.

ABBREVIATIONS

BMI	Body mass index
CI	Confidence interval
FFQ	Food frequency questionnaire
GI	Glycemic index
GL	Glycemic load
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
RR	Relative risk

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Section III

Cancers Specific, Targeted Therapies with Bioactive Foods and Their Products

19 Bioactive Foods and Extracts in Prostate Cancer Prevention

Faysal A. Yafi and Wassim Kassouf

CONTENTS

INTRODUCTION

Prostate cancer (PCa) has the highest incidence (25%) of any male cancer with 192,280 new cases every year in the United States contributing to 27,360 new cancer deaths per year. It also ranks second in cancer-related deaths in American men (9%) (Jemal et al., 2009). One out every six American males will develop PCa during his lifetime (American Cancer Society, 2006), with 91% of these new cases diagnosed at an early stage and amenable to successful management (Jemal et al., 2009). With the discovery of the prostate-specific antigen (PSA) as a screening tool, there has been a visible increase in the rate of early PCa detection and a decrease in prostate-related mortality (31% less deaths per year between 1990 and 2003) (Jemal et al., 2009). However, because of an aging population, the incidence of PCa is on the rise with estimates suggesting more than 300,000 people will be affected by the disease by 2015 (American Cancer Society, 2005). As such, there is a pressing need for methods of prevention of this disease to alleviate the current cost and morbidity associated with both its early detection and treatment.

Chemoprevention is defined as pharmacological intervention with naturally occurring and/or synthetic compounds that may prevent, inhibit, or reverse carcinogenesis or suppress the development of invasive cancer (Sporn, 1976; Sporn and Suh, 2002). The expanded definition of cancer chemoprevention is to inhibit or delay the onset of neoplasia by blocking neoplastic inception as well as reversing the progression of transformed cells before the appearance of malignant lesions (Ansari et al., 2002; Sporn and Suh, 2002; Montironi et al., 2007). This impetus toward attempting to unveil agents that would help in the prevention of prostate cancer stems from the long latency of the disease of about 20 to 40 years with a median age at diagnosis of 72 years, as well as the fact

that it is a multistep process giving us both a significant window of opportunity to impact on the biology of this disease as well as making it susceptible to several preventive agents at different steps of evolution (American Cancer Society, 2006). This is also helped by epidemiological studies that have shown large variations in PCa incidence between different geographical locations (Denis et al., 1999). This may be suggestive of the possibility that nutritional factors may differentially stimulate and inhibit cancer, especially since there is a similar prevalence worldwide of the precursor lesion (Ansari et al., 2002). Further evidence to this theory is the low incidence of PCa in Asian countries where the diets are low in animal fat and rich in plant-based agents while its incidence in the Western world where the diet is typically high in animal fat is much higher (Donaldson, 2004). Notably, reports show that the incidence of PCa in Asian men who immigrate to the West and adopt local diets approaches that of Western men, further evidence to the role of environmental and dietary factors also in the progression of the disease (Moyad and Carroll, 2004).

As such, more than 400 compounds are currently receiving National Institute of Health funding for laboratory research, and more than 20 different clinical trials are being undertaken in the hope of finding efficacious chemopreventive agents (Shukla and Gupta, 2005). As to date, the only agents that have achieved level 1 evidence for decreasing the risk of developing PCa are the antiandrogenic drugs, 5-alpha reductase inhibitors. Finasteride, a selective blocker of type 2 isoenzyme, has shown, through a randomized prospective placebo-controlled clinical trial (the Prostate Cancer Prevention Trial, PCPT) a 24.8% reduction (24.4–18.4%, $p < 0.001$) in PCa risk compared to placebo and an absolute risk reduction of 6% translating to one less cancer for every 16–17 men treated. However, although there were less overall and well-differentiated (Gleason score ≤6) tumors in the finasteride group, there was a significant increase in the frequency of higher grade cancers (tumors with Gleason score >7) ($p = 0.005$) (Thompson, 2003). Based on subsequent studies, others have refuted the findings that finasteride may induce high grade cancer. It was demonstrated that the effect of finasteride in detecting higher grade tumor is due to a sampling artifact; downsizing the prostate with finasteride increases the likelihood of detecting higher grade tumors that were already present in the prostate (Thompson, 2006). Recently, the results of the other randomized prospective placebo-controlled trial (Reduction by Dutasteride of Prostate Cancer Events, REDUCE) were reported and showed similar decrease in PCa risk (23%) with dutasteride, a nonselective blocker of 5-alpha reductase. Importantly, there was no associated increase in risk of developing more aggressive disease (6.8% of men in the placebo group vs. 6.7% of men taking dutasteride) making dutasteride a very promising drug for daily chemoprevention in men at risk (Andriole, 2009).

Among these projects, a number of bioactive foods, extracts, and other dietary components are currently being tested for their potential benefit and have gained widespread attention because of their wide availability, low cost, mostly negligible side effects, and especially the ease with which dietary changes can be implemented. Through different mechanisms of action, multiple dietary phytochemicals are believed to play a role in targeting and potentially inhibiting or slowing the different steps of carcinogenesis. These include numerous agents such as carotenoids, vitamins, dietary fiber, selenium, glucosinolates, indoles, isothiocyanates, flavonoids, phenols, protease inhibitors, plant sterols, and other complementary agents (Shukla and Gupta, 2005).

CAROTENOIDS

Lycopene and β-carotene are carotenoids primarily found in tomatoes and watermelon. They are thought to act as antioxidants preventing DNA damage and therefore protective against PCa development. The mechanisms of action are through the protection of 2-deoxy-guanosine against singlet oxygen damage and the suppression of insulin like growth factor-1-stimulated cell proliferation (Karas et al., 2000). Studies have shown that lycopene is present in high concentrations in prostate tissue, an indication for its role on prostate biology (Giovannucci, 1999). Also, it has been shown to reduce the serum levels of the oxidative damage biomolecular marker thiol in patients with prostate cancer as well as inhibit the growth of prostate cancer cells by way of gap junction

proteins and growth factor signalling (Ansari et al., 2002; Aust et al., 2003; Obermuller-Jevic et al., 2003). Interestingly, similar inhibition of carcinogenesis has also been reported with tomato powder rather than lycopene raising the possibility that inherent compounds other than lycopene may also have anticancer properties (Boileau et al., 2003). In a recent randomized double-blind placebo-controlled study of 40 patients with BPH, daily intake of 15 mg/day of lycopene was associated with significant decrease in PSA levels at six months compared with placebo ($p < 0.05$). Also, while progression of prostate enlargement occurred in the placebo group as assessed by trans-rectal ultrasonography ($p < 0.05$), the prostate did not enlarge in the lycopene group (Schwarz et al., 2008). Furthermore, while some epidemiologic studies have reported no associated benefit to carotenes, there is also compelling evidence showing up to 30–40% PCa risk reduction with tomato intake (Giovannucci, 1999). In the Health Professionals Follow-Up Study (HPFS) which accrued almost 50,000 patients between 1986 and 1994 and surveyed them with regard to dietary habits, a decreased risk of PCa was noted with lycopene intake [relative risk (RR) = 0.84, $p = 0.003$], and more significantly with high tomato intake (RR = 0.77, $p < 0.001$) (Giovannucci et al., 2002). Confirmation of these results is put forth by a large meta-analysis of 21 trials which similarly showed that the RR values for those who consumed high amounts of raw tomato was 0.89 [95% confidence interval (CI) 0.8–1.0], and for cooked tomato products it was 0.81 (95% CI 0.71–0.92) compared with nonfrequent consumers (Etminan et al., 2004). While these studies are very encouraging, in the absence of phase III trials confirming a clear benefit to lycopene, no clear cut recommendations can be made. However, due to its negligible side effects and the fact that it is readily available, it continues to be one of the preferred dietary choices men are adopting to prevent PCa.

VITAMINS

Due to their known antioxidant properties, vitamins A, C, D, and E have been extensively studied for possible roles in chemoprevention of many cancers including PCa.

Vitamin A (retinol) and its analogues work by modulating the growth and differentiation of cancer cells by activating gene transcription via the nuclear retinoic acid receptors alpha, beta, and gamma and retinoid X receptors alpha, beta, and gamma (Sun et al., 2000). Interestingly, one of its analogues, fenretinide lowered the incidence of tumor by 49% and tumor mass by 52% in mice prostates compared with normal fed animals (Slawin et al., 1993). The mechanism of action by which retinoids are thought to prevent PCa is at the promotion phase through inhibition of cell proliferation, induction of apoptosis, and cell cycle arrest (Liang et al., 1999). However, while no studies are yet to show any clear association between PCa risk and vitamin A, one nested case–control study using 692 prostate cancer cases and 844 matched controls from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, showed that serum retinol concentrations showed an inverse association with high-grade disease (Gleason sum >7; $p = 0.01$) (Giovannucci et al., 1995; Schuurman et al., 2002; Bosetti et al., 2004; Schenk et al., 2009). On the other hand, in the recently published multicenter randomized, double-blind, placebo-controlled trial (Carotene and Retinol Efficacy Trial, CARET), results showed that a high dose of β-carotene (30 mg/day) and retinyl palmitate (25,000 IU/day) plus at least one other dietary supplement increases the risk of aggressive PCa (Neuhouser et al., 2009).

Vitamin C is a potent reducing antioxidant capable of scavenging molecular oxygen and free radicals (Hu and Shih, 1997). Vitamin C-rich diets such as fruits and vegetables have been shown to be associated with a lower incidence of esophageal, stomach, colon, skin, lung, and lower urinary tract cancer (Weisburger, 1999; Gaziano et al., 2009). Additionally, in animal studies, vitamin C alone and in combination with vitamin E has been also shown to inhibit prostate cancer cell growth (Nomura et al., 1991; Taper et al., 2001). There are, however, no studies to date showing an association with decreased PCa. Recently, in a randomized, double-blind, placebo-controlled trial of vitamins E and C (the Physicians' Health Study II) in which 14,641 male physicians in the United States

were randomized to receive 400 IU of vitamin E every other day and 500 mg of vitamin C daily or placebo, no difference in the risk PCa was observed (Gunawardena et al., 2004).

Calcitriol (1,25-D3), the active form of vitamin D, is produced in the skin through ultraviolet radiation of 7-dehydrocholestol and in the proximal renal tubules through 1,25-dihydroxyvitamin D3 hydroxylase. Interestingly, residential sunlight exposure has been associated with decreased PCa risk and epidemiological and cross-sectional studies have shown that lower levels of 1,25-D3 were associated with increased risk of PCa (John et al., 2004; Stewart and Weigel, 2004). Furthermore, studies of prostate tissue have shown that calcitriol inhibits the proliferation of PCa cell lines (Stewart and Weigel 2004). This tumor suppressor activity has rendered vitamin D an interesting prospect in the management of advanced prostate cancer. However, recently, in a case–control study (the European Prospective Investigation into Cancer and Nutrition, EPIC) in which serum concentrations of 25-hydroxyvitamin D were measured in 652 prostate cancer cases matched to 752 controls, no significant association was found between 25-hydroxyvitamin D and risk of prostate cancer putting into question the role of vitamin D in PCa prevention (Travis et al., 2009).

Vitamin E has eight stereoisomers with alpha-tocopherol being the most biologically active. Its antioxidant properties stem from its lypophilic character as it accumulates in circulating lipoproteins, cellular membranes and fat deposits, where it reacts very rapidly with molecular oxygen and free radicals (Ansari et al., 2002). Initial interest in alpha-tocopherol was subsequent to the results of the alpha-Tocopherol, beta-Carotene (ATBC) trial which was originally intended to study its effect on lung cancer prevention. By coincidence, its intake was found to result in 32% and 41% reduction in incidence of PCa and prostate-related mortality respectively at six years with an RR of 0.88 (Virtamo et al., 2003). Additionally, in animal studies, supplementation of vitamin E resulted in slowing of prostate cancer growth (Siler et al., 2004). Furthermore, in a large prospective epidemiological study from the University of Basel involving 2974 men, a significant association was noted between low levels of alpha-tocopherol and increased risk PCa (Eichholzer et al., 1999). However, in the aforementioned Physicians' Health Study II, the administration of 400 IU of vitamin E every other day did not decrease the risk of PCa (Gunawardena et al., 2004). Finally, it is noteworthy that high levels of consumption of vitamin E have been associated with increased risk of heart failure and all-outcomes death and as such a safe dose of 400 IU/day should not be exceeded (Lonn et al., 2005; Neil and Fleshner, 2006).

MINERALS

The two main minerals investigated are selenium and zinc. Selenium is an essential trace micronutrient whose concentration in food is dependent on the soil where the food is cultivated. Its mechanism of action on prostate cells seems to be through cell cycle arrest and reduction of apoptosis (Jiang et al., 2000). As with vitamin E, its role in PCa chemoprevention was found coincidentally while investigating a similar role in skin cancer (Duffield-Lillico et al., 2003). While epidemiological studies have supported its preventive role, the strongest evidence comes from the Nutritional Prevention of Cancer Study which accrued 1312 patients and randomized them to receive either 200 μg of selenized yeast or placebo and found that selenized yeast supplements were associated with a 49% reduction in the risk of PCa, most notably in those with low baseline serum selenium levels and those younger than 65 years with a PSA <4 ng/mL (Duffield-Lillico et al., 2003).

SELECT TRIAL

Based on the above observations, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) was established to address the promising roles of both selenium and vitamin E as possible chemoprevention agents for PCa (Klein et al., 2001). It is a prospective, randomized, double-blind, placebo-controlled trial which enrolled 35,534 healthy men with a normal DRE and a PSA <4 ng/mL and randomized them into four arms (selenium + placebo, vitamin E + placebo, selenium + vitamin E,

and placebo). Results showed that there were no statistically significant differences in the rates of prostate cancer between the four groups [placebo, 416 cases (5-year PCa rate of 4.43%); selenium, 432 cases [(4.56%); vitamin E, 473 cases (4.93%); selenium + vitamin E, 437 cases (4.56%)]. Furthermore, vitamin E was slightly associated with an increased risk of PCa and selenium an increased risk of diabetes mellitus and, as such, the recommendations were to discontinue the supplements (Lippman et al., 2009).

Zinc is another known mineral with antioxidant properties. It is present in high concentrations in the prostate and has been shown to counter PCa cell growth in cell cultures (Powell 1999; Liang et al., 1999). In a prospective questionnaire (VITamins And Lifestyle, VITAL) which was completed by 35,242 men, dietary zinc was not found to be associated to PCa risk. However, the risk of advanced PCa decreased with greater intake of supplemental zinc (HR = 0.34, 95% CI = 0.13–1.09, $p = 0.04$) compared with nonusers (Gonzalez et al., 2009). At this point, future research is warranted before conclusions can be made.

FLAVONOIDS

Flavonoids are polyphenolic compounds that possess antioxidative, anti-inflammatory, and possibly anticarcinogenic properties. Those that have been studied in PCa include soy isoflavones, catechins from green and black tea, silymarin from milk thistle, resveratrol, apigenin, and proanthocynadins from grape seed (Shukla and Gupta, 2005).

Soy is an ingredient found abundantly in the Asian diet and it has been hypothesized that this could be one of the reasons for the lower incidence of PCa in Asian men. The two main soy isoflavones are genistein and daidzein. The prior is a phytoestrogen targeting estrogen and androgen receptors and this may explain its effect on PCa cells. Studies in mice have shown decreased PCa incidence and improved survival (Bektic et al., 2004). Epidemiological and case–cohort studies have shown benefit to its use in chemoprevention of prostate cancer (Shukla and Gupta, 2005). A nested case–control study within the Japan Public Health Center (JPHC)-based Prospective Study presented the results of a questionnaire of 14,203 men and showed that plasma genistein level tended to be inversely associated with the risk of total prostate cancer (Kurahashi et al., 2008). This was confirmed in a meta-analysis of patients with large soy consumption, where there was an associated reduction in risk of PCa (RR 0.70, 95% CI 0.59–0.83) (Yan and Spitznagel, 2009). However, in a phase I randomized controlled trial, soy isoflavones failed to decrease PSA levels in men aged 50 to 80 years (Fischer et al., 2004). Hoping to clarify its role, the National Cancer Institute of Canada completed accrual of a randomized, double-blind, placebo-controlled study evaluating the combination of vitamin E, selenium, and soy in men with higher risk for developing PCa. They, however, failed to show less progression to PCa in the group taking supplements (HR =1.03, $p = 0.88$) and no benefit could be demonstrated with soy supplementation (Fleshner et al., 2008).

Tea is among the most popular beverages worldwide and like soy is abundant in the Asian diet. It has been long known that tea, through one of its ingredients, polyphenol, particularly (–)-epigallocatechin-3-gallate has strong antioxidant properties (Fang et al., 2003). While epidemiological studies have supported its role in PCa chemoprevention, this benefit did not translate into clinical studies (Shukla and Gupta, 2005). More recently, however, the aforementioned Japan Public Health Center (JPHC)-based Prospective Study presented the results of a questionnaire-based study spanning 14 years of 49,920 men between the ages of 40 and 69 years. Although they reported no evident correlation between green tea and risk of localized PCa; interestingly, they did notice a dose-dependent decrease in advanced PCa (RR 0.52, 95% CI 0.28–0.96) (Kurahashi et al., 2008). Furthermore, in a prospective trial of 60 volunteers with HGPIN randomized to receive either green tea catechins (GTC) daily or placebo, there was a significant difference in risk of developing PCa between the two arms (3% with GTC vs. 30% with placebo, $p < 0.01$) and this was observed as early as six months after initiation of therapy (Brausi et al., 2008).

Finally, resveratrol (a dietary stilbene), silymarin, proanthocyanidins, and procynadins (found in high concentrations in grape seed extracts) and apigenin are also flavonoids which have shown promise as possible chemopreventive agents. In a study targeting the Simian Virus-40 T-antigen (SV-40 Tag) targeted probasin promoter rat model, resveratrol alone and in combination with genistein successfully suppressed PCa (Harper et al., 2009). Similar results were obtained in other animal studies; however, no clinical evidence as to date has been reported and as such more investigation is warranted.

INDOLES

Indoles are naturally occurring constituents of Brassica vegetables and are readily available in fruit and vegetable-rich diets. Their chemopreventive potential stems from their actions on PCa cell lines by inhibiting cell proliferation and promoting apoptosis as well as from evidence showing them to be strong androgen antagonists and inhibiting PSA production in the prostate (Zhang et al., 2003). While there are promising results as to their role in animal models, there certainly is a need for more advanced investigation and clinical studies.

ISOTHIOCYANATES AND GLUCOSINOLATES

Isothiocyanates are mostly present in cruciferous vegetables and their thiol conjugates have shown promise as chemopreventive agents. Evidence from population-based case–controlled studies has shown a strong association between consumption of cruciferous vegetables and decreased risk of PCa (Cohen et al., 2000). Furthermore, while most prospective studies have failed to show an association with risk of PCa, the study that included the longest follow-up (the Health Professionals Follow-Up Study) showed a significant inverse relationship between men who consumed regular high amounts of cruciferous vegetables and risk of developing PCa (Giovannucci et al., 2003). Finally, in a prospective questionnaire-based study (the EPIC-Heidelberg cohort study), an inverse association between dietary intake of GLS (a secondary plant metabolite occurring in cruciferous vegetables) and the risk of PCa was observed (Steinbrecher et al., 2009).

PHENOLIC ACIDS

Phenolic acids are aromatic plant compounds also known for their antioxidant properties. The most studied phenolic acid is curcumin, a component of the Indian spice turmeric. It has been reported to have inhibitory actions on the proliferation of both androgen-sensitive and androgen-insensitive PCa by inducing apoptosis (Mukhopadhyay et al., 2002). Furthermore, it has shown potential as a radiosensitizating agent in prostate tumor cells (Sah et al., 2003). As such, curcumin is a very interesting prospective PCa chemopreventive agent and more investigation is certainly warranted.

ORGANOSULFUR COMPOUNDS

Organosulfur compounds are the biologically active constituents of allium vegetables such as garlic. Garlic has been historically advertised as a health-promoting nutrient and recent evidence suggests it may have anticarcinogenic activity (Shukla and Gupta, 2005). *In vitro* studies have succeeded in showing its role in inhibition of PCa cell proliferation (Pinto et al., 2000). Furthermore, in a population-based, case–control study out of China which compared 238 men with histologically proven PCa to 471 control subjects, consumption of large amounts of allium vegetables (garlic, scallions, onions, chives, and leeks) was associated with a decreased risk of PCa compared with control (Hsing et al., 2002).

COMPLEMENTARY AGENTS

The National Centre for Complementary and Alternative Medicine (NCCAM) defines complementary and alternative medicine (CAM) as a group of diverse medical and healthcare systems, practices, and products that are not normally considered to be conventional medicine (Shukla and Gupta, 2005). They are becoming increasingly popular in the open market for the prevention of PCa among other diseases.

The most investigated herbal agent to date is PC-SPES which consists of: *Scutellaria baicalensis*, *Glycyrrhiza glabra*, *Ganoderma lucidium*, *Isatis indigotica*, Panax pseudo-ginseng, *Serenoa repens*, *Dendrantherma morifolium*, and *Rabdosia rubescens*. It reduced PSA in select PCa patients enrolled in clinical trials and this could have been secondary to a synergistic effect of its many compounds (Oh et al., 2001). However, some batches were found to contain different compounds such as synthetic estrogens, warfarin, and indomethacin, and the product was recalled. Another CAM that has been studied is the Indian rasagenthi lehyam which has been shown to induce apoptosis in PCa cells (Ranga et al., 2004). *Serenosa repens* (Permixon®), an extract from American dwarf palm fruit has shown dual 5α-reductase inhibitor activity without suppressing PSA expression. It also helped relieve obstructive symptoms in patients with benign prostatic hyperplasia (Al-Shukri et al., 2000). New CAM brands continue to make their way to pharmacies and specialized stores on a daily basis; however, while scientific evidence supporting their efficacy and safety continues to be scarce, recommending dietary changes rather than CAM seems safer and more cost-effective at the present time.

SUMMARY

Prostate cancer has the highest incidence of any male cancer and of every six American men, one will develop prostate cancer during his lifetime. Chemoprevention is defined as pharmacological intervention with naturally occurring and/or synthetic compounds that may prevent, inhibit, or reverse carcinogenesis or suppress the development of invasive cancer. As such, a number of bioactive foods, extracts, and other dietary components are currently being tested for their potential chemopreventive benefit. Tomato products may have a modest preventive role in prostate cancer when large amounts are consumed. Although vitamin E and selenium have shown promise in several case–control and cohort studies, recent level 1 evidence showed no benefit from selenium and vitamin E in their role in PCa prevention. Soy and green tea are an integral part of Asian diets and may explain reasons for the lower incidence of prostate cancer in Asian men. Finally, more investigation is needed to warrant the daily use of some of these bioactive foods as chemopreventive agents for prostate cancer as well as to potentially find new single or combination agents in the future.

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20 Control of Prostate Cancer Proliferation and Gene Expression Using Herbal Supplements

Joseph M. Wu and Tze-chen Hsieh

CONTENTS

INTRODUCTION

Prostate cancer (CaP) is the most commonly diagnosed cancer and produces the second highest cancer mortality rate in U.S. men. According to the American Cancer Society, the expected number of new cases in 2008 was 186,320 with 28,660 deaths. An upward trend in the incidence rate of CaP among adult males in the United States first surfaced in the early 1990s, and was probably linked to an increase in life expectancy and more prevalent use of the PSA test. In recent years, through vigilant screening and early diagnosis, the reported CaP cases have actually decreased slightly; nevertheless, the prognosis associated with metastastic disease remains dismal.

Among factors associated with the high fatality of advanced CaP, androgen and androgen receptor (AR)-mediated signaling are considered of primary importance since they promote the initiation and progression of CaP (Miyamoto et al., 2004; Singh and Agarwal, 2006; Singh et al., 2006; Dehm and Tindall, 2007; Barve et al., 2008; Chen et al., 2008). Thus, CaP rarely occurs in eunuchs or in men with a deficiency in 5α-reductases, the enzymes that convert testosterone to its active metabolite 5α-dihydrotestosterone (Crawford, 2009). Patients diagnosed with localized CaP readily respond to androgen ablation indicating that androgens and androgen-responsive events underpin the survival and expansion of androgen-dependent (AD) CaP cells. The AR also plays an essential role in the establishment of the androgen-independent (AI) state known as hormone refractory prostate cancer (HRPC), which represents an eventual disease outcome following hormonal ablation. Once established, HRPC no longer responds to available therapies and thus is considered fatal with a short survival window for patients averaging only months to less than 2 years (Chung et al., 2005). Because AR contributes to the dismal prognosis of HRPC, intensive efforts have been directed at the design and development of novel anti-HRPC strategies. To these ends, AR-antibodies to block AR function, decoy oligonucleotides containing androgen-responsive element (ARE) acting as AR sequestrants, anti-AR ribozyme, and more recently AR-knockdowns using siRNA have all been proposed and are in different stages of development and evaluation (Zegarra-Moro et al., 2002; Chen et al., 2004; Tran et al., 2009). A similar consideration and rationale may apply to efforts spent in discovering and identifying agents from botanical sources for the suppression of AR levels and AR-mediated, ligand-dependent and ligand-independent signaling pathways, as they too are deemed attractive targets in the prevention and management of CaP, especially HRPC. The continuous efforts in seeking unconventional and often unproven alternative therapies, by scientists and patients alike, under the overarching rubric of complementary and alternative medicine (CAM), underscore the short-duration effectiveness as well as the overwhelmingly toxic side effects of existing therapies for CaP and HRPC.

Much of CAM therapies are based on the use of herbs, rooted in the practice of oriental or traditional Chinese medicine (TCM), which is a holistic, artful form of medical practice with a history down the millennia (Ferro et al., 2007; Lee et al., 2008). Even in China today, the majority of the population still relies on TCM, applied alone or combined with Western medicine for treating various ailments including cancer. Interestingly, in Western countries including the United States, CAM and TCM usage and clinics are gaining popularity in recent years because of their perceived safety and efficacy (Eisenberg et al., 1993, 1998; Kessler et al., 2001; Tindle et al., 2005; Chen et al., 2007; Conboy et al., 2007). By extrapolation, therefore, herbs, herbal supplements, and herbal medicine, as the mainstay of TCM, are also becoming more widely accepted and adopted among patients and healthcare professionals. Furthermore, because TCM-based herbs and herbal supplements are derived from plants found in countries ouside the United States, they are conjectured to possibly contain novel, naturally occurring bioactive agents having the potential to target the AR and its downstream signaling pathways, and thus possibly proffering therapeutic indications for HRPC (Paterson and Anderson, 2005; Ehrman et al., 2007a,b,c). In support of this possibility, it has been noted that a large percentage of anticancer drugs in use today has been discovered and developed from botanical sources, fostering the belief that the latter may represent significant untapped resources for discovering new herbal medicines (Nielsen, 2002; Butler, 2005; Newman and Cragg, 2007).

Despite the positive attributes of low toxicity and high complementarity in TCM herbs and botanical-based bioactive therapeutic agents, significant limitations are found in the TCM practice of disease management, notably, largely anecdotal evidence of efficacy and not quantifiable data obtained through randomization and blind studies, lack of documentation on compliance and outcome, insufficient standardization of preparation, safety, and potential for adverse interaction with widely prescribed medications in the United States.

Are herbal supplements and extracts of benefit, and considered efficacious and even therapeutic for CaP? The first part of this chapter will summarize various stages of CaP and treatment challenges that remain, as introduction to the prevalent use of herbs and herbal preparations in treating CaP. In Part B, some of the rationale for and against the use of herbal supplement in the management of CaP will be reviewed. Lastly, our own studies on extracts prepared from a polyherbal formulation called Equiguard™ will be described.

PART A: AN OVERVIEW OF CAP STAGING AND ANTI-CAP STRATEGIES

AD AND HRPC

Two broad categories of clinical CaP have been identified. The AD is under the control of androgens and therefore responds readily to radical prostectomy, radiation, and androgen blockade. Despite initial success evident by clinical remission seen in most treated patients, relapse of the disease occurs frequently and is accompanied by emergence of the HRPC. Expansion of the HRPC clones eventually establishes the AI or HRPC state, which is hormone-independent and thus can coexist with the continual administration of androgen deprivation therapies (Feldman and Feldman, 2001; Hsieh et al., 2007; Steinkamp et al., 2009). HRPC is characterized by increased prostate-specific antigen (PSA), robust cell growth concurrent with drastic diminution of androgens to essentially undetectable levels, and propensity for metastasis to sites beyond the confines of the prostate gland. HRPC patients show high fatality rates, with a median survival of ~18 months due to lack of life-prolonging curative therapies (Chung et al., 2005).

Genetic and epigenetic changes contribute to HRPC. First, proliferation of HRPC cells may be attributable to germ line or somatic AR mutations and/or gene amplifications, and to the interplay of AR with growth factors and cytokines. Amplification and mutations of the AR, exemplified by expansion of CAG (glutamine) repeats in exon 1, changes in the ligand-binding domain and in regions flanking the activation function 2 (AF-2), domain binding site (Dehm and Tindall, 2006), have been reported in prostate specimens obtained from HRPC patients. Secondly, HRPC cells also show prominent presence of growth indolent yet metabolically active neuroendocrine cells, which are capable of secreting cellular effectors that fuel the proliferation of interspersed, neighboring HRPC cells (Komiya et al., 2009). These genetic/metabolic changes may allow the HRPC cells to remain viable in a low androgen state by altering ligand specificity promulgating cell growth using nonclassical ligands, and even paradoxically switching antiandrogens from acting as therapeutic inhibitors to functioning as HRPC proliferation stimulators (Gao et al., 2001; Krishnan et al., 2002; Urushibara et al., 2007). Because there is a significant time gap before the androgen deprivation-responsive AD progresses to the incurable HRPC state, CaP is amenable to preventive therapies. Moreover, since HRPC is considered heterogeneous, it is more likely to respond to combination and/or sequential treatment strategies rather than single agent therapies.

ANTI-CAP STRATEGIES

Targeting the Central Nervous System (CNS)-Adrenal-Testicular Axis to Block Androgen Synthesis

Functioning of the prostate depends on the availability of androgens, whose synthesis and release is under the control of CNS-adrenal-testicular axis (Figure 20.1). The CNS neurons induce the release

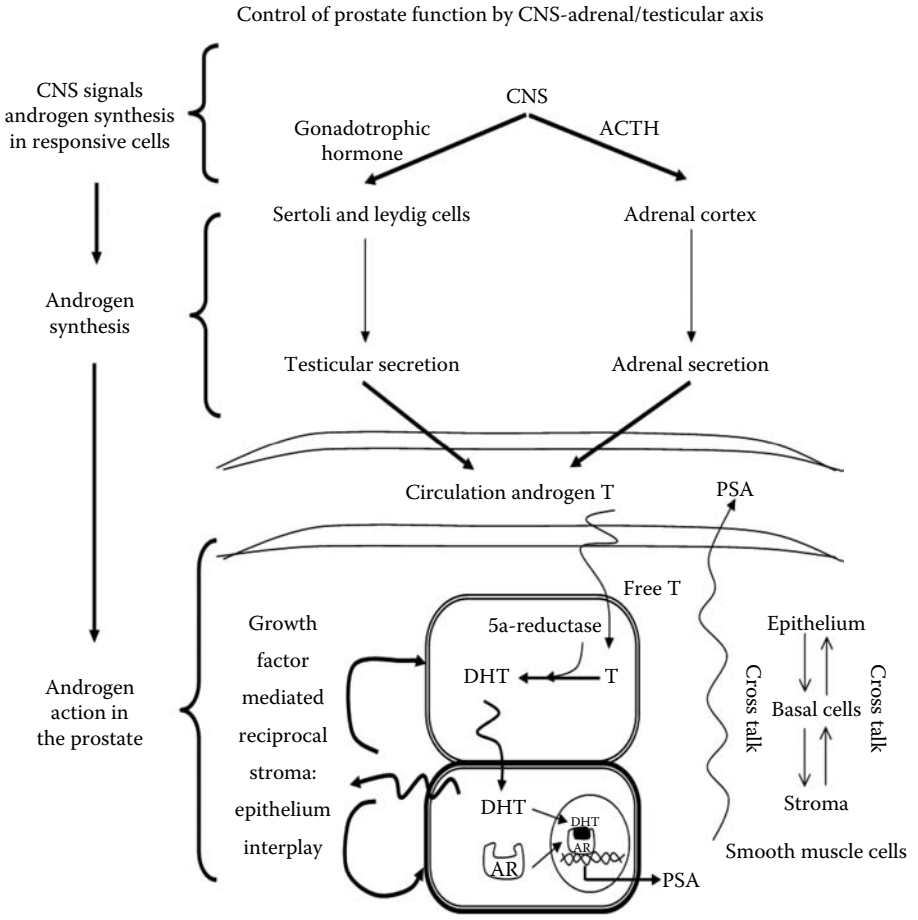


FIGURE 20.1 Control of prostate function by the CNS-adrenal-testicular axis. Neuronal activity from the CNS induces the pulsatile release of LH-RH from medial region of the hypothalamus, followed by LH-RH binding to cognate receptor located in the anterior pituitary. This triggers the release of LH and ACTH into circulation. Systemic LH is delivered to Leydig and Sertoli cells of the testis while ACTH acts on the adrenal gland, both eliciting synthesis and release of testosterone. Circulatory testosterone affects the prostate microenvironment, organized into the three adjoining compartments of epithelium, extracellular matrix (ECM), and the stroma. Differential interplay between the stroma and epithelium in the prostate microenvironment controls normal prostate growth as well as abnormal epithelial proliferation in prostate cancer.

of gonadotrophic hormone and adrenocorticotropic hormone (ACTH), impinging on the testis and the adrenal cortex, and signaling the synthesis and release of testosterone. The synthesized testosterone is delivered by circulation to the prostate epithelial and basal cells, where it is enzymatically converted to dihydrotestosterone (DHT) by 5-α-reductase isoenzyme type II. The synthesized DHT diffuses into neighboring stromal cells where it binds the AR to transcriptionally stimulate the *de novo* synthesis of autocrine- and paracrine-acting growth factors by the stromal cells (Coffey and Isaacs, 1981; Gonzalgo and Isaacs, 2003). Reciprocal interplay between the stroma and epithelium collectively drive growth and development of both epithelial and the stromal cells as a key event in prostate carcinogenesis (Wong et al., 2003; Jin et al., 2004; Bhowmick and Moses, 2005).

A major anti-CaP strategy focuses on the CNS-adrenal-testicular axis to suppress the synthesis of testosterone and inhibit its conversion to DHT. The former aspect can be met by orchiectomy, and pharmacological intervention strategies, for example, administration of diethylstilbestrol (DES), lutenizing hormone-releasing hormone (LH-RH) agonists, and antiandrogens, to induce an androgen

deprivation state and in majority of cases a clinically favorable response. Unfortunately for most patients, hormonal therapies will result in relapse of the disease and additionally are complicated by severe pain and untoward quality of life issues, thus compelling the need to consider alternative modalities.

Targeting Cell Proliferation Inhibition and Inducing Cell Cycle Arrest

Eukaryotic cell division proceeds through an orderly sequence of events known as cell cycling. The transition of cell cycle phases is controlled by two precisely regulated checkpoints, G_1/S and G_2/M. Each checkpoint is regulated by positive factors, which drive the cycle forward, and negative factors, which induce cycle arrest. Numerous proteins are involved in this progressive process, acting in a dominant (oncogene products) or recessive (tumor suppressor gene products) manner.

To drive the expansion of AD and HRPC cells, proliferation must be coordinated with cell cycle phase transition. Thus, proteins with pivotal roles in regulating checkpoint control are reasonable candidates for developing anti-CaP strategies. The function and efficacy of such anti-CaP candidates may be analyzed by flow cytometry in combination with biochemical dissection of the spatiotemporal changes in the expression of relevant cell cycle regulatory proteins. For instance, demonstration of a significant increase in the percentage of cells in G_1 phase, based on flow analysis, in cells exposed to a putative anti-CaP agent may reflect a blockade in the cell transition from G_1 to S phase. This cellular dysfunction, in turn, could result from G_1/S checkpoint regulation secondary to an inhibition of phosphorylation of the retinoblastoma gene (Rb), concomitant with a reduction in the expression/activity of cyclin-dependent kinase 4 (cdk4):cyclin D protein complex, which plays an essential role in Rb phosphorylation. Natural products have been found to be good sources of inhibitors of cell cycle phase transition (Hung et al., 1996); determination of the effectiveness of an agent in downregulating the expression and/or suppressing the activity of the relevant proteins, therefore, would provide useful information on its potential as anti-CaP candidate (Figure 20.2a).

Apoptosis as a Target for Developing Anti-CaP Agents

An additional target for the design of anti-CaP agents and strategies exploits the induction of apoptosis. Onset and execution of apoptosis can be validated by using several assays including: (1) flow cytometry showing fragmented DNA as a sub-G_1 peak, (2) DNA laddering pattern as revealed by agarose gel electrophoresis, (3) immunohistochemical staining *in situ* measuring the digoxigenin-tagged nucleotide incorporation onto the free 3′-ends of DNA (TUNEL assay), (4) processing of actin to yield a truncated 15-kDa product, and (5) cleavage of poly(ADP-ribose) polymerase (PARP) from its native 133-kDa molecular weight to an 89-kDa fragment (DiPietrantonio et al., 1998, 2000a).

Suppression of AR Expression and Function

The AR plays an integral role in the establishment of HRPC, driving cell proliferation and increases in PSA. The PSA expression is under the control of AR, both as the wild-type or mutated/amplified protein. Binding of AR by androgens is concomitant with a change in its conformation and dissociation of heat shock protein chaperones, following which dimerization, phosphorylation, and nuclear translocation of AR occurs. Nuclear AR interacts with the *cis*-acting AREs located on the promoter of the PSA gene, and occurs simultaneously with the cooperative and sequential binding of other *trans*-acting factors and the RNA polymerase II transcription machinery, leading to the transcription of PSA (Sadar, 1999; Sadar et al., 1999; Sadar and Gleave, 2000). Important PSA gene *cis*-acting elements include: AREs located at –170/–156 and –4148/–4134; androgen response region (ARRs) located at –395/–376; and additional distinct consensus sequences (Sadar, 1999; Sadar et al., 1999; Sadar and Gleave, 2000; Jia et al., 2003; Cinar et al., 2004; Kim et al., 2005). These *cis*-acting elements are recognized by *trans*-acting factors, notably, transcription factors AP-1 (activator protein 1) and CREB (cAMP response element binding protein), the retinoblastoma gene Rb, cyclins E and D (Yamamoto et al., 2000; Hofman et al., 2003; Petre-Draviam et al., 2003; Wang et al., 2004) (Figure 20.2b). Thus,

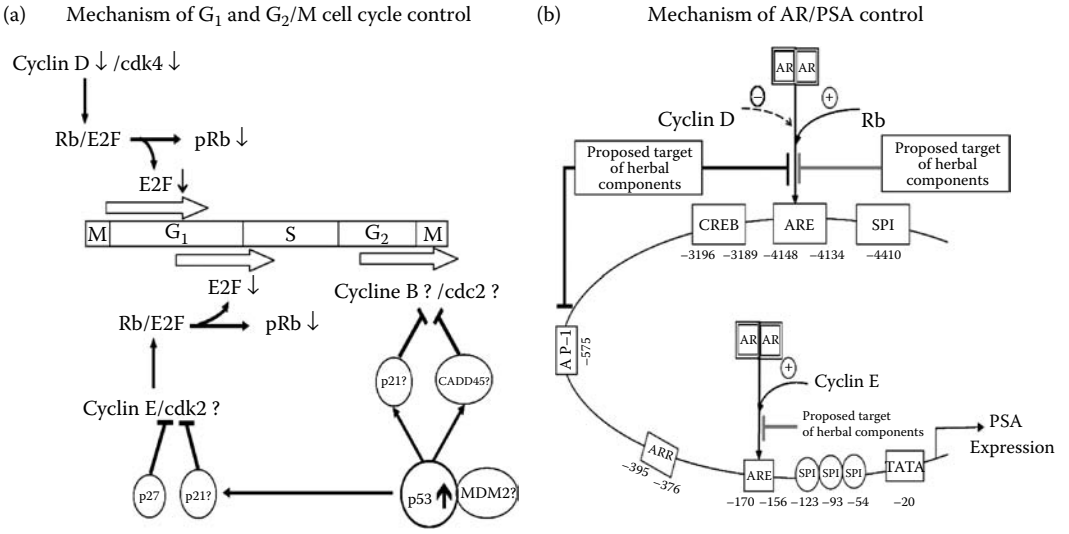


FIGURE 20.2 Schematic model showing the regulation of cell cycle checkpoint and control of AR and PSA, by herbals. (a) Herbals regulate the expression of cyclins D/E and Rb playing a pivotal role in G_1/S cell cycle traverse. Herbal supplements are hypothesized to contain multibioactive components (chemicals, small peptides), each present at subpharmacological concentrations capable of reducing the expression of cyclins D/E and Rb as well as suppressing Rb phosphorylation. These changes collectively induce dysfunctional G_1/S transition and cell growth arrest. (b) Interrelationship between cyclin D/E and Rb expression and control of PSA by herbal components. Bioactive components present in herbal formulations act to lower AR expression, and/or its translocation, and/or its interaction with AR-responsive elements, resulting in suppression of androgen-responsive genes such as PSA. Likewise, cyclins D/E and Rb are AR-comodulatory proteins; their reduced expression by herbal-derived components will indirectly control PSA transcription mediated by the AR.

AR comodulatory proteins, notably, cyclins D/E and Rb are good targets for discovering and identifying anti-CaP agents.

Disrupting the Interplay between Prostate Stroma and Epithelium

The prostate microenvironment is organized into adjoining epithelium, extracellular matrix (ECM), and the stroma. Because the majority of CaP in humans consists of adenocarcinomas arising from the epithelial cells lining the glands and ducts of the prostate, and since genetic insults to the epithelium are considered the initiation events of CaP, focus of research has mostly been on prostate epithelial cells. Recent evidence increasingly shows that the stroma has a role beyond a supporting scaffold, by actively regulating prostate functions and prostate carcinogenesis (Tuxhorn et al., 2001, 2002; Chung et al., 2005). The emerging role of a reactive stroma, activated by neighboring epithelial carcinoma cells, is evident even during the PIN stage of CaP; moreover, during CaP progression, the stroma undergoes serial and sequential alterations to develop a phenotype that cooperates with the tumorigenic epithelial cells to further drive epithelial cell proliferation, motility, and invasiveness (Tuxhorn et al., 2001). Anti-CaP strategies may therefore consist of identifying agents capable of cotargeting the proliferation of both stromal and epithelial tumor cells as well as disrupting their reciprocal, dynamic interplay.

PART B: HOW WELL DO HERBAL THERAPIES FIT AS ANTI-HRPC STRATEGIES?

Alternative Treatment and Management Strategies for Malignant Diseases

A major challenge in the treatment and management of malignant diseases is the continuous need for discovering and identifying novel antineoplastic compounds. This challenge stems from the fact

that a malignant tumor is heterogeneous in nature, composed of genetically and phenotypicaly diverse cell types, and may be undergoing complex, multistage, clinically silent, evolution conducive to drug resistance and difficult to simulate with short-term *in vitro* procedures, and impractical to be screened using *in vivo* assays.

CAM has been gaining momentum and attention in the United States for treating malignant diseases (Eisenberg et al., 1993, 1998). It is estimated that their use by patients increased from 34% in 1990 to 42% in 1997, and ranged from 50–69% in adult cancer patients (Eisenberg et al., 1993, 1998). Because this number is still rising, it is projected that there will be more visits to alternative health practitioners than total visits to all primary care physicians.

Why? The complex nature of human cancer suggests that alternative management may be appropriate to improve the therapeutic efficacy of conventional treatment and/or the quality of life for cancer patients. Specifically, the perception is that herbal therapies within the rubric of CAM can expand options for preventing and treating cancer, by intrinsically present novel antitumor activities, by increasing the sensitivity of neoplastic cells to chemotherapeutic agents, and also by reducing toxic and adverse effects induced by chemotherapy, without interfering with their tumoricidal effects. One might therefore envisage medicinal herbs as specialized botanicals and as valuable sources of new therapeutic candidate compounds merely manifesting the tremendous chemical diversity intrinsically found in innumerable species of plants. This role of botanicals as a source for remedies has been recognized since ancient times; plant-derived active principles and their semisynthetic and synthetic analogs have always served as one of the major sources for discovery of new anticancer drugs.

Herbal Medicine Offers New Opportunities in CaP Treatment

Treatment modality for patients diagnosed with localized or metastatic CaP primarily consists of prostastectomy, radiation, chemotherapy, and androgen ablation by combined androgen blockade. With passing time, however, the cancer becomes refractory to hormone ablation, leaving patients with little other therapeutic options. This is not particularly surprising because of the multifactorial, multistage, and heterogeneous nature of CaP, suggesting that use of single agents for effective treatment of CaP will be challenging. Faced with the threat of imminent finality of life, these patients often seek unconventional "alternative" and/or "complementary" treatments, most commonly available in the form of phytotherapies using herb-based products. Use of herbals may be considered an atypical alternative, complementary form of treatment akin to combination and/or sequential therapies.

What considerations and rationale can be brought to bear on using alternative herbal therapies for CaP? Epidemiologic studies have consistently shown that age-adjusted incidence and mortality rates for clinical CaP display significant geographic variations and ethnic/racial group differences. In addition to genetic and epigenetic factors and their interplays that are thought to contribute to the observed variable incidence (Hsing and Devesa, 2001; Hsing et al., 2001), diet and nutrition may also exert promoting as well as protecting roles in the progression and establishment of clinical CaP (Doll and Peto, 1981; Ames, 1983; Ames and Gold, 1997). Moreover, culture and regional customs, such as food and other lifestyle preferences, such as use of medicinal herbs for general health maintenance may also act to subsume potentially metastatic CaP in the latent, subclinical state (Darzynkiewicz et al., 2000; Hsieh et al., 2002b). In contrast to Western countries, in eastern Asia, particularly in China, medicinal herbs are frequently consumed seasonally as health preventive measures, a practice which has been maintained throughout centuries.

This type of lifestyle habits and customs underscores significant differences existing between West and Oriental cultures and medical professionals in regard to causes, management, and treatment of chronic diseases including cancer. Classical medicine in China (traditional Chinese medicine, TCM) stems from a 3000-year-old discipline that is rooted in the cosmological concept of *ying–yang* and the *Five Elements*. According to TCM, illnesses reflect imbalance or disturbance resulting

from human and universal processes; accordingly, treatment requires a restoration of the balancing activities, which can best be achieved by using a formulation containing a combination of active, counteractive, and balancing ingredients (usually derived from botanicals and herbs). Of note, medicinal plants represent a still largely untapped source of structurally novel compounds that might serve as leads for the development of novel drugs.

Herbal therapies are embodied in traditional Chinese medical practices, where disease treatment approach using a "holistic/integrative" orientation is quite distinct from the "pharmaceutical" approach of Western medicine. Typically, Chinese herbal prescriptions comprise mixtures which, if properly prepared, deliver multiple bioactive agents to target cells/organs. Because this "integrative" strategy emphasizes application of the total spectrum of bioactive ingredients present in a herbal mixture and evaluates success based on the "well being/curing" of the patients as a whole, its concoction is not absolutely dependent upon precise knowledge of the specific defect/derangement in target cells. Moreover, the same beneficial clinical outcome may be achieved using formulations with no apparent identity. Therefore, conceivably, for some diseases, Chinese herbs may increase the effectiveness of modern drug treatments, reduce their side effects, and under the best circumstances replace them completely. TCM-based herbal supplement uses medicinal plants basically in two different ways: (1) as complex mixtures containing a broad range of constituents (extracts, tinctures, essential oils, and infusions), and (2) as pure, chemically defined active principles. Pure compounds are generally employed when the active principles of a medicinal plant exhibit strong, specific activity and/or have a small therapeutic index, requiring accurate and reproducible dosage. Herbal therapies rely upon presentation of aggregate, ill-defined combinations of bioactive, inactive, and counteractive agents, with the aim that their collective manifestation results in reduced toxicity and appearance of new/novel activities. These features are considered important in cancer prevention/treatment since they may serve to counteract and circumvent overlapping molecular pathways, which typically characterize malignant states and which oftentimes impede success in cancer treatment. It is also notable that TCM is widely practiced and is often used in the treatment of cancer either alone or in combination with Western medicine. Many important chemotherapy agents have been derived from TCM and other natural products (Darzynkiewicz et al., 2000). For instance, the diterpenoid taxol, isolated from the stem bark of the Pacific Yew, has become one of the most promising lead compounds to emerge from the antitumor screening of natural products in recent years. During the period 1983 to 1994, of the 520 new chemical entities introduced into the world market, 203 were derived from natural products (Harvey, 1999, 2007, 2008). Natural products and compounds derived from natural products continue to provide many therapeutic drugs. In 1991, 16 out of 42 new chemical entities introduced into the world market as drugs were derived from natural products, and in 1992, 18 out of 43 were also derived from natural products. A recent review indicated 46 natural products under current clinical investigation in the areas of infectious disease, neurological disease, cardiovascular and metabolic disease, immunological and inflammatory disease, and oncological disease (Shu, 1998). Indeed, survey shows that the vast majority of the globally diverse plant species are yet to be tested for agents with pharmaceutical potentials.

Positive and Negative Attributes of Herbals as Anti-CaP Agents

As mentioned, cancer is now recognized to be a constellation of diseases with multiple genetic, cellular, and biochemical aberrations, capable of elaborating functionally overlapping molecular pathways that effectively counteract activity of single agents widely used to treat malignancies. The conventional approach of treating cancer with single agents has met with limited success, due to toxicity at the effective dosage, and also due to lack of balancing chemicals commonly found in the traditional remedy. Single agents are not sufficiently broadbased in their mechanism of action as to effectively control all of the underlying complexities of malignant cells described. Greater emphasis has been placed on "combination" or "sequential" approaches, wherein putatively effective agents, at suboptimal concentrations, are either presented as a group or are given in a particular sequence,

to target cells (Kern et al., 1997). A variation of the "combination" theme is to apply whole herbal preparations as "alternative" disease treatment modalities. Herbal therapies differ from a single agent approach, the mainstay of conventional therapies, in that aggregate phytochemicals are used. Moreover, to date, less than 0.5% of the higher plants on this planet have been analyzed as drug candidates (Hsieh et al., 1997a,b); the vast majority of the globally diverse plant species has yet to be tested for agents with pharmaceutical potentials, making it likely that new leads will be forthcoming from systematic screening of extracts prepared from natural plant species.

Numerous issues and limitations still exist in herbal therapies. Foremost among them is poor to inadequate characterization of the active ingredients. Even in cases where active ingredients have been identified, reconstituting individual components to the same potency as the crude preparation has been challenging. Moreover, little is known about the quality, safety, effectiveness, mode of action, side effects, and interactions between active components within herbal mixtures. There is also lack of quality control and standards, and inadequate information on manufacturing process. Thus, there is no assurance of safety or effectiveness, or information on risk:benefit ratio. Also, the mechanism of action of herbs unclear and not systematically studied, and possible interaction with prescribed medicines unknown.

PART C: STUDIES OF POLYHERBAL FORMULATIONS

Polyherbal formulations are frequently used in the practice of TCM and are based on the premise that treatment of human diseases, especially those of a chronic nature, can best be met through the increased probability of functional synergism by the potential belying the distinct combinatorial interaction intrinsically present within the diverse bioactive components in natural products. Polyherbal formulations exert their effectiveness in disease treatment through a multitude of mechanisms, including expanded specificity, reduced toxicity, and improved bioavailability, modulation of influx and efflux of active components, among others.

Overview on Equiguard™

With aging of the U.S. population and an increase in the elderly worldwide, kidney and urinary/urological diseases are becoming significant public healthcare issues. The urinary system is composed of the organs, muscles, and nerves that work together to produce, store, and eliminate urine. By controlling urine flow, the urinary system maintains proper water and salt balance throughout the body. Included in this system are the kidneys, ureters, and the bladder and, additionally, the prostate gland and sex organs in men. Severity in urinary system disorders range from easy-to-treat to life-threatening. Examples include kidney stones, polyuria, renal failure, urinary incontinence, urinary tract infections, prostatitis, benign prostatic hyperplasia, and prostate cancer.

The Equiguard™ is a dietary supplement composed of standardized extracts from nine Chinese herbs, respectively, *Epimedium braevicornum* Maxim, *Morinda officinalis* How, *Rosa laevigata* Michx., *Rubus chingii* Hu, *Schisandra chinensis* [Turcz.] Baill., *Ligustrum lucidum* Ait., *Cuscuta chinensis* Lam., *Psoralea corylifolia* L., and *Astragalus membranaceus* [Fisch.] Bge. (Hsieh et al., 2002a). This proprietary preparation was formulated to relieve polyuria and incontinence by maintaining and restoring kidney harmony and hence functions in adults. The efficacy of Equiguard™ for the urological symptoms is supported by limited studies using animals and healthy elderly men and women (Hsieh et al., 2002a; Xiong et al., 2008).

According to TCM, polyuria and incontinence are symptoms frequently associated with age-related decline in kidney and bladder functions. The role of the kidneys in these disorders is significant since, in the conceptual framework of TCM, the kidneys are considered as the "fountain of life" which implicitly suggests that the two kidneys are bestowed with distinct primordial *ying-yang* functions absent from other organs. Importantly, therefore, harmony in the kidneys promotes wholesomeness in health while failure, attributable to dysfunction of the primordial *ying-yang*, will

result in physiological disintegration and ultimately havoc in the individual. Since the prostate is considered an integral component of the urological system, we performed *in vitro* studies to test the effects of extracts of Equiguard™ to modulate prostate growth and gene expression. These studies used CaP cells mimicking the AD and HRPC states.

Hypothesis

Our hypothesis is that Equiguard™ contains bioactive ingredients, each present at subpharmacological concentrations that interact by synergy or by creating novel activities, to elicit gene changes that affect cell cycle control and PSA expression.

Issues on Polyherbal Formulation Studies

Studies of herbal formulations face a daunting challenge, namely, reference standard for batch-to-batch comparison and quality in manufacturing. Innumerable factors could affect the concentration of active herbal components: weather conditions, plant maturity, and contamination by other plants, suggesting that batch-to-batch and lot-to-lot variations might be expected and likely for all herbal formulations.

Another issue relates to standardization of Equiguard™ extracts; its availability would provide an objective basis for comparison of data from different laboratories. One approach used in our laboratory involves suspending 350 mg Equiguard™ capsule in 1 mL ethanol:water (0–100%) with ethanol increased in 10% increments, and the mixture shaken at 150 rpm, for one hour at room temperature. Clear extract was obtained by centrifuging and filtering the suspension. The biological activity of the extract was assayed by adding appropriately diluted Equiguard™ ethanol extracts to medium of LNCaP cells and determining suppression of cell proliferation and reduction of PSA. These experiments empirically showed that 70% ethanol extract gave the most consistent biological activity. Quality assurance of Equiguard™ was also determined by demonstration of a reproducible high pressure liquid chromatography (HPLC) elution profile.

Testing Anti-CaP Activities of Equiguard™

To characterize Equiguard™ extract, dose- and time-dependent experiments were performed on the cell lines representing different stages of CaP, respectively, LNCaP (AD) and DU145, PC-3 and JCA-1 (HRPC) as detailed (Hsieh et al., 2002a; Lu et al., 2003, 2004; Hsieh and Wu, 2008). These studies have shown that 70% ethanol extracts of Equiguard™ decreased CaP cell growth in cells tested (Figure 20.3a) and also reduced AR and PSA expression in LNCaP cells (Figure 20.3b).

Modulation of LNCaP Cell Growth and Cell Cycle Regulatory Protein by Equiguard™

Dissemination of tumor cells from the primary cancer site often involves their metastasis to lymph nodes. Accordingly, we investigated the effects of 70% ethanol extracts of Equiguard™ on growth of LNCaP cells which are derived from an individual whose cancer metastasized to the lymph nodes, and which have been used as a model system for investigating parameters connected with androgen-responsiveness characteristic of CaP cells (Hsieh et al., 2002a).

We first investigated whether Equiguard™ affected the proliferation of cultured LNCaP cells. A dose- and time-dependent reduction in cell growth was observed. In control cells, proliferation was preceded by lag on day 1, while cells treated with Equiguard™ showed little growth, and at the highest dose tested, less cells were found on day 3 than on day 1 (Figure 20.3a). The colony formation assay showed that clonogenicity of LNCaP cells was markedly suppressed by ethanol extracts of Equiguard™.

Cell cycle analysis was performed to better understand the antiproliferative effects of Equiguard™. These studies showed that Equiguard™ induced dose-dependent G_1/S arrest and apoptosis (Hsieh

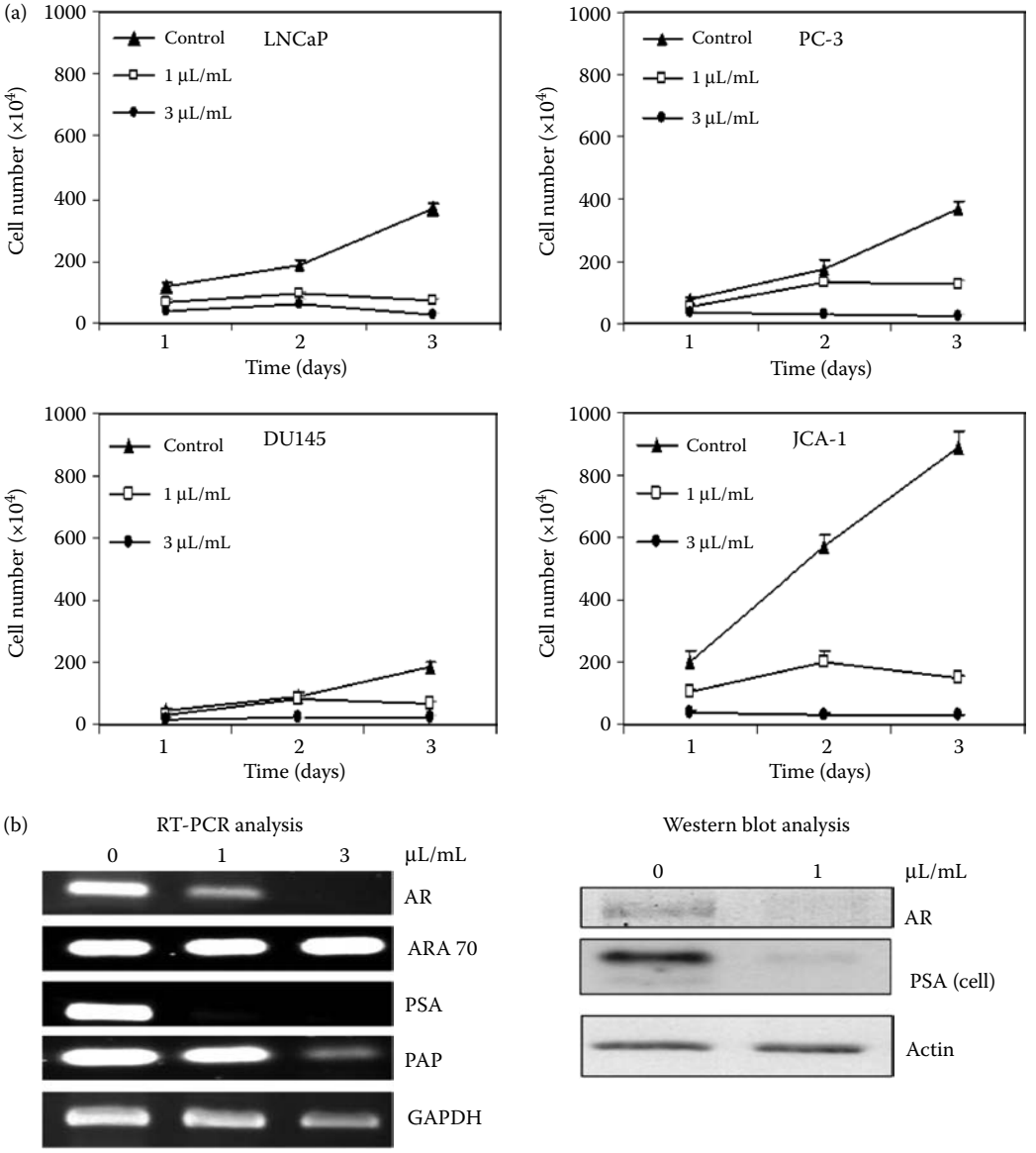


FIGURE 20.3 Effects of ethanolic extracts of Equiguard™ on proliferation and gene expression of AD (LNCaP) and HRPC (PC-3, DU145, and JCA-1) prostate cancer cells. (a) Suppression of proliferation of AD and HRPC cells by increasing dose of Equiguard™. (b) Suppression of AR and AR-coactivator expression and PSA levels by 70% ethanolic extracts of Equiguard™.

et al., 2002a). LNCaP cells treated with Equiguard™ extracts for three days showed that the percentage of cells in G_2 and M phase of the cell cycle remained unchanged, whereas cells in S phase decreased concomitant with G_1 phase increase. At higher concentrations an additional peak (sub-G_1 cells), characteristic of cells undergoing apoptosis, was also observed. To obtain information on the cell cycle effects of Equiguard™, changes in expression of cell cycle regulatory proteins were assayed. We found that LNCaP cells treated with Equiguard™ showed a dose-dependent increase in the expression of tumor suppressor gene p53 as well as elevation in serine-15 phosphorylation (Lu et al., 2003b, 2004), suggesting that this herbal supplement contains active components that increase the stability of the p53 and in turn control surveillance and maintenance of integrity of the DNA.

Another protein playing a pivotal role in controlling the G_1/S cell cycle checkpoint is Rb that functions as an on/off switch for binding to transcriptional factor E2F via a cyclin-dependent kinase (cdk)-mediated phosphorylation mechanism (Taya, 1997; DiPietrantonio et al., 1998; Harbour et al., 1999; Qu et al., 2003; Lu et al., 2003a). Therefore, we tested whether extracts of Equiguard™ contain chemicals capable of specifically inhibiting Rb phosphorylation. A dose-dependent inhibition in Rb expression was clearly evident in treated cells, accompanied by a reduction in hyperphosphorylated Rb and increase in hypophosphorylated Rb providing a molecular explanation on how Equiguard™ might restrict cell progression from G_1 to S phase (Figure 20.2a). Since distinct sites of Rb become phosphorylated in a cell cycle stage- dependent manner by protein kinase complexes such as cyclin D/cdk4/6 and cyclin E/cdk2 (Hsieh et al., 1999; Murillo et al., 2001), the effects of Equiguard™ on the expression of cyclins D1, E and cdk4, was determined. The level of all three proteins was reduced in treated cells (Lu et al., 2004). Since the phosphorylation of Rb may be affected by upstream signaling events, for example, mediated by NF-kB. The levels of NF-kB and its control by IkB in treated cells were assayed by Western blot analysis. Both proteins were down-regulated (Hsieh and Wu, 2008). Since CDKIs might also affect control of Rb phosphorylation by Equiguard™, we also checked changes in specific CDKIs and found that treatment caused a dose-dependent increase on the expression of Kip1/p27 without changing Cip1/p21 (Lu et al., 2004).

In summary, upregulation of Kip1/p27 by Equiguard™ might restrict cyclin D1/cdk4 activity, inhibiting Rb phosphorylation and inducing accumulation of hypophosphorylated Rb (Figure 20.2a) (Lu et al., 2004); in sequence, hypophosphorylated Rb binds to transcription factor E2F involved in the control of S-phase transition. Collectively, these changes contribute to G_1/S block in cells treated with Equiguard™.

Studies with HRPC DU145 and PC-3 Cells

In its advanced stages, prostate tumor often ends in distant target tissues such as the brain and bone. Accordingly, we investigated the effects of 70% ethanol extracts of Equiguard™ on growth of DU145 cells, which were derived from brain metastasized CaP cells. Both growth and clonogenicity were significantly inhibited by ethanol extracts of Equiguard™ (Figure 20.3a) (Hsieh et al., 2002a). However, flow cytometric analysis did not reveal an arrest in G_1 nor an induction of apoptosis; rather, an increase in proportion of S-phase cells and a decrease in G_2/M was seen at 1 μL/mL concentration (Hsieh et al., 2002a). The differential cellular responses of LNCaP cells and DU 145 cells support the contention that multiple bioactive ingredients are present in Equiguard™. The presence of a large array of diverse active ingredients in Equiguard™ attests to their potential for treating CaP, which is known to be heterogeneous.

A major complication of CaP is metastasis to bone. Therefore, we investigated the growth response of PC-3 cells—derived from an individual whose cancer disseminated to the bone—to ethanol extracts of Equiguard™. Time and dose-dependent growth suppression was observed in PC-3 cells after Equiguard™ treatment (Figure 20.3a) (Hsieh et al., 2002a). Thus, compared with the other CaP cell lines, PC-3 cells were affected to the greatest degree by Equiguard™ (Hsieh et al., 2002a). The basis of this growth disruption likely is attributed to a suppression of cell progression through S and G_2 + M phases, and additionally, the induction of apoptosis. Contrary to LNCaP cells, however, which responded to Equiguard™ by being arrested in the G_1/S phase of the cell cycle, flow cytometry analysis of DU145 and PC-3 cells treated with Equiguard™ showed an inhibition in G_2/M traverse (Hsieh et al., 2002a).

Induction of Apoptosis in Equiguard™ Treated LNCaP Cells

Since flow cytomety analysis showed induction of apoptosis in Equiguard™-treated cells, we tested changes in apoptogenic candidate proteins. Translocation of cytochrome *c* from mitochondria to the cytosol occurs during apoptosis, activating caspase 3 and resulting in PARP cleavage to distinct PARP degradation products (DiPietrantonio et al., 1999, 2000a,b). We found a dose-dependent accumulation of cytochrome *c* in the cytoplasm, after a three-days' treatment with Equiguard™; however, no change

in caspase 3 was detected (Lu et al., 2004). The treatment also downregulated PARP expression without increasing the PARP cleavage product. Thus, treatment by Equiguard™ may release cytochrome *c* from the mitochondria, with a transient activation of caspase 3 and PARP cleavage.

Effect of Equiguard™ on Prostate Specific Gene Expression in LNCaP Cells

Another feature of the LNCaP cells is their ability to synthesize and secrete PSA, a 34-kDa tissue-specific glycoprotein with kallikrein-like serine protease activity, which is produced almost exclusively in epithelial cells lining the acini and ducts of the prostate, and is expressed in normal, benign prostate hyperplasia (BPH), and primary/metastatic prostate tissues. In normal prostate, serum PSA ranges from 0–4 ng/mL; elevated PSA (higher than 5 ng/mL) accompanies prostate carcinoma, benign prostate hyperplasia or prostatitis. PSA has been used as a serum marker to evaluate stages of CaP, and for monitoring responses and progress of patients to different therapies. The regulation of PSA is frequently coordinated with changes in its transcription factor AR (Hsieh et al., 1997). Treatment of LNCaP cells by Equiguard™ decreased intracellular and secreted PSA, which was statistically correlated with the suppression of cell proliferation by Equiguard™ (correlation coefficient $r = 0.9459$) (Figure 20.3b) (Lu et al., 2003b). Equiguard™ also reduced AR, in correlation with altered PSA at the protein but not at the RNA levels suggesting that within a concentration range this herbal supplement controlled PSA by a mechanism only partially dependent on the expression of AR (Figure 20.3b) (Lu et al., 2003b). We also screened for changes in RNA expression of ARA70 and prostate acid phosphate (PAP) to determine whether they participate in the molecular action of Equiguard™. Expression of these genes was investigated in control and Equiguard™-treated LNCaP cells using RT-PCR and results in Figure 20.3b show that expression of AR and PSA show a copious and coordinated decrease with the low dose of Equiguard™. At the high dose of Equiguard™, PSA did not decrease whereas AR did, suggesting that a complex mechanism may be involved in the control of PSA by the AR. By day 3, even the expression of PAP, another CaP marker was significantly reduced. In contrast, ARA70 expression was not affected (Figure 20.3b).

Control of IL-6 Signaling in Equiguard™ Treated CaP Cells

IL-6 is a pleiotropic cytokine having profound effect on various cell types. IL-6 is thought to play a role in the control of AD → HRPC transition. Elevated serum IL-6 is significantly correlated with and partly predictive of shorter survival and higher morbidity rates in AI patients; IL-6 drives neuroendocrine cell differentiation through PI3K-ETK-STAT-mediated signaling; IL-6 also activates MAP kinase either through JAK-Ras-MAPK or via Erb-B2; IL-6 cross-talks with STAT-3 and AR to control prostate cell growth and AR ligand-independent PSA expression; IL-6 also enhances cell survival through control of anti-apoptotic proteins bcl-2/bcl-xL via the JAK-STAT-3 or PI3K-AKT pathways (Culig et al., 2005; Yuan et al., 2007; Wegiel et al., 2008).

Functions of IL-6 are mediated by two membrane receptor components, a ligand-binding receptor IL-6R and a protein gp130 that serves as signal-transducing molecule for IL-6, and a number of other cellular effectors. Binding of IL-6 to IL-6R facilitates consequent binding of cell-surface attached gp130 and its dimerization. Dimerized gp130 activates JAK family tyrosine kinase, by tyrosine phosphorylation in *trans* of the JAK kinases, associated with cytoplasmic tail of gp130 (George et al., 2005; Palmer et al., 2005). The activated JAK kinases, in turn, carry out tyrosine phosphorylation of up to five discrete "docking" sites in the cytoplasmic tail of gp130, which are considered crucial in the subsequent recruitment of STAT-3 (Figure 20.4a).

To test the hypothesis that Equiguard™ restricts AD → HRPC transition by disrupting IL-6:IL-6 receptor interaction by modulating the expression and/or function of either/both components, and the expression of the transducing molecule gp130 and/or its interaction with IL-6:IL-6R, we performed semi-quantitative RT-PCR to monitor mRNA expression of these genes in control and Equiguard™ treated cells. Results in Figure 20.4 show that IL-6R, gp130 and LIFR were substantially reduced following a three-day treatment with 3 μL/mL Equiguard™ in LNCaP (Figure 20.4b) and DU145 cells (Figure 20.4c) whereas no significant changes were observed in JCA-1 and PC-3

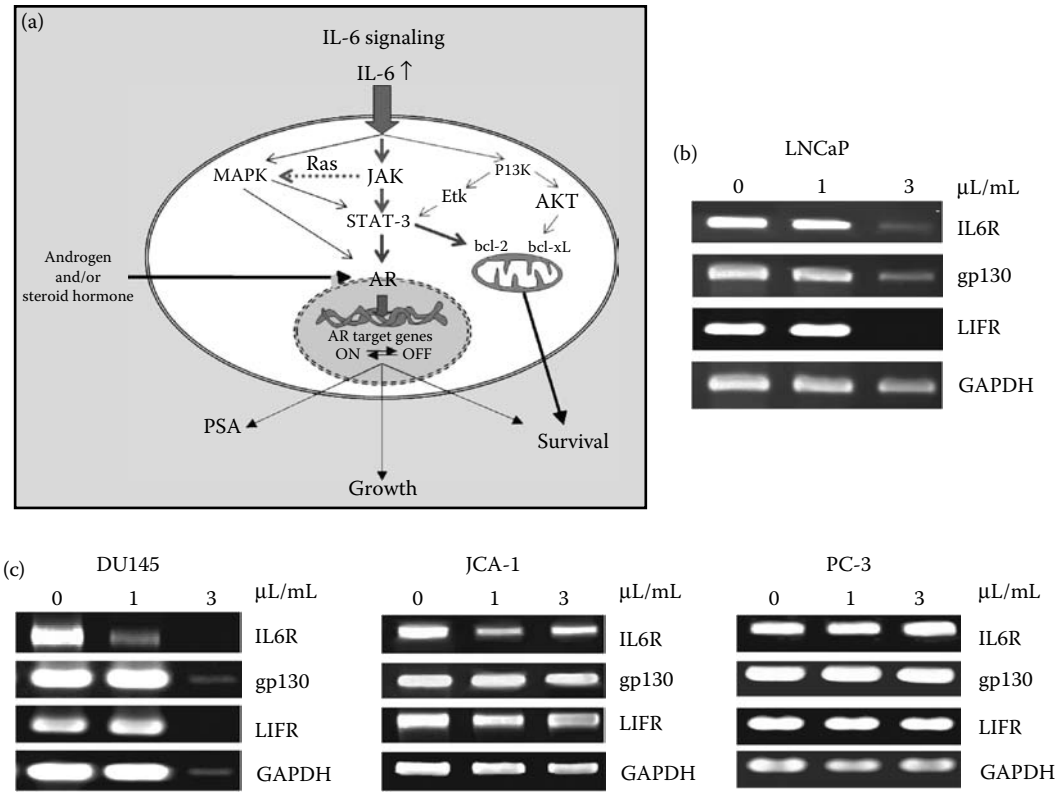
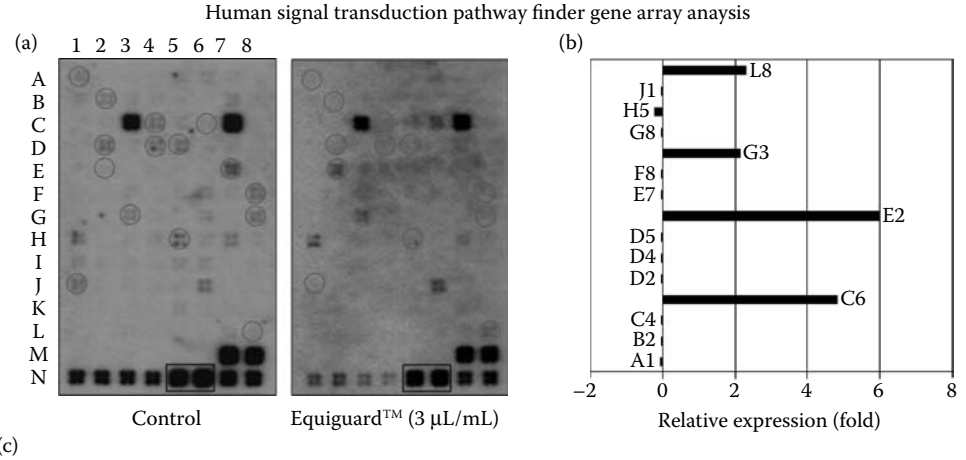


FIGURE 20.4 Control of IL-6 and IL-6-mediated signaling by ethanolic extracts of Equiguard™. (a) Scheme showing binding of IL-6 to IL-6R facilitating dimerization of gp130, its activation of the JAK/STAT signaling pathway, culminating in the increased expression of PSA, and increased cell survival and proliferation. IL-6 drives neuroendocrine cell differentiation through PI3K-ETK-STAT-mediated signaling; IL-6 also activates MAP kinase either through JAK-Ras-MAPK or via Erb-B2; IL-6 cross-talks with STAT-3 and AR to control prostate cell growth and AR ligand-independent PSA expression; IL-6 also enhances cell survival through control of antiapoptotic proteins bcl-2/bcl-xL via the JAK-STAT-3 or PI3K-AKT pathways. (b and c) Control of expression of IL-6 and IL-6 interacting proteins at the mRNA level by increasing doses of Equiguard™. Note that the herbal supplement had minimum effects on PC-3 and JCA-1 cells, in contrast to the pronounced effect it shows in LNCaP and DU145 cells.

cells (Figure 20.4c). These results suggest that Equiguard™ has the potential to control neuroendocrine cell differentiation, a distinct marker of HRPC.

Investigation of the Anti-CaP Activities of Equiguard™ Using Target-Specific Arrays

The target array approach is a panoramic analysis of gene expression based on binding of mRNAs prepared from control and treated cells to an array of known cDNA molecules immobilized on glass or nylon (Lu et al., 2003a,b). This method was used in our study to identify existing genes modulated by Equiguard™, as an approach that could be applied to the standardization of Equiguard™ extracts. To determine the validity and application of this method, samples from 48 hour control and Equiguard™ treated LNCaP cells were analyzed by using the "Human Cancer PathwayFinder GEArray Q series" obtained from SuperArrays (SuperArray, Bethesda, MD). Each gene presented in this array appears as four printed spots. In addition, RPL13A (as positive controls) and negative controls are also included to facilitate data normalization using the software provided by the manufacturer, and to compare results from different experiments. Figure 20.5 shows the effects of



(c)

Position	Gene name	Description	Fold difference
A1	Akt-1	v-akt murine thymoma viral oncogene homolog 1	0.05 ↓
B2	Survivin	Apoptosis inhibitior 4	0.03 ↓
C4	cdk4	Cyclin-dependent kinase 4	0.02 ↓
C6	**p27Kip1**	**Cyclin-dependent kinase inhibitor 1B**	**4.80 ↑**
D2	b catenin	Catenin (cadherin-associated protein), beta 1	0.02 ↓
D4	EGFR	Epidermal growth factor receptor	0.03 ↓
D5	erb-2	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2	0.03 ↓
E2	**c-fos**	**Human cellular oncogene c-fos**	**6.00 ↑**
E7	IGF-1	Insulin-like growth factor 1	0.02 ↓
E8	Integrin b1	Integrin, beta 1	0.02 ↓
G3	**c-jun**	**v-jun avian sarcoma virus 17 oncogene homolog**	**2.15 ↑**
G8	Mdm2	Mouse double minute 2	0.03 ↓
H5	MUC-18	Homo sapiens MICA gene, allele MUC-18	0.21 ↓
J1	DNA-PK	Protein kinase, DNA-activated, catalytic polypeptide	0.03 ↓
L8	**VEGF**	**Vascular endothelial growth factor**	**2.29 ↑**
N5, N6	RPL 13A	Ribosomal protein L 13a	Positive control

>2.0 and <0.5 are significant

FIGURE 20.5 Array analysis of gene expression in control and Equiguard™ treated LNCaP cells. (a) Total RNA was isolated from control and treated cells using Trizol reagent, after possible contaminating DNA was removed with DNase. Four micrograms of RNA was reverse transcribed to biotinylated cDNA, which was hybridized to immobilized gene-specific cDNAs using "Human Cancer PathwayFinder GEArray Q series" The signals were detected by chemiluminescence, as detailed by the manufacturer. The software provided by the manufacturer was used for the data analysis. Four positive controls (β-actin, GAPDH, cyclophilin A and ribosomal protein L13a) and negative controls (pUC18 DNA or blank) are included to facilitate data normalization and to compare results from different experiments. (b and c) Genes differentially expressed in Equiguard™-treated cells.

treatment with Equiguard™, compared with untreated LNCaP cells. It is evident that such treatment results in increases, decreases, and unchanged gene expression. Composite results of several arrays were combined and among genes increased are: p27, c-fos, c-jun and VEGF (Figure 20.5c). Since p27 is a checkpoint kinase inhibitor, these results support cell cycle arrest by Equiguard™ (Lu et al., 2004). Among suppressed genes are: Akt, survivin (API4), cdk4, beta-catenin, EGFR, erb-2, IGF-1, integrin-B1, mdm2, MUC-18, and DNA-PK (Figure 20.5c). Downregulation of some of these genes support the finding that Equiguard™ induces G_1/S checkpoint arrest by reducing cdk4 (Lu et al., 2004). Results of the array analyses support the proposition that the multitude of cell, biochemical, and gene responses elicited by Equiguard™ can serve as markers for its use as a reference standard for further characterizing the anti-CaP activity of extracts of Equiguard™.

Studies of Composition Herbs of Equiguard™ in AD and HRPC Cells

Since Equiguard™ is a herbal preparation comprising nine different herbs, we tested whether individual herbs of Equiguard™ may exert different anti-CaP properties by monitoring the activity of individual herbs in reducing cell growth and affecting PSA expression in LNCaP cells. These studies, summarized in Figure 20.6, showed that extracts derived from herbs #6 (*Ligustrum lucidum* Ait.) and #8 (*Psoralea corylifolia* L.) had more pronounced activity. Similar experiments were also performed with the other AI CaP cell lines including DU145 and PC-3 (Figure 20.6a). They all confirmed that herb #6 (*Ligustrum lucidum* Ait.) is particularly effective in reducing cell growth (Figure 20.6a) and in inducing apoptosis (data not shown). These results raise the distinct

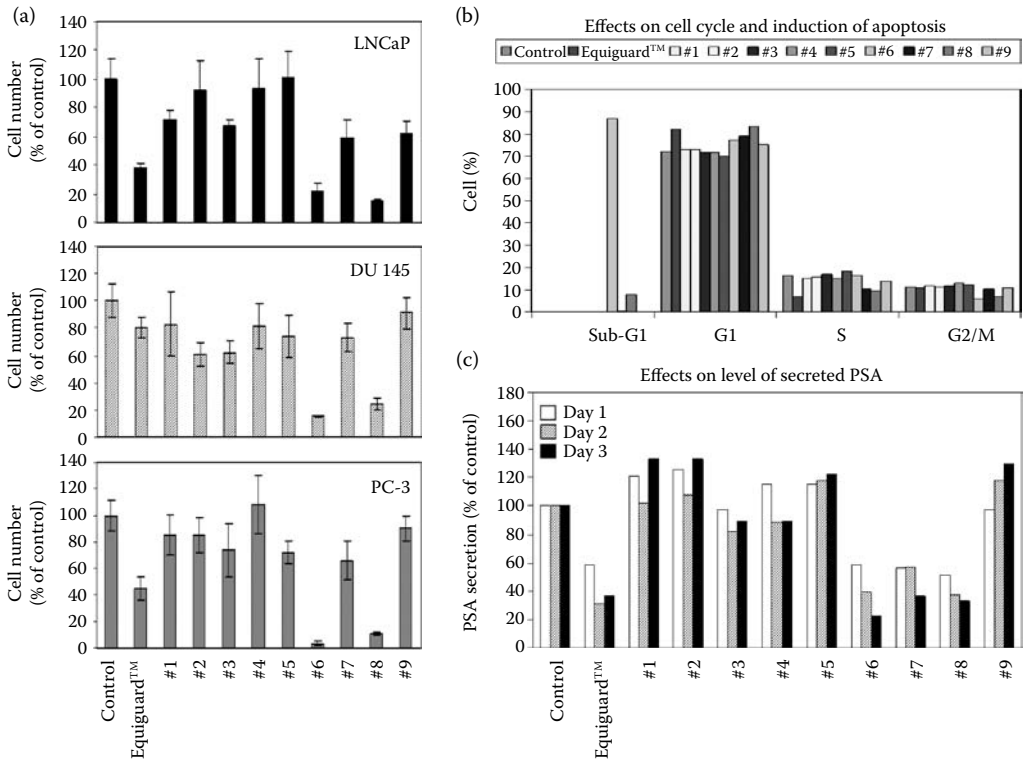


FIGURE 20.6 Effects of individual herbs of Equiguard™ on cell growth and PSA expression, showing that herb (*Ligustrum lucidum* Ait.) and #8 (*Psoralea corylifolia* L.) had more pronounced activity in suppressing AD and HRPC cell proliferation (a), and cell cycle phase transition (b), and PSA levels (c) in LNCaP cells.

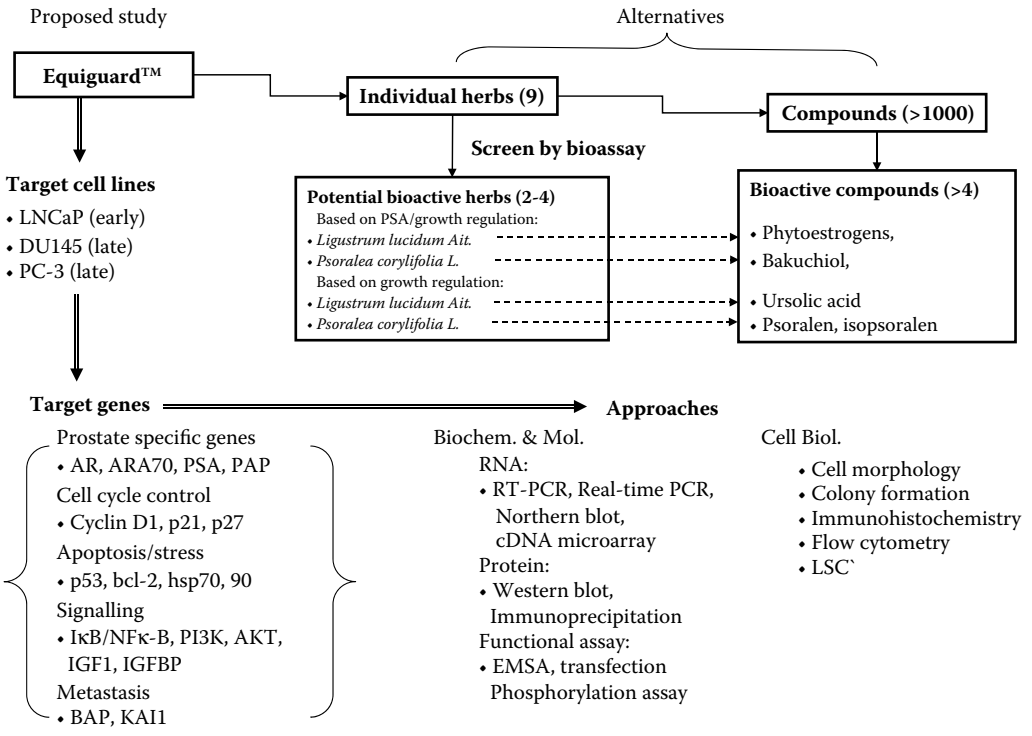


FIGURE 20.7 A scheme that illustrates a plan of attack in dissecting the anti-CaP activities of herbal supplements from the stage of formulation, to individual herbs, to bioactive components contained in individual herbs.

possibility that further fractionation and characterization of these herbs following the scheme in Figure 20.7 might reveal novel anti-CaP activities with pronounced inhibitory effects on both AD/HRPC cells.

Mechanistic Framework of Antiprostatic Carcinogenic Effects of Equiguard™

A popular concept of the 1970s and early 1980s that cancer could be eradicated using a tumor-specific "magic bullet" has been supplanted by the recognition that cancer requires 20–40 years for clinical presentation and hence is a chronic disease amenable to prevention. The consumption of botanical extracts for preventing and treating human diseases is entrenched in more than 80% of all human cultures, and is indisputably supported by decades of epidemiological research, mostly in developed countries, which report lowered risk of a variety of cancers in populations consuming diets high in fruits and vegetables. These observations have provided the impetus to identify and characterize a complex mixture of botanical extracts and active components they contain with disease prevention properties. We suggest that Equiguard™ may be considered a prototype of such a botanical mixture, and that it exerts potent anti-CaP against both AD and HRPC cell lines.

Although the mechanism of action of Equiguard™ remains to be fully understood, its ability to regulate cell proliferation and PSA/AR gene expression and control of other genes in AD and HRPC may be readily comprehended based on the scheme shown in Figure 20.2. In line with TCM principles, efficacy of Equiguard™ relies upon combining multiple herbs to both enhance primary pharmaco-activity as well as to mitigate the toxicity of components present in the mixture. The success of Equiguard™ in treating both AD and AI CaP cells may be due to unique combinatorial sets

of active ingredients intrinsically present in Equiguard™, capable of efficiently targeting multiple pathways, which functionally overlap to provide growth stimulatory advantage to prostate cells at different stages of carcinogenesis.

In summary, the *in vitro* efficacy of Equiguard™ may be attributed to the fact that its formulation follows TCM principles encompassing features, such as: (i) minimum toxicity as cytotoxic agents are offset by counteracting and balancing ingredients; (ii) broadbased mechanistic platform with overlapping and distinct antitumor activities that can be directed to the primary as well as metastatic malignant sites; and (iii) circumvention of drug resistance development. These features presumably underlie its ability to negate and modulate the multistep, multipath, multifocal and highly heterogeneous nature of CaP. It is worth noting that these very same features were also actively exploited in the early days of oncology, as seen in the combined use of chemotherapeutic agents (combination polychemotherapy), and more recently in combining antibodies to simultaneously target multireceptors to enhance antitumor activity.

CONCLUSION

Natural botanicals have been the primary feeder of modern day medicine. Healthcare professionals who utilize botanicals for treating diseases cannot afford to indulge in a clinically ineffective ideology; they use botanicals because they work. Carefully selected, specifically indicated plant medicines, when prescribed for patients by trained practitioners, can be highly effective and safe. Crude plant and whole plant extracts work differently in the body than single constituent pharmaceuticals.

In conclusion, there is no need for the question mark on the *in vitro* efficacy of herbal extracts for CaP. The reemergence of plant medicines has an integral role in U.S. healthcare. Herbal medicines, although under recognized and underutilized, offer vital answers to the quest to reduce the pain and suffering engendered by disease and to promote optimal health. The hope offered by botanical medicines is worthy of further attention through significant and substantial research.

SUMMARY POINTS

Strategies for Treatment of CaP

Blockade of androgen production by inhibiting the CNS-adrenal-testicular axis and steroid synthesis
Suppression of cell proliferation and induction of cell cycle checkpoint arrest
Induction/activation of apoptosis
Suppression of androgen receptor expression and function
Disruption of the interplay between stroma and epithelium in the prostate microenvironment

Positive and Negative Attributes of Herbals
Positive Attributes

- Multiple components with distinct and overlapping antitumor activities
- Better potential for overcoming heterogeneity within tumors
- Obviate toxic side effects of current therapies
- Minimize development of drug resistance

These features are important in combating cancer, now recognized to be a constellation of diseases with multiple genetic, cellular, and biochemical aberrations, capable of elaborating

functionally overlapping molecular pathways that effectively counteract the activity of single agents widely used to treat malignancies.

Negative Attributes

- Lack of quality control and standards
- Insufficient information on effectiveness, mechanism of action, interaction between components in herbal mixtures
- Risk:benefit ratio not well defined
- Possible interference and interaction with prescribed medicine

Mechanism of Anti-CaP Activities of Herbal Supplements

Functional synergy among bioactive components in herbs may result in a broadened anti-CaP and anti-HRPC preventive and therapeutic index marked by increased distinct anticancer activities and reduced untoward effects.

ABBREVIATIONS

AD	androgen-dependent prostate cancer
AI	androgen-independent prostate cancer
AP-1	activator protein 1
AR	androgen receptor
ARE	androgen-response element
CAG	codon for amino acid glutamine
CAM	complementary and alternative medicine
CaP	prostate cancer
CDKI	cyclin-dependent protein kinase inhibitors
cdks	cyclin-dependent protein kinases
CNS	central nervous system
CREB	cAMP response element binding protein
DES	diethylstilbestrol
DHT	dihydrotestosterone
ECM	extracellular matrix
G_1/S and G_2/M	phases of the cell cycle subject to restrictive control by cyclin-dependent protein kinases
gp130	a signal-tranduction component of IL-6R
HRPC	hormone-refractory prostate cancer
IL-6	cytokine interleukin-6
IL-6R	interleukin-6 receptor
JAK	Janus kinases
LH-RH	lutenizing hormone-releasing hormone
LIFR	leukemia inhibitory factor
PAP	prostate acid phosphatase
PARP	poly(ADP-ribose) polymerase
PIN	prostate intraepithelial neoplasia
PSA	prostate specific antigen
Rb	retinoblastoma
siRNA	small inhibitory RNA
STAT	signal transducer and activator of transcription
TCM	traditional Chinese medicine

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We dedicate this chapter to the fond memories of Professor Wen-hsien Chou of Suzhou University, China. Professor Chou was a champion for advocating support of basic research in TCM, particularly in regard to the use of herbal preparations in general health maintenance and chronic disease prevention. He was a visionary voice of inspiration and unique insight in this worthwhile cause, cheering us on with his towering enthusiasm, generous personal attention and guidance, and unshakable commitment in the sharing of ideas and resources. We shall miss him.

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21 Plant-Derived Antioxidants and Use in Prevention and Treatment of Prostate Cancer

Carlie Margaret Abersold and Simin Bolourchi-Vaghefi

CONTENTS

INTRODUCTION

Prostate cancer is the most common cancer diagnosed in American men after skin cancer. Approximately one in six men will have prostate cancer during his lifetime. The American Cancer Society estimates 217,730 new cases of prostate cancer diagnosed and 32,050 deaths may occur in the United States in 2010 due to this disease (http:\\www.cancer.org).

Multiple risk factors associated with the development of prostate cancer include advancing age (the strongest risk factor), African American ethnicity, and family history of prostate cancer. Additional risk factors linked with the pathogenesis of cancer are lifestyle and behavior factors, including tobacco use, diet, physical activity, and obesity (American Cancer Society, http://www.cancer.org). Dietary habits may contribute as much as 35% to one's risk factor of cancer, according to researchers, while an estimated 5% may be due to genetics (Doll and Peto, 1981; Ray, 2005; Stoeckli and Keller, 2004; World Cancer Research Fund, 1997). Epidemiological studies indicate an inverse relationship with high intakes of vegetables, fruits, and other plant-based micronutrients and cancer risks. In contrast, high dietary fats, specifically fats in red animal meats and animal products, are associated with an increased incidence of several leading cancers including breast, colon, prostate, ovarian, endometrial and pancreas (Ray, 2005; Thomas et al., 2007; World Cancer Research Fund, 1997).

Studies show the incidence and mortality of prostate cancer differs greatly by geographic regions supporting the association with dietary consumption of the population. In industrialized nations

with diets containing as much as 30–40% of calories from fat (much of it animal fat), the incidence of prostate cancer is high. In Asia, where diets are usually low in animal fats and high in soy protein intake, prostate cancer incidence is low. Epidemiological observations indicate that diet may be the significant environmental and lifestyle factor leading to an increased prostate cancer risk in American men (Basu and Imrhan, 2005). Considering the costs for prostate cancer screening, diagnosis, and therapy as well as the negative effects of treatments and interventions, dietary approaches that slow or delay progression of this disease may significantly enhance the quality of life for men who are often diagnosed over the age of 50. Nutritional intervention may provide a safe and natural strategy for prevention and management of prostate cancer as an alternate or adjunct to medical treatments and medications (Clinton, 2005).

CAUSES AND MECHANISM OF ACTION

A review of studies on prostate cancer concludes, "At this point, prostate cancer epidemiology, genetics, pathology, and molecular biology are converging on the hypothesis that prostate cancer develops as a consequence of genome damage, inflicted by reactive oxygen and nitrogen species elaborated by inflammatory cells and by ingested carcinogens likely present in red meats, in the setting of inadequate cellular defenses against reactive chemical species" (Nelson, 2004, p. 3212S).

Plants and herbs have been used for prevention and treatment of cancer as well as other chronic diseases throughout history. Plants contain many naturally occurring substances that possess antioxidant and anti-inflammatory properties, which act as chemopreventive agents for cancers of the breast, colon, and prostate. Studies identify many specific dietary micronutrients including carotenoids (lycopene), retinoids, and vitamin A, vitamin E, vitamin C, selenium, and phenols for their role as antioxidants and their ability to inhibit the development and progression of prostate cancer (Basu and Imrhan, 2005). In addition to the antioxidant properties, carotenoids and vitamins A, E, C, and selenium are important cofactors for specific enzymes necessary for optimal function and health within the body and at the cellular level.

Consumption of chemical carcinogens formed on foods containing creatinine such as fish and meats during broiling, grilling or frying, has been linked to prostate, colon, breast, and pancreas cancer. The formation and action of these heterocyclic amines that are carcinogens interfere with normal metabolism of cells and organs in the body by releasing reactive oxygen species (ROS) are believed to be inhibited by antioxidants.

Epidemiological research indicates a lower incidence of many chronic diseases in populations who regularly consume primarily a plant-based diet with plenty of vegetables, fruits, red wine, and tea. Consumption of vegetables, fruits, nuts, seeds, red wine, and tea—rich sources of antioxidants and micronutrients including vitamin C, E and beta-carotene—is believed to reduce oxidation reactions in the body and therefore delay disease progression (Weosbirger, 2000). Diets deficient in trace elements, antioxidants, phytochemicals, and vitamins may lead to an increase risk or progression of prostate cancer (Thomas et al., 2007).

Over the last decade, studies have indicated and continue to report how dietary components work at the molecular and cellular level causing or inhibiting the growth of tumor cells leading to apoptosis, depending on their effect, by targeting one or more signaling intermediates. The developing data supports dietary interventions for use in the prevention and management of cancer (Khan et al., 2007).

REVIEW

In this chapter we try to provide information based on studies illustrating the role of some of the micronutrients as well as nonnutritive components of vegetables and fruits and other botanicals in prevention of carcinogenesis and possible role in prevention of tumor formation.

Lycopene

Supporting data from epidemiological, *in vitro*, animal, and small clinical studies over the past 10 years acknowledge that consumption of tomatoes and tomato products are associated with a reduced risk of prostate cancer. The developing hypothesis is that lycopene, the most abundant carotenoid in tomatoes and the red pigment of tomatoes, watermelon, papaya, pink grapefruit and guava, may be the phytochemical in red fruits and vegetables known to be a potent antioxidant in the prevention of prostate cancer. The Health Professionals Follow-up Study (HPFS), a cohort study of more than 47,000 men, indicated an intake of two to four servings per week of raw tomatoes was associated with a 26% reduced risk of prostate cancer compared with no servings per week (Campbell et al., 2004). Consumption of more than 10 servings per week of all combined dietary tomato sources was associated with a 35% reduced prostate cancer risk compared with less than 1.5 servings per week.

Another HPFS cohort follow-up confirmed findings of the earlier study with a 23% decreased prostate cancer risk when two or more servings were compared with less than one serving per week (Campbell et al., 2004). A nested case–control study within this cohort observed a significant inverse association between high plasma lycopene concentrations and a lower prostate cancer risk evident in men >65 years of age and participants with no prior family history of prostate cancer. The findings support the hypothesis that lycopene intake may offer better protection in sporadic prostate cancer cases rather than cases associated with genetics.

The diet of healthy subjects supplemented with tomato products for 15 days exhibited a significant increase in *ex vivo* lipoprotein oxidation lag period, and additional studies indicated that tomato and tomato juice consumption was followed by decreased lymphocyte DNA damage. Protection from *in vivo* oxidative damage shown by tomato consumption may potentially prevent mutations associated with cancer initiation and progression (Campbell et al., 2004).

In a small clinical trial, 32 prostate cancer patients consumed tomato-sauce-based pasta dishes containing lycopene every day for three weeks prior to scheduled radical prostatectomy. The group consuming tomato products exhibited a decrease in serum PSA levels. Leukocyte oxidative DNA damage was significantly reduced following tomato consumption than before the dietary intervention. In addition, compared with men randomly selected, the men who were given tomato products showed less oxidative DNA damage of prostate tissue after the intervention (Canene-Adam et al., 2005).

In separate trials, there was no association of prostate cancer risk with dietary or supplemental intake of beta-carotene, vitamin E or C, although supplemental beta-carotene was effective in reducing prostate cancer risk when given to men with decreased dietary beta-carotene intake. The authors concluded that the results did not indicate recommendation of high-dose antioxidant supplementation for the general population for prevention of prostate cancer. They do recommend a beta-carotene supplement to reduce the risk of prostate cancer in men with low beta-carotene intakes (Kirsh et al., 2006).

A pilot study investigated the effects of lycopene supplementation in elderly men diagnosed with benign prostate hyperplasia (BPH) (Schwarz et al., 2008). A nonmalignant enlargement of the prostate gland, BPH is caused by excessive growth of prostatic nodules. On microscopic examination of the prostate, BPH is found in 70% of the men by the age of 60 and 90% of the men by the age of 70, making it the most common benign neoplasm of aging men. It is also a risk factor for developing prostate cancer later in life. The study concluded that lycopene inhibited disease progression and may improve symptoms in patients with BPH. Other benefits mentioned are that lycopene supplements are safe and well tolerated, and lycopene does not interfere with PSA levels (Schwarz et al., 2008).

A meta-analysis of 11 case–control and 10 cohort or nested case–control studies reporting on the effect of vegetables and fruits on the risk of prostate cancer was conducted. The investigators suggested that components of vegetables and fruit may play a role in reducing the risk of prostate cancer. These studies suggest that phytochemicals and nutrients in foods that appear to promote health benefits warrant further research to understand this relationship. Additional studies indicate

that consumption of tomato products increases blood and prostate lycopene while favorably influencing markers of oxidative stress, prostate-specific antigen, or tissue biomarkers. Ongoing research indicates various factors can influence the absorption and distribution of lycopene to human tissue including age, food source, processing, cooking, and mastication, additional dietary components in the meal, hormonal status, and possibly medications and supplements (Clinton, 2005).

Lycopene has been shown to be one of the most potent *in vitro* antioxidants of the carotenoids in a variety of epidemiological trials and researchers have determined the antioxidant properties are responsible for the positive health benefits and disease prevention. Erdman et al. (2009) suggest that little evidence exists to support the antioxidant theory for lycopene mechanism of action in preventing prostate cancer because he believes that the level of lycopene in the tissue is too low. That research indicates that the lycopenoids or other metabolic products of lycopene are factors that cause some of the reported bioactivity of lycopene. In preliminary studies, tomato polyphenols, specifically quercetin, kaempferol, and naringenin, have decreased cancer cell growth *in vitro*. Combinations of these polyphenols present in whole foods may have additive effects in decreasing cancer proliferation. In addition to the bioactivity of lycopene, plant products containing lycopene also contain many important nutrients, including folate, vitamin C, potassium, some vitamin A and E, and several other carotenoids and polyphenols, having benefits beyond any single component which may also be associated with lower cancer risk (Campbell et al., 2004).

Pomegranate Fruit and Extract

Pomegranate fruit extract (PFE) was shown in a recent study to reduce cell growth and initiate apoptosis of extremely aggressive human prostate carcinoma cells (Malik and Mukhtar, 2006). The outcome of this study suggests that pomegranate consumption may slow prostate cancer progression. Pomegranate is a relatively new fruit introduced to the population of the Western world. As such research studies using this fruit or its juices in health promotion and disease prevention are limited. As time progresses and with more research effort investigating properties of pomegranate it may prove to be a natural healthful addition to other antioxidant, phytochemical-loaded foods available. This natural approach may offer additional options for prostate cancer patients with a desire to improve treatment outcomes and extend life (Malik and Mukhtar, 2006).

Green Tea and Black Tea

Tea, both green and black, has been in use for more than 5000 years in East Asia as both refreshment and for the medicinal properties. Tea has an estimated consumption averaging 120 mL per day per capita and is cultivated in more than 30 countries, which makes it the second most consumed drink after water worldwide. After tea was introduced to Japan in the early thirteenth century, Eisai, a Buddhist monk considered to be the "Father of Tea" in Japan, declared, "Tea is a miraculous medicine for the maintenance of health. Tea has an extraordinary power to prolong life."

Of the more than 2000 components in tea, the flavanoids are considered to be the most abundant and bioactive. These low molecular weight antioxidants are found in the leaves, flowers, and other parts of the plants. Epidemiological studies have shown that the antioxidant and anti-inflammatory actions of tea flavanoids are partly responsible for the chemopreventive activity of tea against chronic diseases, specifically their anti-carcinogenesis activity (Wheeler and Wheeler, 2004). Studies have also shown that epigallocatechin gallate (EGCG) which is the polyphenolic antioxidant present in tea, prevents cancer initiation in the cells as well as progression of initiated cells. The hypothesis that EGCG, being a powerful antioxidant, can trap peroxyl radicals preventing and protecting cell membrane from oxidative stress, has been put forward (Saffari and Sadrzadeh, 2004).

Study of green tea (GT) and black tea (BT) offer promise in preventing prostate cancer. Research has shown that the components of GT and BT to be bioavailable and bioactive in the prostate where they may be active in the prevention of prostate cancer. A study was under taken with a total of 20

men scheduled for surgical prostatectomy (Henning et al., 2006). These men were randomly assigned to three groups and, respectively, had to consume GT, BT, or a caffeine-matched soda control (SC) daily for five days prior to radical prostatectomy. Tea polyphenols were found to be higher in prostate tissue of men consuming GT and BT than in men consuming SC. Proliferation of prostate cancer cells was inhibited when cells were grown in media consisting of serum from the patient obtained after consumption of GT and BT relative to baseline serum. The study found that GT and BT polyphenols and theaflavins are bioavailable in the prostate and may function in the prevention of prostate cancer. GT and BT are derived from the leaves of the Camellia sinensis plant which is available worldwide. The authors believe that chemoprevention using natural, nontoxic plant components can prevent the development of cancer or its progression. These natural nontoxic nonnutrients from plant sources can be used as an effective approach in reducing the incidence of prostate cancer and related mortality. Data indicates that greater than 25% of prostate cancer patients use alternative or nonprescription therapies including tea and its extracts (Henning et al., 2006). Experimental and epidemiological studies reviewed have indicated catechins and other polyphenol components of tea are bioactive compounds that can protect against prostate cancer in humans (Siddiqui et al., 2006).

Soy

Epidemiologic and case–control studies indicate consumption of soybeans and soy-containing foods are associated with reduced prostate cancer risk. The soy isoflavonoids, plant-based compounds known as phytoestrogens, are believed to be the active nutritional components of soy that lower the risk of cancer. A 16-year-long prospective health study of 13,855 Seventh Day Adventist men produced strong evidence for the benefits of soy. Men that consumed more than one glass of soy milk daily had a 70% lower risk of prostate cancer compared with participants that did not consume soy milk (Hedlund et al., 2005).

A study was designed to observe the effect of dietary soy intake and diadzein metabolism, and the potential prevention of prostate cancer. Diadzein can be converted by the intestinal flora to form metabolites with different bioactivities. Healthy 19–65 years old Caucasian men were followed with a secondary goal to compare plasma and prostate fluid concentrations of five isoflavonoids: genistein, diadzein, equol, dihydrodiadzein, and *O*-desmethylangolensin. The study concluded that long-term soy consumers of at least two years show significant changes in intestinal metabolism of the soy isoflavone diadzein. Also, it appears likely that the high concentrations of isoflavonoids in prostate fluid may inhibit cellular proliferation or act as antioxidants to reduce the toxic byproducts of oxidation that build up over time (Hedlund et al., 2005).

A thorough review of clinical studies on the soy isoflavone genistein suggests that genistein may be effective against prostate cancer cells *in vitro* targeting the regulation of cell cycle to initiation of apoptosis. The idea that genistein might be appropriate in human cancer therapy has been supported by well-designed animal experiments. However, it is difficult to make conclusions regarding the clinical efficacy of genistein because of the variations of study protocols: few participants, less than six months duration, and not using a standardized drug formulation. The presented data potentially allow recommending patients to use soy products containing genistein in a preventive setting. While at present there is not sufficient evidence that genistein is effective in prostate cancer treatment, the authors express that the data indicate that it is appropriate to recommend genistein to patients as a preventive approach (Perabo et al., 2008).

Reports have suggested soy isoflavones, specifically genistein, can initiate genetic damage in cultured cells of humans. In contrast, subjects treated with a purified soy unconjugated isoflavone mixture including genistein did not show chromosomal damage caused by genistein even at high concentrations. With the objective of assessing the genetic toxicity of a purified soy unconjugated isoflavone mixture, Miltyk et al. (2003) treated 20 patients with prostate cancer for 28 days with 200 mg of genistein daily and then for another 56 days with 600 mg daily. The authors conclude

that even though genetic damage *in vitro* can be induced by isoflavones, the subjects treated with a purified soy unconjugated isoflavone compound did not experience this outcome. Dietary soy phytochemical concentrate and soy protein isolate were evaluated to determine their role on prostate cancer tumorigenesis in mice. Evaluation of tumor tissue after consumption of soy products revealed considerable reduction in tumor cell proliferation, greater apoptosis and microvessel density reduction. Mice fed soy protein and phytochemical concentrate had a reduction of the angiogenic protein insulin-like growth factor-I in the circulation. Dietary soy products may therefore reduce growth of experimental prostate tumors by directly targeting tumor cells and indirectly increasing tumor vasculature (Zhou et al., 1999).

Grape Seeds

Grapes (*Vitis vinifera*) are considered to be one of the most consumed fruits worldwide. Grape consumption is associated with a reduced risk of cancer. Grapes and grape seed extract have increasingly gained interest and have been studied for many years as a dietary approach to prevent, slow, or reverse prostate cancer. Agarwal et al. (2000) conducted studies using a polyphenolic fraction derived from grape seeds (GSP) rich in procyanidins. The authors conclude that GSP is effective against tumor growth in a murine skin model and may have an anticarcinogenic effect against prostate cancer by possible involvement of several cell processes.

In another study, Agarwal et al. (2002), using human prostate carcinoma cell line DU145, cultured cells in RPMI 1640 with 10% bovine serum under standard culture conditions. The cultured cells were treated with a polyphenolic extract derived from grape seeds extract (GSE). The results showed that GSE causes a dose-dependent and time-dependent apoptosis in DU145 cells. On the basis of the results of their studies the authors concluded that GSE is effective against tumor growth in the same skin model and may show to have an anticarcinogenic effect against prostate cancer by multiple cell processes involving signaling, regulation, inhibition, and death. In this study, GSE used contained catechin and epicatechin along with procyanidins and other polyphenolic compounds. These authors suggest that additional *in vivo* preclinical studies are needed to further assess the effectiveness of GSE against advanced prostate cancer before recommending use in human prostate cancer patients.

Dietary resveratrol is a main component in various plant products. Resveratrol is found in high concentrations in grape skins. Resveratrol is recognized as a superior cancer chemopreventive after safe and effective testing of carcinogenesis in animal studies. Stewart et al. (2003) evaluated the appropriateness of resveratrol use for prostate cancer prevention. The authors hypothesized that resveratrol may be especially suitable to prevent prostate cancer due to its ability to inhibit the multiple steps of carcinogenesis, reduce androgen receptor activity, and suppress androgen receptor expression. They further suggest that the use of resveratrol may have potential preventive value in addition to an adjuvant treatment for advanced prostate cancer which is hormone-dependent.

Grape seed extract (GSE) is marketed for its health-related benefits and is one of the most commonly consumed dietary supplements in the United States. Epidemiological studies indicate dietary supplements such as herbal extracts as well as GSE are used by one in five people and are used by as many prostate cancer patients as an adjunct to their current medications. In advanced prostate cancer cells, resistance is developed against apoptosis reducing the effectiveness of therapeutic treatments. New strategies must be developed to stimulate apoptosis to reduce proliferation while also controlling invasiveness of the disease. This has encouraged evaluation of the anticancer effects of many plant-derived compounds for their possible use in the prevention and therapy of specific cancers including prostate cancer.

Vitamins, Minerals, and Other Supplements

Vitamin E and beta-carotene supplementation in men with low dietary beta-carotene intakes was associated with reduced risk of prostate cancer. However, the results do not indicate recommendation

of high-dose antioxidant supplementation for the general population for the prevention of prostate cancer (Fisher, 2000). No overall association between dietary or supplemental vitamin E intake and prostate cancer risk was observed in the prospective analysis from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial or HOPE-TOO trial and several other observational studies. However, a subgroup of men who were current smokers or who had quit within the past 10 years, given supplemental vitamin E in excess of 400 IU per day, had a statistically significant 71% reduction in risk of advanced prostate cancer compared with those who did not take supplemental vitamin E (Lee et al., 2006).

In 1994, the Alpha-Tocopherol, Beta Carotene (ATBC) Cancer Prevention Trial reported 35% fewer cases of prostate cancer in men taking supplemental vitamin E. Two years later the Nutrition Prevention of Cancer trial reported 65% fewer cases of prostate cancer in men taking selenium supplements. These trials appeared to indicate that men at risk of prostate cancer could prevent the disease by consuming supplemental antioxidants. Twelve years later with the expectation of repeating the previous results, two major trials found supplementation of selenium or vitamin E alone or in combination did not reduce incidences of prostate or other cancers. Epidemiology can have one of three possible explanations for every statistical association: bias, chance, and cause. The earlier results shown on prostate cancer prevention trials appear likely to be due to *chance* (Gann, 2009).

Dietary Fiber

Dietary fiber is defined as food that comes mainly from the plant cell wall that is not easily digested by the human digestive tract. At one time fiber was considered inedible and food processing began to eliminate it. Dietary fiber originates in fruits, vegetables, and whole grains, and including them in the diet provides important vitamins and minerals which are eliminated in refined or processed plant products. Epidemiological studies and animal experiments as well as human studies have shown that dietary fiber is an essential part of a healthy diet, reducing the risk of almost all of the chronic diseases including gastrointestinal (GI) problems, obesity (the root of many cancers), type 2 diabetes, and cardiovascular diseases.

Studies have shown that including dietary fiber in the diet assists in the prevention of obesity due to decreasing hunger by delaying the absorption of nutrients and keeping blood glucose levels stable until the next meal. Fiber increases the transit time of digested food in the GI tract facilitating elimination of waste products of digestion thus preventing colon and rectal problems including cancers. Diabetes is prevented by gradual absorption of glucose and its gradual release in the blood stream. Fiber also has protective effects against cancers of the breast, colon, and prostate (Ray, 2005). Additionally, dietary fiber protects against cardiovascular disease by assisting in lowering cholesterol. Consuming a plant-based diet high in fruits, vegetables, and whole grains ensures adequate dietary fiber to promote health and prevent chronic diseases.

CONCLUSION

Chronic diseases related to diet are considered to be the major individual cause of morbidity and mortality in Western countries including the United States. Rarely observed in early civilizations, these chronic diseases strongly associated with diet now affect from 50% to 65% of adults in modern Westernized societies (Cordain et al., 2005). Cancer is a severe threat to public health as the second leading cause of death in the United States accounting for 25% of all deaths. Approximately one-third of all cancer deaths are a result of nutritional factors, including obesity, according to the American Cancer Society (American Cancer Society, 2004). It has been shown that a plant-based diet with a high intake of vegetables and fruits providing adequate antioxidants and phytochemicals can prevent chronic diseases. Studies reviewed above strongly suggest many advantages of consuming a plant-based high-fiber diet consisting of a variety of fruits and vegetables.

Dietary research since 1982 has concluded that necessary nutrients should be obtained from food. Nutrients within food work synergistically with other components in food. Isolating one nutrient and assigning it a dose considered optimal for everyone may cause physiological imbalances and may be toxic. Although supplements have been indicated to have some positive effects on cancer prevention and risks, possible harm has been shown in numerous studies using supplements. For example, high levels of multivitamin use were associated with a higher risk of advanced and fatal prostate cancer in men with a history of prostate cancer according to a 2007 study (Brower, 2008).

Including a variety of fruits and vegetables in the daily diet offers protection against cancer and other chronic diseases by reducing the intake of foods associated with increased cancer risks. Fruits and vegetables have numerous vitamins and minerals, and hundreds of phytochemicals as well as other unidentified or unknown components that may be responsible for their role in cancer prevention.

Reducing medical costs, extending life as well as the quality of life, avoiding disease and hospital stay are some of the benefits of good health. A healthy lifestyle includes daily consumption of fruits, vegetables, and plant-based foods, in addition to regular exercise to maintain a healthy body weight. A Greek adage says "it is the function of medicine to help people die young as late as possible" (Weisburger, 2000, p. 948).

SUMMARY

Consumption of a plant-based diet has been shown by scientific research to reduce the development and progression of many types of cancer, specifically prostate cancer. Many lifestyle and risk factors including age and race cannot be changed. Diet, however, is one major risk factor that can be changed or improved to extend and enhance the quality of life.

Recommendations to individuals wanting to increase their ability to prevent the onset of prostate cancer, strengthen the immune system to fight against the progression, or prevent reoccurrence of the disease and have better outcomes before, during, and after treatment are as follows:

1. Obtain regular physicals and preventive healthcare as recommended by your healthcare provider.
2. Consume a plant-based diet with a variety of fruits and vegetables to ensure adequate dietary intake of vitamins, minerals, micronutrients and fiber while also preventing nutritional deficiencies.
3. Attain a healthy body weight by avoiding excess fat, especially *trans* fat and saturated animal fat, salt, sugar, and processed foods.
4. Drink plenty of low-calorie, low sugar or zero calorie, no-sugar beverages daily including water to ensure proper hydration.
5. Participate in regular activities and exercise to maintain a healthy body weight.

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22 Cruciferous Vegetables and Their Components in the Prevention of Breast Cancer

Susan E. Steck

CONTENTS

INTRODUCTION

Breast cancer is the leading incident cancer and the second leading cause of cancer death among American women (Jemal et al., 2009). An American woman has a one in eight chance of being diagnosed with breast cancer over her lifetime (Mahoney et al., 2008). Established risk factors for breast cancer include age, family history, early age at menarche, late age at menopause, nulliparity, late age at first live birth, no breastfeeding history, increased breast density, and BRCA1 and BRCA2 mutations (Mahoney et al., 2008). Many of these are related to lifetime estrogen exposure which is believed to be causally related to breast carcinogenesis. However, most of these risk factors are not amenable to interventions as a means to prevent breast cancer occurrence.

Some lifestyle factors, such as sedentary behavior, alcohol intake, and use of exogenous hormones, are also positively associated with breast cancer risk (Mahoney et al., 2008). Body mass index (BMI) is associated with increased risk of breast cancer in postmenopausal women, but decreased risk in premenopausal women. Other lifestyle factors, such as dietary intake of macronutrients, micronutrients, and phytochemicals have been studied, but results from human studies are inconclusive. Migrant studies, in particular, point to the role of the environment, including food intake, on impacting cancer risk. For example, studies of women who have migrated from Asia, where breast cancer incidence rates are low, to the United States, which has one of the highest breast cancer incidence rates in the world, show that Asian women who migrate to the United States tend to develop the breast cancer rates of their host country within one to two generations (Stanford et al., 1995; Lee et al., 2007). Because genetic factors cannot account for the rapid shift

in cancer rates among immigrants, environmental factors, such as diet, are thought to play an important role in breast cancer etiology.

One important difference in the diet of Asian populations compared with American populations is the higher amount of cruciferous vegetables consumed. Cruciferous vegetables, also called *brassica* vegetables, are of the plant family Brassicaceae (or alternatively, Cruciferea), which includes broccoli, bok choy, cabbage, cauliflower, watercress, Brussels sprouts, kale, collard greens and mustard, among others. Eastern populations are known for having higher intakes of cruciferous vegetables, in particular cabbages (among other dietary differences), than Western populations, and their rates of breast cancer are much lower. In this chapter, the evidence for a role of cruciferous vegetables in breast cancer etiology is reviewed, with attention to the biologic mechanisms and a review of the epidemiologic studies that have examined the association between cruciferous vegetable intake, interactions between cruciferous vegetables and genetic polymorphisms, and breast cancer risk. The focus is on results from human studies of cruciferous vegetable intake, although some animal and cell culture studies are discussed.

ANTICANCER CONSTITUENTS OF CRUCIFEROUS VEGETABLES

Cruciferous vegetables contain a variety of phytochemicals and nutrients that may have anticancer activity, such as fiber, folate, carotenoids, vitamin C, and chlorophyll. In addition, cruciferous vegetables contain phytochemicals called glucosinolates which are unique to this family of vegetables. Upon chopping or chewing of the vegetables, glucosinolates are broken down into other phytochemicals called isothiocyanates and indoles by an enzyme called myrosinase which is present in a separate cellular compartment of the plant. There is evidence from experimental studies (animal models and cell culture studies) that isothiocyanates and indoles can beneficially affect processes involved in carcinogenesis, and can reduce mammary tumor incidence (Verhoeven et al., 1997).

Many different isothiocyanates exist and have been studied for their anticancer properties. Different cruciferous vegetables contain a different array of glucosinolate precursors (more than 100 have been identified in plants), and thus, some vegetables are better sources of certain isothiocyanates than others. By far the most widely studied isothiocyanate is sulforaphane of which broccoli is the major source. Other isothiocyanates include phenethyl isothiocyanate (PEITC; from, e.g., Chinese cabbage, radishes, and watercress), benzyl isothiocyanate (BITC, from, e.g., cabbage and garden cress) and allyl isothiocyanate (AITC; from, e.g., mustard, collard greens and kale). Glucobrassican is a glucosinolate found in all *brassica* vegetables that forms an unstable isothiocyanate which is further broken down into indole-3-carbinol (I3C) (Kushad et al., 1999). I3C has different activity than isothiocyanates, such as its effect on estrogen metabolism as described below.

The amount of different glucosinolates and their isothiocyanate hydrolysis products can vary up to 10-fold within and between cruciferous vegetables (Finley, 2005). Significant amounts of isothiocyanates are released upon consumption of cruciferous vegetables (Chung et al., 1992), and cooking can denature myrosinase, thus reducing the amount of available isothiocyanates (Conaway et al., 2000). However, myrosinase activity of the gut microflora can result in small amounts of isothiocyanates being released even when myrosinase in the plant has been inactivated by heat (Krul et al., 2002). Isothiocyanates are metabolized by the mercapturic acid pathway and are rapidly excreted in the urine as *N*-acetylcysteine (NAC) conjugates (mercapturic acids) (Mennicke et al., 1983; Ye et al., 2002). Urinary levels of mercapturic acids (commonly called urinary isothiocyanates) can be measured and serve as a useful biomarker of recent intake of cruciferous vegetables (Chung et al., 1998). In addition, a study of eight women undergoing reduction mammoplasty found that sulforaphane reaches the human mammary gland after a single dose of a broccoli sprout preparation (Cornblatt et al., 2007), thus, providing further evidence that isothiocyanates may play a role in breast health.

Overview of Biologic Mechanisms

Administration of cruciferous vegetables or phytochemicals from cruciferous vegetables reduces mammary tumor incidence and size in animal studies (Zhang et al., 1994; Fahey et al., 1997; Verhoeven et al., 1997). The primary mechanism to explain this effect has centered around the ability of isothiocyanates and I3C to inhibit phase I metabolizing enzymes and induce phase II metabolizing enzymes (Hecht, 1999; Talalay and Fahey, 2001; Lampe and Chang, 2007; Clarke et al., 2008). However, there is now evidence from cell culture studies that isothiocyanates and I3C also have beneficial effects on inflammation, cell cycle regulation, epigenetic modulation, angiogenesis and apoptosis, all of which are processes involved in carcinogenesis (Gamet-Payrastre et al., 2000; Chiao et al., 2004; Keum et al., 2004; Zhang, 2004; Zhang et al., 2006). Table 22.1 summarizes the potential mechanisms by which components of cruciferous vegetables may impact breast carcinogenesis.

Effects on Phase I Metabolizing Enzymes and Estrogen Metabolism

One means of by which cruciferous vegetables may affect breast cancer risk is by manipulating estrogen metabolism. The 16 alpha-hydroxyestrone (16-OHE) metabolite has estrogenic properties, while the 2-hydroxyestrone (2-OHE) has a low affinity for the estrogen receptor (Bradlow et al., 1985; Osborne et al., 1993; Zhu and Conney, 1998). Multiple enzymes involved in estrogen metabolism have been shown to be induced or inhibited by phytochemicals found in cruciferous vegetables. In cell culture and animal studies, administration of I3C has been shown to induce CYP1A1, the phase I enzyme responsible for 2-hydroxylation of estradiol (Stephensen et al., 2000; Horn et al., 2002). This is in direct contrast to the action of the isothiocyanate, sulforaphane, which has been shown to inhibit CYP1A1 in cell culture. The interactive effects of I3C and sulforaphane on CYP1A1

TABLE 22.1
Potential Anticarcinogenic Mechanisms of Cruciferous Vegetable Constituents

Constituent	Mechanism	Reference
Isothiocyanates	Induce phase II metabolizing enzymes	Dinkova-Kostova et al. (2002); Riedl et al. (2009); Verhagen et al. (1997)
	Inhibit carcinogenic activation by CYP enzymes	Conaway et al. (2002); Hecht (2000)
	Induce cell cycle arrest	Zhang (2004)
	Induce apoptosis	Hecht (2000)
	Inhibit angiogenesis	Myzak and Dashwood (2006)
	Reduce inflammation	Gerhauser et al. (2003); Heiss et al. (2001); Myzak and Dashwood (2006)
	Modulate epigenetic mechanisms	Dashwood et al. (2006); Lea et al. (2002); Myzak et al. (2004, 2006a,b)
Indole-3-carbinol	Induce phase II metabolizing enzymes	Chen et al. (1996); Nho and Jeffery (2001); Staack et al. (1998)
	Induce CYP enzymes involved in estrogen metabolism; increase urinary 2-OHE and urinary 2-OHE:16-OHE ratio	Fowke et al. 2000; Michnovicz et al. (1997); Zhou et al. (2007)
	Induce cell cycle arrest	Chinni et al. (2001); Cover et al. (1998)
	Induce apoptosis	Chen et al. (2004); Chinni et al. (2001); Hong et al. (2002); Howells et al. (2002)
	Inhibit angiogenesis	Chang et al. (2005); Meng et al. (2000)
	Modulate epigenetic mechanisms	Yu et al. (2006)

levels following ingestion of whole broccoli has not been well studied, but appears to result in CYP1A1 induction.

Consumption of *brassica* vegetables daily for four weeks increased the ratio of 2-OHE to 16-OHE in 34 healthy postmenopausal women (Fowke et al., 2000). Similarly, supplementation with 400 mg I3C daily for 2 months resulted in increased urinary excretion of the C-2 estrogens in another study (Michnovicz et al., 1997). An increase in the 2-OHE/16-OHE ratio may be favorable because it reduces the exposure to active estrogen, which is the leading risk factor for breast cancer to date. However, associations between urinary 2-OHE/16-OHE ratio and breast cancer risk have been inconsistent in the epidemiologic literature (Schneider et al., 1982; Kabat et al., 1997; Ho et al., 1998; Meilahn et al., 1998; Ursin et al., 1999; Muti et al., 2000; Cauley et al., 2003).

Modulation of other enzymes involved in estrogen metabolism, CYP1A2, CYP1B1 and CYP3A4, has also been linked to cruciferous vegetables. CYP1A2 is induced upon intake of cruciferous vegetables (Pantuck et al., 1979; Vistisen et al., 1992; Kall et al., 1996; Lampe et al., 2000b), and there is evidence of a gene-gene-diet interaction in that frequent consumers of cruciferous vegetables with the null genotype for *GSTM1* had higher CYP1A2 activity compared with *GSTM1* non-null individuals (Probst-Hensch et al., 1998). Women with the *CYP1A2*F AC* genotype had a lower mean ratio of 2-OHE/16-OHE compared with women with AA or CC genotypes (Lurie et al., 2005). In addition, induction of the CYP3A4 enzyme, primarily responsible for C-16 hydroxylation of estrogen, has been found to be suppressed by sulforaphane (Zhou et al., 2007). Another cell culture study reported that an isothiocyanate found in Spanish black radish induced CYP1A1, CYP1A2, as well as CYP1B1 (Hanlon et al., 2007), and a polymorphism in *CYP1B1* has been associated with lower 2-OHE and 16-OHE levels in women at high risk for breast cancer (Greenlee et al., 2007). CYP1B1 is involved in the conversion of estradiol to the potentially procarcinogenic 4-hydroxy estrone.

EFFECTS ON PHASE II METABOLIZING ENZYMES

Phase II metabolizing enzymes, such as glutathione-*S*-transferase (GST), NAD(P)H:quinone oxidoreductase (NQO1), UDP-glucuronosyl transferase (UGT), and heme-oxygenase (HO1) are responsible for deactivating carcinogens and protecting cells from DNA damage by suppressing oxidative stress. Isothiocyanates or cruciferous vegetables have been shown to increase activity of these enzymes in a limited number of human studies, and in cell culture studies through antioxidant response element (ARE)-mediated transcription of the genes encoding these enzymes (Dinkova-Kostova et al., 2002). I3C has also been found to increase phase II enzyme activity in animals (Nho and Jeffery, 2001). An increase in phase II metabolizing enzyme activity may result in decreased risk of cancer by increased deactivation and elimination of carcinogens from the body and reduced DNA damage from reactive oxygen species (Kensler, 1997). The GST family of enzymes, which consists of 7 classes and at least 17 subtypes, is the most well-studied of these enzymes in relation to cruciferous vegetables. Epidemiologic studies of the interaction between *GST* polymorphisms and cruciferous vegetable intake in relation to breast cancer are summarized below in the section entitled Gene-Diet Interactions.

A few trials in humans have found evidence for increased activity of phase II metabolizing enzymes upon cruciferous vegetable intake. Consumption of 300 g/day Brussels sprouts for two weeks in one study (Bogaards et al., 1994) and for six days in another study (Nijhoff et al., 1995b) increased plasma GST-alpha in males. However, urinary GST-alpha and urinary and plasma GST-pi levels were not changed in the Nijhoff et al. (1995b) study, and no effects were observed in women. In another study, GST-alpha activity increased in blood from women with the null genotype for *GSTM1* after consuming *brassica* vegetables for six days, but no effect was observed among *GSTM1*-positive women or men of either genotype (Lampe et al., 2000a). In a study of smokers, UGT activity (as measured by urinary excretion of glucuronidated nicotine metabolites) increased after consumption of 170 g/day watercress for three days (Hecht et al., 1999).

Blood or urinary markers of enzyme activity may not reflect the target tissue activity of enzymes, and so other studies have examined GST activity in tissue samples following cruciferous intake. GST-alpha and GST-pi levels were increased in rectal biopsies upon intake of 300 g/day Brussels sprouts for seven days in a small crossover feeding study (Nijhoff et al., 1995a). In another study, significant increases in *GSTM1*, *GSTP1*, *HO1*, and *NQO1* gene expression were observed in nasal lavage cells from subjects consuming 200 g/day of broccoli sprout homogenate for three days (Riedl et al., 2009). Another study, in which perfusion of the proximal jejunum was performed in six subjects, found that mRNA expression of GSTA1 and UGT1A1 increased after perfusion of an onion/broccoli extract (Petri et al., 2003). In contrast, no change in expression of GSTs in human gastric mucosa was found after one dose of standard broccoli or high-glucosinolate broccoli in another study (Gasper et al., 2007). No studies have examined changes in enzyme activity in breast tissue in humans after cruciferous vegetable intake; however, one study did report NQO1 and HO1 enzymatic activity in breast tissue from women who had undergone reduction mammoplasty and consumed a broccoli sprout preparation prior to surgery (Cornblatt et al., 2007). While it is impossible to causally link the enzyme activity to the broccoli sprout intake in this study because no preconsumption samples were taken, the authors also performed a study in rats demonstrating that NQO1 and HO1 activity increased substantially in rat mammary epithelium after dosing with sulforaphane (Cornblatt et al., 2007).

Other Anticancer Mechanisms

In addition, and sometimes as a result of, phase I and phase II enzyme modulation, isothiocyanates and I3C have been shown to have other beneficial effects on processes involved in carcinogenesis, as reviewed in (Aggarwal and Ichikawa, 2005; Higdon et al., 2007; Juge et al., 2007) and summarized in Table 22.1. Sulforaphane has antiproliferative effects in human breast cancer cells (Jackson and Singletary, 2004a) and is involved in cell cycle regulation (Zhang, 2004). Apoptosis is a tightly regulated process of programmed cell death, and disruption of apoptosis is implicated in carcinogenesis. Both sulforaphane (Jackson and Singletary, 2004b) and I3C (Howells et al., 2002) administration *in vitro* has been shown to enhance apoptosis in breast cancer cells. Angiogenesis is the growth of new blood vessels by tumors to provide fuel and nutrients for growth. There is limited but suggestive evidence that isothiocyanates and I3C can inhibit angiogenesis and inhibit tumor invasion in breast cancer models (Meng et al., 2000; Chang et al., 2005; Myzak and Dashwood 2006).

Inflammation is recognized as a risk factor for cancer because of its ability to promote cellular proliferation and inhibit apoptosis. There is evidence that some isothiocyanates may reduce the inflammatory response and decrease pro-inflammatory transcription factor binding to DNA (Heiss et al., 2001; Gerhauser et al., 2003). Epigenetic mechanisms are increasingly being recognized as critical to cancer development, and histone deacetylation is a form of epigenetic silencing of gene expression. Overexpression of histone deacetylase (HDAC) is present in many malignancies, and sulforaphane has been shown to inhibit HDAC in human breast cancer cells (Pledgie-Tracy et al., 2007), colon and prostate cancer cells (Myzak et al., 2004, 2006b), as well as in peripheral blood mononuclear cells of humans after ingestion of 68g of broccoli sprouts (Myzak et al., 2007). Thus, there are a number of promising areas of research that provide mechanistic evidence for a role of cruciferous vegetables and their bioactive phytochemical constituents in breast cancer prevention.

EPIDEMIOLOGIC STUDIES OF BREAST CANCER

Cruciferous Vegetable Intake

Intake of cruciferous vegetables has been examined in epidemiologic studies of cancer for a large number of organ sites (van Poppel et al., 1999; Talalay and Fahey, 2001; Lampe and Peterson, 2002; Seow et al., 2005; Higdon et al., 2007; Jeffery and Keck, 2008; Lam et al., 2009). The association

between cruciferous vegetable intake and breast cancer risk, in particular, has been examined in epidemiological studies of different designs with varying results (Table 22.2). Of three studies reviewed by Verhoeven et al., in 1996 (Verhoeven et al., 1996), no association of *brassica* vegetable intake on breast cancer risk was observed in one (Graham et al., 1982); while an inverse association of raw cabbage intake, but not of cooked cabbage, broccoli or cauliflower was found in another (Katsouyanni et al., 1986); the third study found a significant 50% reduction in risk for those subjects in the highest tertile of *brassica* vegetable intake versus the lowest tertile (Levi et al., 1993).

A second review paper published in 2007 highlighted results from four additional studies (Higdon et al., 2007). In a case–control study of participants from New York, significant decreased risk was observed for premenopausal women consuming the highest amounts of broccoli, but no significant associations were observed among postmenopausal women (Ambrosone et al., 2004). A study of participants from Shanghai, China, found that urinary isothiocyanate concentration (as a biomarker of intake) was inversely associated with breast cancer, while *brassica* intake as assessed in a food frequency questionnaire was not (Fowke et al., 2003). In one study of a Swedish population, a significant 24% reduction in risk of breast cancer was observed in those subjects in the highest quartile (median intake = 1.1 servings/day) compared to the lowest quartile (median intake = 0.1 servings/day) of *brassica* vegetable intake (Terry et al., 2001). When the data were further divided into deciles of *brassica* vegetable consumption, an even greater reduction in risk was observed comparing the highest decile to the lowest (RR 0.58, 95% CI 0.42–0.79). In contrast, the Pooling Project of Prospective Studies of Diet and Cancer, which included data from seven cohort studies and over 7000 breast cancer cases, found no association between cruciferous vegetable intake and breast cancer risk (Smith-Warner et al., 2001). On further analyses, however, nonsignificant reduced risk with broccoli and Brussels sprout intake (RR 0.86; 95%CI 0.72, 1.02 and RR 0.67; 95%CI 0.35, 1.27, respectively) but not cabbage intake (RR 1.05; 95%CI 0.85, 1.29) were reported.

A few other studies have examined cruciferous vegetable intake and breast cancer. Hebert and Rosen performed an ecological study of nutritional, socioeconomic and reproductive factors using data from 59 countries and found that *brassica* vegetable consumption had the strongest protective association with breast cancer risk on a per-calorie exposure compared with other factors examined (Hebert and Rosen, 1996). In a large case–control study, the Long Island Breast Cancer Study Project, postmenopausal women consuming six or more servings of cruciferous vegetables per week were at reduced risk of breast cancer compared with women consuming zero or one serving per week, while an opposite effect was observed in premenopausal women (Gaudet et al., 2004a). The increased risk observed in premenopausal women is unexplained as it is inconsistent with other studies and may possibly be due to chance given the large number of comparisons made in the study. By contrast, Potischman et al., (1999) found no association between cruciferous vegetable intake and breast cancer risk in a U.S. population of women aged 20–44 years at diagnosis, and Shannon et al., (2005) found no association between cruciferous vegetable intake and breast cancer risk in a case–control study in Shanghai. Thus, the data regarding cruciferous vegetable intake and breast cancer risk from epidemiologic studies are mixed.

Inconsistencies across epidemiologic studies of nutrition and cancer are not uncommon, and may be related to the use of different dietary assessment tools in different studies. Given the low intake of cruciferous vegetables in the United States [fewer than 20% of Americans in the Continuing Survey of Food Intakes by Individuals reported consuming a cruciferous vegetable (broccoli, cauliflower, kale or Brussels sprouts) in the two-day reporting period, and only 3% reported consuming broccoli on at least one of the two days (Johnston et al., 2000)], it is also possible that narrow range in intake limits the ability to observe an association if one exists in these and similar populations (Hebert and Miller, 1988; Hebert, 2005). Finally, given the known biologic interaction with phase I and phase II metabolizing enzymes, it may be necessary to examine effects stratified by genetic polymorphisms in genes encoding these enzymes in order to more clearly observe an effect of cruciferous vegetable on breast cancer risk. This has been done in studies of cancer at other organ sites, such as lung (London et al., 2000; Spitz et al., 2000; Zhao et al., 2001; Lewis et al., 2002; Wang

TABLE 22.2
Epidemiologic Studies of Cruciferous Vegetable Intake, Urinary Isothiocyanates and Female Breast Cancer

Author, Date	Location	Sample Size (Number of Cases)	Exposure	Frequency	Result	Covariates
Dietary intake						
Graham et al., 1982	New York, USA	2024	*Brassica* vegetables	≥20 compared to 0–3 times/month	OR = 1.0, not significant	
Katsouyanni et al., 1986	Greece	120	Cabbage, raw		χ^2 trend = 2.6 ($p < 0.05$)	Age, interviewer, years of schooling
			Cabbage, cooked		χ^2 trend = 1.32	
			Broccoli		χ^2 trend = 0.26	
			Cauliflower		χ^2 trend = 1.77	
Levi et al., 1993	Vaud, Switzerland	107	Brassica vegetables		OR = 0.5, $p < 0.05$	Age, education, energy intake
Potischman et al., 1999	USA	568	Cruciferous vegetables	≥3.5 compared to <1.4 times/wk	OR = 0.95 (95%CI = 0.7, 1.3)	Age, study site, ethnicity, education, age at first birth, alcohol intake, oral contraceptive use, smoking status
Terry et al., 2001	Sweden	2832	*Brassica* vegetable	1.1 compared to 0.1 servings/day (medians)	OR = 0.76 (95%CI = 0.62, 0.93), p for trend = 0.01	Age, height, BMI, smoking, SES, alcohol intake, high-fiber grains and cereals intake, fatty fish intake, multivitamin use, parity, hormone replacement therapy, history of benign breast disease, family history, type of menopause, age at menopause, age at menarche, age at first birth
Smith-Warner et al., 2001	Pooling Project (USA, Canada, Netherlands, Sweden)	7057	Cruciferae	100 g/day intake increment	RR = 0.96 (95%CI = 0.87, 1.06) p for heterogeneity = 0.95	Age at menarche, parity × age at first birth interaction, oral contraceptive use, history of benign breast disease, menopausal status, postmenopausal hormone use, family history, smoking status, education, BMI, BMI × menopause interaction, height, alcohol intake and energy intake
Fowke et al., 2003	Shanghai, China	341 total cases	*Brassica* vegetables	Quartile 4 compared to quartile 1	OR = 0.8 (95%CI = 0.5, 1.3), p for trend = 0.79	Soy protein, fibroadenoma, family history, leisure activity, waist to hip ratio, BMI, age at menarche, number of children

continued

TABLE 22.2 (continued)
Epidemiologic Studies of Cruciferous Vegetable Intake, Urinary Isothiocyanates and Female Breast Cancer

Author, Date	Location	Sample Size (Number of Cases)	Exposure	Frequency	Result	Covariates
		122 premenopausal	*Brassica* vegetables	Quartile 4 compared to quartile 1	OR = 0.4 (95%CI = 0.1, 1.1), *p* for trend = 0.07	Same as above
		219 postmenopausal	*Brassica* vegetables	Quartile 4 compared to quartile 1	OR = 1.0 (95%CI = 0.5, 1.8), *p* for trend = 0.79	Same as above
Ambrosone et al., 2004	New York, USA	301 premenopausal	Cruciferous vegetables	>2041 compared to ≤809 g/mo	OR = 0.7 (95%CI = 0.5, 1.2)	Age, education, age at menarche, age at first pregnancy, family history, BMI
			Broccoli	>1024 compared to ≤305 g/mo	OR = 0.6 (95%CI = 0.4, 1.0)	Same as above
		396 postmenopausal	Cruciferous vegetables	>1879 compared to ≤658 g/mo	OR = 0.8 (95%CI = 0.6, 1.2)	Age, education, age at menarche, age at first pregnancy, family history, BMI, age at menopause
			Broccoli	>800 compared to ≤140 g/mo	OR = 1.0 (95%CI = 0.7, 1.4)	Same as above
Gaudet, et al., 2004a	Long Island, NY, USA	456 premenopausal	Cruciferous vegetables	≥6 compared to 0–1 servings/wk	OR = 1.76 (95%CI = 1.18, 2.61) *p* for trend = 0.03	Age, energy intake
		961 postmenopausal	Cruciferous vegetables	≥6 compared to 0–1 servings/wk	OR = 0.80 (95%CI = 0.60, 1.05), *p* for trend = 0.12	Same as above
Shannon et al., 2005	Shanghai, China	378	Cruciferous vegetables	≥1.04 servings/day compared to ≤3.1 servings/wk	OR = 1.08 (95%CI = 0.62, 1.89), *p* for trend = 0.83	Age, total fruit and vegetable intake, breast-feeding

Van Gils, 2005 (van Gils et al., 2005)	EPIC cohort (Europe)	2550	Cabbages	>43 compared to ≤3 g/day	RR = 1.18 (95%CI = 1.01, 1.38), *p* for trend = 0.11	Age, center, energy intake, alcohol intake, saturated fat intake, height, weight, age at menarche, parity, oral contraceptive use, hormone therapy use, menopausal status, smoking status, physical activity, education
Lee, 2008 (Lee et al., 2008)	Shanghai, China	3452 total cases	Isothiocyanate[a]	Quintile 5 compared to quintile 1	OR = 0.82 (95%CI = 0.70, 0.96), *p* for trend = 0.007	Age, education, age at menarche, age at live birth, BMI, family history, regular exercise, energy intake, study phase
		2086 premenopausal	Isothiocyanate[a]	Quintile 5 compared to quintile 1	OR = 0.91 (95%CI = 0.74, 1.13), *p* for trend = 0.46	Same as above
		1366 postmenopausal	Isothiocyanate[a]	Quintile 5 compared to quintile 1	OR = 0.68 (95%CI = 0.53, 0.87), *p* for trend < 0.001	Same as above
Urinary biomarker						
Fowke, 2003 (Fowke et al., 2003)	Shanghai, China	337	Urinary isothiocyanates	Quartile 4 compared to quartile 1	OR = 0.5 (95%CI = 0.3, 0.8), *p* for trend < 0.01	Soy protein, fibroadenoma, family history, leisure activity, waist to hip ratio, BMI, age at menarche, number of children
		122 premenopausal		Quartile 4 compared to quartile 1	OR = 0.6 (95%CI = 0.2, 1.7), *p* for trend = 0.38	Same as above
		219 postmenopausal		Quartile 4 compared to quartile 1	OR = 0.5 (95%CI = 0.2, 0.9), *p* for trend = 0.01	Same as above

[a] Estimated dietary isothiocyanate exposure calculated by using food-frequency questionnaire data and food-specific isothiocyanate concentrations.

et al., 2004; Brennan et al., 2005), colon and colorectal (Lin et al., 1998; Slattery et al., 2000; Seow et al., 2002), prostate (Joseph et al., 2004), head and neck (Gaudet et al., 2004b), andrenal cell (Moore et al., 2007), and in a few studies of breast cancer as summarized in the following section.

Gene-Diet Interactions

In addition to inducing GSTs, isothiocyanates are metabolized by GSTs prior to being excreted in the urine. *GSTs* are polymorphic in humans, and it has been hypothesized that carriers of the null or less active genotypes for *GST*s may be at reduced risk of cancer due to slower elimination of protective isothiocyanates from the body. This has been supported by studies in Asian and Eastern European countries where cabbage is the major cruciferous vegetable consumed (London et al., 2000; Zhao et al., 2001; Seow et al., 2002; Moore et al., 2007). However, studies in the United States tend to find the opposite, that individuals with the present or more active polymorphic forms of *GST*s and high intake of cruciferous vegetables (mostly broccoli), are at reduced risk of cancer (Spitz et al., 2000; Joseph et al., 2004; Wang et al., 2004). Thus, it is speculated that differences in the isothiocyanate content of different cruciferous vegetables and the different affinities of GSTs for specific isothiocyanates may explain these discrepant findings across different populations (Gasper et al., 2005; Steck et al., 2007a), though further research is needed in this area to explain the complex relationships.

For breast cancer, a few studies have examined the interaction among *GST* polymorphisms and cruciferous vegetable intake, or the biomarker of urinary isothiocyanates. Of five studies examining interaction between cruciferous vegetable intake and polymorphisms in *GSTM1*, *GSTT1*, *GSTP1*, and/or *GSTA1*, none found statistically significant interaction in relation to breast cancer risk in either a Chinese population (Fowke et al., 2003; Lee et al., 2008), or women from New York (Ambrosone et al., 2004; Ahn et al., 2006; Steck et al., 2007b). However, in the only study that examined *GSTA1* polymorphisms, a significant trend was observed such that women with the **B/*B* genotype were at increased risk for breast cancer, and this risk was ameliorated with higher cruciferous vegetable intake (Ahn et al., 2006). The study of gene-diet interactions is complex, and will require further work to elucidate the role of genetic susceptibility in predicting individual response to dietary factors.

SUMMARY

Cruciferous vegetables contain a number of phytochemicals and nutrients with anticarcinogenic properties that make them promising targets for intervention studies of breast cancer. In particular, isothiocyanates and I3C, byproducts of glucosinolate hydrolysis, have been shown to modulate phase I and phase II metabolizing enzymes, affect estrogen metabolism, induce cell cycle arrest and apoptosis, modulate epigenetic mechanisms, inhibit angiogenesis, reduce inflammation, and reduce mammary tumor incidence and size in animal models. Epidemiologic studies have provided less consistent evidence for protection by cruciferous vegetables against breast cancer in humans, and results from studies of gene–cruciferous interactions and breast cancer are preliminary at this time. It has been proposed (Ambrosone and Tang, 2009) that small-scale intervention studies are needed in this area to improve our understanding of the effects of cruciferous vegetable intake on biologic processes and the role of genetic susceptibility in mediating these effects.

ABBREVIATIONS

AITC: allyl isothiocyanate; ARE: antioxidant response element; BITC: benzyl isothiocyanate; BMI: body mass index; GST: glutathione-*S*-transferase; HDAC: histone deacetylase; HO1: heme-oxygenase; I3C: Indole-3-carbinol; NAC: *N*-acetylcysteine; NQO1: NAD(P)H: quinine oxidoreductase; 2-OHE: 2-hydroxyestrone; 16-OHE: 16 alpha-hydroxyestrone; PEITC: phenethyl isothiocyanate; SES: socioeconomic status; UGT: UDP-glucuronosyl transferase.

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23 The Role of Flavonoids in Fruits and Vegetables Related to Breast Cancer Prevention

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CONTENTS

INTRODUCTION

Breast cancer is the leading cause of cancer-related death in women worldwide (Hery et al., 2008). Globally, more than 1.1 million women are diagnosed each year, representing approximately 10% of all newly diagnosed cancer cases (Anderson et al., 2005). Breast cancer is more common in North America and Western Europe than it is in most of Asia and Africa. Furthermore, the mortality rate for premenopausal breast cancer is nearly four times greater in the Western world, compared with Far East Asian nations (Limer and Speirs, 2004). In the past, health initiatives have focused primarily on two approaches for battling breast cancer: utilizing effective methods of early detection and providing standardized treatment to cure the disease. While advancements in research and technology have improved survival rates, there have not been similar advancements with regard to prevalence (Anderson et al., 2005). Furthermore, the systemic toxicity of current chemotherapeutic drugs is a significant limitation to their effectiveness in advanced stage cancer (Singh et al., 2002). This, coupled with a growing incidence of drug-resistant breast cancer, initiated a search for new strategies in disease management (Long et al., 2008) and more importantly, in cancer prevention.

Breast cancer is largely influenced by environmental factors while genetics are thought to play a much smaller role (WHO, 2003; Key et al., 2004). The World Health Organization (WHO) reports that nearly 30% of cancer deaths are related to dietary factors. While confounding factors such as age at menarche and parity, tobacco use, and physical activity are of significance, ecological observations continue to suggest that diet is a key variable in prevention. Interestingly, cancer rates have

been observed to change in populations as they move between countries adopting different dietary behaviors (WHO, 2003; Key et al., 2004). Studies of migrant populations have revealed a significant increase in breast cancer rates among those moving from low-risk areas such as Japan to higher-risk areas such as the United States (Escrich et al., 2006). As such, the novel concept of chemoprevention through dietary constituents has gained attention (Singh et al., 2002). International variations in breast cancer rates and dietary patterns helped formulate various hypotheses regarding potential preventative dietary factors.

Consumption of fruits and vegetables is widely advocated as part of a healthy diet. Specifically related to breast cancer, a high intake of yellow-orange and dark green fruits and vegetables has been associated with reduced breast cancer risk (Malin et al., 2003). The Joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) recommend a minimum intake of 400 g of fruits and vegetables per day for the prevention of chronic diseases such as cardiovascular and other degenerative diseases, as well as a number of different cancers (WHO, 2003). The National Cancer Institute recommends 5–9 servings of fruits and vegetables daily for cancer prevention (National Cancer Institute, 2009). Advancements in pharmaceutical sciences have aided in isolating many of the bioactive components in a fruit and vegetable rich diet (Singh et al., 2002). Among these, flavonoids have been identified as a large family of potentially chemopreventive compounds that are widely distributed in plant foods (Spencer, 2007). This chapter reviews the literature on flavonoids in fruits and vegetables, provides an overview of absorption, metabolism and bioavailability, as well as a description of biological actions. In addition, information is provided on food preparation methods and the effect they have on flavonoid content, as it relates to breast cancer prevention.

BACKGROUND

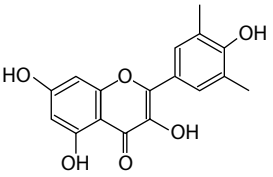
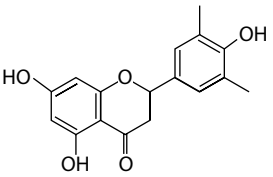
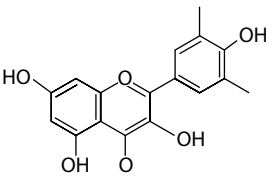
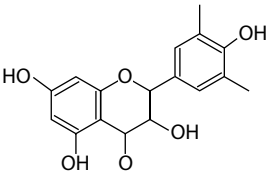
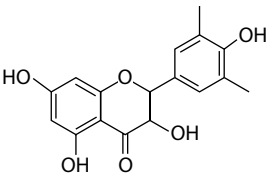
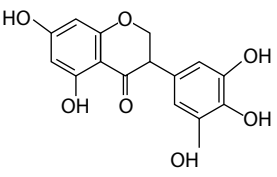
Flavonoids have been known as a plant pigment for over a century and constitute the largest and most important class of polyphenolic compounds (Ross and Kasum, 2002; Brusselmans et al., 2004). Beyond pigment, they contribute to the smell, flavor, and bitterness of a variety of plant foods. Their molecular structure consists of two aromatic carbon rings, linked by a three-carbon bridge (Brusselmans et al., 2004). To date, more than 5000 different flavonoids have been identified; however, at least six of them are common in the diets of humans (Aherne and O'Brien, 2002). Flavonoids are further subdivided into six classes, depending on their oxidation state and functional groups: flavonols, flavones, isoflavones, flavanones, flavanols (catechins), and anthocyanidins (Aherne and O'Brien, 2002; Spencer, 2007). The International Food Information Council (IFIC) and the U.S. Department of Agriculture (USDA) have extensive databases on food sources for the various classes of flavonoids. Table 23.1 provides the list of the most common flavonoids as well as their bioactive compounds and food sources (IFIC, 2009a,b; USDA, 2009; Wang et al., 2009).

The first observation of the biological actions of flavonoids was made in the early 1930s. At this time, researchers proposed that flavonoids should be classified as vitamins. In the early 1980s, researchers noted that a reduced risk in breast cancer did not correlate with traditional nutrients alone (Ross and Kasum, 2002). Attention has since focused on nonnutrient, bioactive food components of which flavonoids constitute one family (Harnly et al., 2006).

ABSORPTION AND METABOLISM

After ingestion flavonoids undergo extensive metabolism resulting in a diverse family of bioactive molecules (Spencer, 2007; Hackman et al., 2008). Structurally flavonoids most often occur in plants and other foods as glycosides; flavonoids bound to one or more sugar molecules (Ross and Kasum, 2002; Walle, 2004). Flavonoids that are not attached to a sugar molecule can also occur in food items although to a much lesser extent and are known as aglycones. Aglycones are more commonly identified as the metabolites produced from the degradation or hydrolysis of glycosides (Justesen et al., 2000). In the past, it was generally accepted that flavonoids traveled through the digestive tract

TABLE 23.1
Common Flavonoids, their Chemical Structure, Bioactive Compounds, and Food Sources

Flavonoids	Chemical Structure	Bioactive Compounds	Food Sources
Flavonols		Quercetin, kaempferol, myricetin	Apples, onions, broccoli, berries, tea, red wine
Flavones		Apigenin, luteolin	Grains, leafy vegetables, parsley, thyme, celery, hot peppers
Anthocyanindins		Cyaniding, delphinidin, malvindin	Berries, cherries, red/ purple grapes, red wine
Flavanols		Epicatechin, epicatechin gallate (EGC), epigallocatechin (EGCG), catechins	Tea, chocolate, apples, berries, grapes, apricot
Flavanones		Hesperetin, naringenin, eriodictyol	Citrus fruits and juices
Isoflavones		Daidzein, genistein, glycitein	Soy beans, soy-based foods

Source: Reprinted from Ramos, S. 2007b. *J Nutr Biochem* 18(7): 427–442. With permission from Elsevier.

to the colon where they would undergo degradation by faecal flora; however, current research has shown this is merely one of a number of routes through which the body processes flavonoids including the stomach, small intestine, and the liver (Nemeth et al., 2002).

Numerous sites and modes of action regarding the biological fate of flavonoids have been investigated (Ramos, 2007a). There seems to be a correlation between the structure of a particular flavonoid and the extent to which it is available for absorption. For example, flavonoid aglycones (quercentin, hesperetin, naringenin, and epicatechin) may be absorbed in the stomach while their

respective glucosides are not due to the nature and amount of glycosylation (Nemeth et al., 2002). However, other aglycones can be further metabolized to glucuronides, sulphates and *O*-methylated metabolites and absorbed in the small intestine and to phenolic compounds in the colon to be metabolized in the liver (Williams et al., 2004). Laboratory experiments have demonstrated hydrolysis of anthocyanins as well as other flavonoid glucosides can occur in the oral cavity (Walle, 2004).

In the small intestine, brush border enzymes and membrane transporters have been demonstrated to play a significant role as mediators in absorption and bioavailability (Ross and Kasum, 2002). A study of the interactions between intestinal enzymes and flavonoids was conducted *in vitro*, using both sheep and human small intestine enzymes. Cytosolic β-glucosidase (CBG) and lactase-phlorizin hydrolase (LPH) are two β-glucosidases present in the small intestine. Both hydrolyze a broad range of flavonoid glycosides. CBG is found within the cell and would require active transport of glucosides into the cell for hydrolysis (Nemeth et al., 2002). LPH is exposed in the lumen and therefore acts to release aglycones for passive diffusion across the cell membrane followed by further absorption (Nemeth et al., 2002).

Bioavailability seems to vary considerably between different flavonoids as well as between different food sources of the same flavonoid (Ross and Kasum, 2002; Hackman et al., 2008). There are notable discrepancies between many *in vitro* and *in vivo* studies, thus exact mechanisms of absorption and metabolism remain somewhat elusive. A plausible theory to help explain these discrepancies *in vivo* resides in the many variations that exist from one human body to the next. The differences in microflora or enzyme activity may alter a person's ability to produce specific flavonoid metabolites and therefore alter biological actions within the cell. When these metabolites are inside the cells, they can be part of different metabolic activities as antioxidants, modulators of cell signaling, or in cell proliferation and apoptosis.

BIOCHEMICAL ACTIONS

Flavonoids may exert potential benefit in cancer prevention through three major roles: as antioxidants and free radical scavengers, as modulators of cell signaling pathways, and as antiestrogenic compounds. Table 23.2 indicates bioactive components of flavonoids and mechanisms of action for breast cancer chemoprevention.

Cell signaling modulation seems to be the preferred and the most studied mechanism for chemoprevention. It seem that flavonoids can affect different steps in the signaling pathway cascades that will result in the activation of apoptotic pathways in damaged cells, inhibition of antiapoptotic proteins in cells, and proliferation of normal cells. Recent data is focusing on the effects of flavonoids as antiestrogenic compounds. Figure 23.1 shows the possible mechanisms by which isoflavones can block one or more steps in tumorigenesis at the initiation, promotion, and progression stages of cancer development.

ANTIOXIDANT AND FREE RADICAL SCAVENGING

In the human body, oxidative stress is initiated by free radicals despite natural antioxidant defense systems. These volatile molecules seek stability through electron pairing with biological macromolecules such as DNA, proteins and lipids (Ross and Kasum, 2002; Hazra et al., 2008). Ironically, many free radicals are oxygen-derived byproducts of normal cellular functions such as aerobic respiration. Studies conducted *in vitro* using flavonoid extract and reactive oxygen species (ROS) have shown that the structural characteristics of flavonoids support free radical scavenging and antioxidant functions (Hazra et al., 2008). Furthermore, flavonoids have also displayed the ability to protect and enhance endogenous antioxidants, providing support to the body's natural defense systems (Ross and Kasum, 2002).

Scavengers influence the course of a chemical reaction by combining with free radicals, resulting in a more stable molecule. This action can be viewed as the self-sacrificing ability of antioxidants.

TABLE 23.2
Bioactive Components in Flavonoids and Mechanisms of Action for Breast Cancer Chemoprevention

Flavonoid	Model	Protective Effect	Mechanism of Action in Cell Signaling Pathways	Reference
Flavanols: epicatechin gallate (EGC)	HBC, breast cancer cells	Inhibit cell growth and proliferation	Inhibition of signaling pathways at ERK, inhibition of NFκB-inducing kinase, inhibition of apoptotic protein kinases	Ramos, 2007a,b
Flavanols: epigallocatechin gallate (EGCG)	MCF7, breast cancer cells; mammary cancer cells	Activate apoptotic pathways	Inhibition of cell signaling pathway via inhibition of BcL-2 (antiapoptotic protein), increasing Bax (pro-apoptotic protein), activation of caspase 8 and 10 activities (pro-apoptosis)	Ramos, 2007a,b; Bigelow et al., 2006
Flavones: apigenin	MCF7 cells (human breast cancer cells)	Inhibit cell proliferation	At low dose: antiestrogenic via inhibition of estrogen receptor; at high dose: inhibit DNA synthesis via protein kinase pathway	Long et al., 2008
Flavonols: quercetin	MCF7 cells (human breast cancer cells)	Activate apoptosis	Activate MAPK, caspases, inhibit Bcl-2 (antiapoptotic)	Hakimuddin et al., 2004
Isoflavones: genistein	Breast cancer cells; mice	Activate apoptosis; reduce number of tumors	Inhibition of protein kinase activity and estrogen-induced cell proliferation	Dampier et al., 2001; Messina and Wu, 2009; Park et al., 2009

Various flavonoids have been shown to act as potent scavengers in a number of damaging oxidation species including hydroxyl radicals, superoxide anions, and lipid peroxidation, to name a few (Hazra et al., 2008). This action is an important step in preventing the progression of various oxidative stresses which have been implicated in the initiation stage of breast cancer development.

A study investigating the antioxidant capacity of onions examined the effect on plasma levels in six healthy women (Boyle et al., 2000). Onions, a food source of the flavonol quercetin, were found to protect against DNA damage by decreasing DNA strand breaks and preserving DNA stability. An increase in the plasma level of flavonol glucosides, quercetin-3-glucoside and isorhamnetin-4′-glucoside, was observed in all subjects after onion consumption (Boyle et al., 2000). When onion consumption was combined with tomatoes, plasma quercetin levels were significantly higher than with the consumption of onions alone (Boyle et al., 2000). This is important as consuming combinations of fruits and vegetables may have a different or even synergistic effect in addition to what *in vitro* or *in vivo* studies of isolated nutrients may predict.

Flavonoids have also been shown to employ their antioxidant abilities through the upregulation of other endogenous antioxidants. Specifically, a number of flavonoids have been shown to alleviate oxidative stress by inducing glutathione *S*-transferase (GST). GST is an enzyme that has been speculated to play a role in the inhibition of cancer formation by detoxifying various mutagenic compounds like carcinogens (Ross and Kasum, 2002; Hazra et al., 2008). Experiments conducted with flavonoid-rich extracts in cell culture have shown reductions in reactive oxygen species (ROS) and lipid peroxidation, in conjunction with increased cellular glutathione pools (Hackman et al., 2008). By enhancing the body's

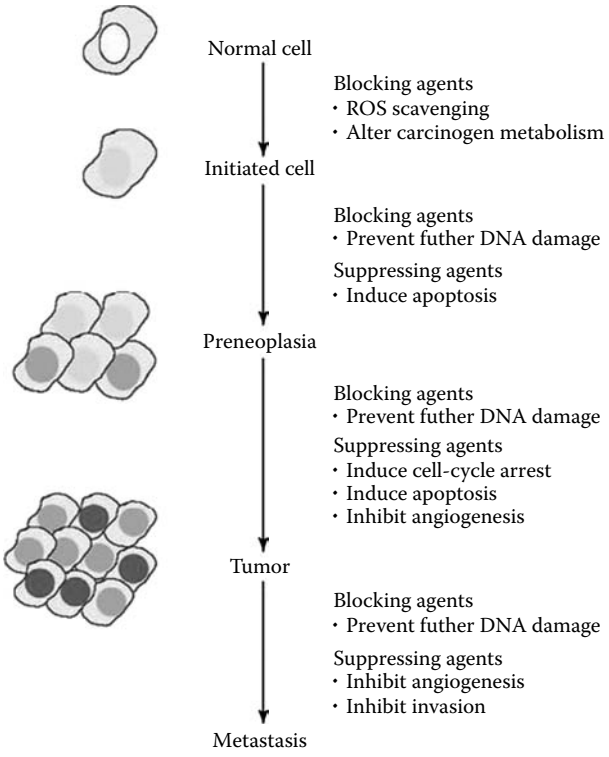


FIGURE 23.1 Possible mechanisms by which isoflavones can block one or more steps in tumorigenesis at the initiation, promotion, and progression stages of cancer development. (Reprinted from Ramos, S. 2007b. *J Nutr Biochem* 18(7): 427–442. With permission from Elsevier.).

natural defenses, flavonoid antioxidants may inhibit many stages of cancer development including initiation, promotion, and progression as well as delaying tumorigenesis (Ramos, 2007a).

CELL SIGNALING AND CELL CYCLE REGULATION

Flavonoids have been shown to act in both estrogen-dependent and estrogen-independent signaling pathways. This special feature is of great interest to breast cancer researchers for a number of reasons. Drug-resistant breast cancer is on the rise and blocking both pathways may be necessary to stop proliferation of antiestrogen-resistant breast cancer cells (Long et al., 2008). Also, women who are at an increased risk of developing breast cancer may opt to initiate tamoxifen treatment in hopes of prevention. Thus, flavonoids have been a focus in the search for a nontoxic, chemopreventive agent.

Investigations on the biological actions of flavonoids have shown their ability to modulate cell signaling and plasma estrogen levels as well as inhibit carcinogenesis (Murray et al., 2006; Yang et al., 2007). By upregulating or downregulating key signaling proteins via methylation, flavonoids can induce apoptosis and inhibit cancer cell proliferation (Murray et al., 2006; Ramos, 2007b). Additionally, their cytotoxic effects on cells appear to be limited to those that are damaged while their cytoprotective effects are seen in healthy cells (Ramos, 2007a).

Damage to the genetic material of cells causes disruption in normal cell cycle progression and division (Ramos, 2007b; Meeran and Katiyar, 2008). The cell cycle refers to a cascade of events in which the genetic contents of the cell are duplicated, resulting in subsequent cellular division. Progression of this cycle is monitored and regulated by cell cycle checkpoints. These checkpoints ensure proper completion of one step, such as replication or mitosis, before moving on to the next

(Meeran and Katiyar, 2008). Damage checkpoints serve to repair DNA damage or allow for the activation of pathways that lead to apoptosis, if damage is irreparable (Singh et al., 2002; Spencer, 2007). Cells have developed a number of defense mechanisms in order to cope with exogenous and metabolic damages; however, they are not infallible and cancer formation still occurs.

The ability to interfere in cancer cell cycle progression has been observed in cell culture with a number of flavonoids. After exposure to luteolin, quercetin, and kaempferol, each for a 24-hour period, breast cancer cell growth in culture was halted and apoptosis was initiated (Brusselmans et al., 2004). Additional research in breast cancer cell lines has investigated the affects of galangin, an herbal flavonoid-therapeutic used in parts of Asia (Murray et al., 2006). Galangin was shown to be a potent inhibitor of breast cancer proliferation by down regulating various signaling proteins. This was especially evident in cyclin inhibition. Cyclins are proteins that regulate the transition of cells through the different phases of the cell cycle; dysregulation is associated with mammary tumorigenesis. Cyclin D3 has been known to play a role in breast cancer development; however, it has not yet been targeted by chemotherapeutics (Murray et al., 2006). Furthermore, overexpression of cyclins downstream from D3, such as cyclin E, are characteristic of the breast cancer cell cycle (Meeran and Katiyar, 2008). Galangin-treated cells were assayed for cyclins D1, D3, A, and E expression after 18 hours of exposure. Results were not significant for cyclin D1; however, D3 expression was nearly undetectable and expression of cyclins A and E was significantly reduced (Murray et al., 2006). As cyclins A and E are downstream from D3, it can be surmised that this flavonoid is a potent downregulator of various cyclins, through the inhibition of D3. In addition, some studies support the hypothesis that the cytotoxic effects are selective for malignant or damaged cells rather than healthy cells (Brusselmans et al., 2004; Murray et al., 2006).

Flavonoids are also involved in the activation of apoptotic pathways of the cancer cells. Activation of apoptosis pathways occurs at different sites: activation of JNK (pro-apoptotioc proteins), activation of caspases, inhibition of Bcl-2 (anti-apopototic proteins), release of cytochrome *c*, inhibition of of NF-κB, and MAPK cascade. However, these findings were reported in studies of cultured cells and unfortunately the effect *in vivo* has not been completely elucidated or supported (Ramos, 2007b).

ESTROGEN RECEPTOR MODULATORS

While some flavonoids are able to act through estrogen-receptor-mediated mechanisms, they are generally considered to be nonestrogenic themselves (Ross and Kasum, 2002). The exception to this is the flavonoid subclass, isoflavones. They are found primarily in legumes and soybeans as well as soy-derived products, and are a principal group of phytoestrogens; plant-derived hormones. They have a structure that resembles endogenous estrogen and are capable of exerting estrogen-like actions within the body (Limer and Speirs, 2004).

Isoflavones (genistein and daidzein) are a point of interest for their association with a reduced prevalence of breast cancer in areas where they are a known dietary staple. Specifically, Asian diets are known to contain considerable amounts of soy products, lentils, and chickpeas (Breinholt et al., 2000; Peterson et al., 2003; Bosetti et al., 2005). Additionally, serum concentrations of 17β-oestradiol, or endogenous estrogen, are approximately 40% lower in these women compared with their Caucasian counterparts (Limer and Speirs, 2004). The exact role of phytoestrogens in breast cancer initiation and development is unclear. However, researchers suspect the chemotherapeutic effects are dependent on lifelong exposure. Isoflavones are detectable in breast milk following soy consumption, indicating the lower breast cancer incidence in Asian countries may be related to exposure from birth via breastfeeding (Limer and Speirs, 2004). Additionally, studies have suggested a dual chemoprotectant mechanism of isoflavones from soy; they've been shown to suppress steroid hormone biosynthesis while inducing the metabolism of oestradiol to 2-hydroxyoestrone, a known anticancer metabolite of 17β-oestradiol (Limer and Speirs, 2004).

Increased protein kinase activity has been shown to increase estrogen-stimulated breast cancer growth and genistein seems to modulate estrogen receptor activity (Long et al., 2008). Apigenin has

been speculated to inhibit the phosphorylation of kinases as well as induce their degradation. Additionally, apigenin has been shown to reduce estrogen receptor protein levels, thus inhibiting breast cancer cell progression (Long et al., 2008). Flavonoids present in green tea have also been linked with a decreased risk of breast cancer formation (Yang et al., 2007). Plasma estrone levels were significantly lower in regular green tea drinkers suggesting its ability to modulate estrogen levels (Yang et al., 2007).

Recently, a study in rats reported a reduction in tumor development and proliferation following the consumption of isoflavones in addition to chemopreventive effects. Rats fed with isoflavones (50 mg/kg body weight—a high dose) before cancer induction developed less tumors, with smaller sizes and in some cases, prevented tumor growth when compared with controls. These benefits were due to the activation of apopototic pathways on the tumor cells such as caspases activation and NF-κb inactivation (Park et al., 2009).

SPECIFICITY, TIMING, AND DOSE RESPONSE TO ISOFLAVONE INTAKE

Studies *in vitro* using MCF7 cell lines have shown a differential dose–response in cell proliferation to varying levels of genistein. Increased estrogen-dependent tumor cell proliferation occurred at the lowest doses of genistein while the higher doses were associated with inhibition of estrogen-dependent cell growth via estrogen receptor inhibition or increasing apoptosis (Dampier et al., 2001). Animal data has also shown that early intake of genistein is protective against breast tumor development (Lamartiniere et al., 2000).

Human studies also demonstrated that the effects of isoflavones depend on the amount and the developmental stage when soy products are consumed. A correlation between high consumption of soy products and a lower risk of breast cancer has been observed when comparing Asian with non-Asian female subjects (Zhang et al., 2005; Trock et al., 2006; Wu et al., 2008). Asians have lower rates of breast cancer as compared with non-Asians and this decrease has been attributed to the higher consumption of soy foods. Isoflavone intake in non-Asians is about 1 mg/day compared with 30 mg/day in Asians (Messina et al., 2006). Interestingly, a protective effect of soy on breast cancer risk in premenopausal women and a detrimental effect on breast cancer risk in postmenopausal women consuming soy has been reported (Ju et al., 2006). Long-term high soy consumption starting early in life appears beneficial in reducing breast cancer risk in women; however, in women with low estrogen levels or with previous estrogenic tumors such as in postmenopausal women, the consumption of soy appears related to increase tumorigenesis (Ju et al., 2006). The American Cancer Society currently recommends that women diagnosed with breast cancer can safely consume soy containing foods up to three servings per day (Doyle et al., 2006).

Synergism

There may be a synergistic effect of flavonoids with other fruit and vegetable components as well as with commonly used synthetic medications such as tamoxifen used for treating breast cancer by inducing apoptosis (Ramos, 2007b). This synergistic effect has also been demonstrated in studies of other cancers such as lung, oral, pancreatic, bladder, skin, and colon rather than in breast cancer (Khafif et al., 1998; Suganuma et al., 1999; Sakamoto, 2000; Tamura et al., 2003; Hwang et al., 2005; Sharma et al., 2005; Mohammad et al., 2006).

Preparation Methods

Cooking plant foods can significantly alter their chemical composition and thus influence the concentration and bioavailability of various compounds. This has been of increasing interest for food scientists and food manufacturers alike. While the consumption of raw plant foods has been

advocated as an integral part of a healthy diet, there is growing evidence that may broaden recommendations to encourage the consumption of minimally processed plant foods (Miglio et al. 2008). Naturally occurring protective compounds may be enhanced or made more readily available for absorption, depending upon the preparation method used.

A recent study on carrots, zucchini squash (courgettes), and broccoli examined their physicochemical characteristics prior to and after preparation (Miglio et al., 2008). The total phenol content and total antioxidant capacity (TAC) was determined for each of the vegetables while raw, and after being boiled, steamed and fried. The results are summarized in Table 23.3. The TAC was obtained using three different assays: Trolox equivalent antioxidant capacity (TEAC) assay, total radical-trapping antioxidant parameter (TRAP) assay, and ferric reducing antioxidant power (FRAP) assay.

Interestingly, TAC significantly increased in each vegetable after preparation, while the total phenol content diminished. The decline in flavonoids accompanied by a spike in TAC is thought to be the result of the conversion of polyphenols into chemical species not yet identified (Miglio et al., 2008). Softening of the vegetable matrix during cooking increases the extractability of antioxidants, which may result in the formation of new, very active compounds, adding protective benefits for chemoprevention (Miglio et al., 2008). These results indicate that vegetables are higher in nutritional quality when minimally processed.

Another study examining the effect of processing on vegetables looked at onions, green beans, and peas (Edwald et al., 1999). The array of cooking methods used included blanching, boiling, microwaving, pan-frying with rapeseed oil and pan-frying with butter. Additionally, this study looked at the effects of warm holding for one and two hours after the vegetables were boiled for three minutes.

TABLE 23.3
Methods of Preparation and Antioxidant Content of Three Common Vegetables

	TEAC	FRAP	TRAP	
	(mmol of Trolox/100 g)	(mmol of Fe^2/100 g)	(mmol of Trolox/100 g)	Total Phenolic Compounds[a]
		Carrots		
Raw	0.40 ± 0.01	0.68 ± 0.03	0.03 ± 0.00	69.6 ± 0.8
Boiled	0.83 ± 0.01	1.45 ± 0.02	0.20 ± 0.00	ND
Steamed	0.70 ± 0.02	1.23 ± 0.038	0.04 ± 0.00	39.6 ± 3.2
Fried	1.05 ± 0.02	3.25 ± 0.05	0.56 ± 0.02	48.0 ± 2.0
		Zucchini Squash (Courgettes)		
Raw	0.80 ± 0.00	2.79 ± 0.11	0.20 ± 0.02	59.0 ± 1.1
Boiled	1.53 ± 0.11	6.32 ± 0.86	0.29 ± 0.03	17.9 ± 1.0
Steamed	1.40 ± 0.04	5.92 ± 0.08	0.36 ± 0.01	35.3 ± 1.5
Fried	1.64 ± 0.04	7.97 ± 0.12	0.75 ± 0.01	21.9 ± 1.5
		Broccoli		
Raw	1.10 ± 0.05	5.23 ± 0.56	1.61 ± 0.04	99.8 ± 2.4
Boiled	2.17 ± 0.17	8.91 ± 0.57	1.95 ± 0.09	27.0 ± 3.4
Steamed	3.51 ± 0.15	11.98 ± 0.74	3.59 ± 0.21	61.8 ± 8.2
Fried	2.88 ± 0.32	9.02 ± 1.68	2.41 ± 0.26	40.3 ± 0.7

Source: From Miglio, et al. 2008. *J Agric Food Chem* 56: 139–147. With permission.

[a] Total phenolic compounds include quercetin and kaempferol.

ND = not detected.

Interestingly, onions experienced the most significant flavonoid loss during the peeling and trimming process. Thus, the outermost layers of an onion are thought to be the most nutrient-dense part of the vegetable (Edwald et al., 1999). For all three vegetables, boiling and warm holding for one hour yielded the highest flavonoid losses among the cooking methods tested. There was no significant difference between warm holding for one hour or two. All three vegetables experienced their highest flavonoid content after being pan-fried and microwaved. The results from this study also support minimal processing of vegetables; however, they suggest some methods are preferential over others (Edwald et al., 1999).

CONCLUSIONS

Flavonoids have been shown to have a positive role in breast cancer chemoprevention. Much of the evidence supporting this positive role of flavonoids is derived from animal models and *in vitro* studies. There are many reasons for this benefit including the role of flavonoids as antioxidants and modulators of cell signaling via apoptosis of damaged cells and increased proliferation of normal cells. The WHO, IFIC, and the National Cancer Institute along with many other global breast cancer organizations agree that a balanced diet with adequate consumption of fruits and vegetables (at least 400 g/day or five to nine servings depending on the vegetable or fruit consumed) along with physical activity, limiting tobacco, and alcohol consumption is protective for reducing breast cancer risk. Aspects of flavonoids dose and timing of consumption in the life cycle have been associated with breast cancer prevention. In cell culture, exposure to higher doses of flavonoids early in life was found to be protective whereas exposure along with low estrogen, as would be the case in postmenopausal women, was associated with stimulation of estrogenic tumor growth. Similar findings were observed in postmenopausal women with a history of estrogen sensitive breast cancer. Consequently, the American Cancer Society recommends that consuming up to three servings of soy-containing foods can be safe for postmenopausal women. Future research is needed focusing on the synergistic benefits of consuming different flavonoids, the timing of intake and recommended amounts of flavonoid food sources and preparation methods for vegetables and fruits to maximize the desired protective effect in prevention of breast cancer.

ABBREVIATIONS

CBG	cytosolic β-glucosidase
EGCG	epigallocatechin-3-gallate;
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ERK	extracellular regulated kinase
IAP	inhibitor of apoptosis protein
JNK	c-Jun N-terminal kinase
GST	glutathione S-transferase
IFIC	International Food Information Council
Joint FAO/WHO	Joint Food and Agriculture Organization/ World Health Organization
LPH	lactase-phlorizin hydrolase
MAPK	mitogen-activated protein kinase
MCF-7	Michigan Cancer Foundation-7
MIF	migration-inhibitory factor
MMP	metalloprotease
NF-κB	nuclear factor κB
ROS	reactive oxygen species
USDA	United States Department of Agriculture

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24 Chemopreventive Properties of Ginseng Compounds on Colon Cancer

Chong-Zhi Wang and Chun-Su Yuan

CONTENTS

INTRODUCTION

Colorectal cancer accounts for 10% of the overall cancer cases in the United States, and the expected number of new patients in 2009 is 106,100 with 49,920 deaths (Jemal et al., 2009). Although early diagnosis with rigorous screening may have reduced its incidence compared to a few years ago, the prognosis associated with metastatic disease continues to remain bleak. Current treatment of this cancer generally employs surgical resection combined with chemotherapy using cytotoxic drugs and radiation therapy. Because this therapy is only moderately successful for late stage cancers, novel approaches to the treatment of this cancer are required. Several controlled clinical trial data supported a multimodal and multidisciplinary approach, including combination of treatments and schedule in which they are administered, in treating both early and advance stage colorectal cancers (Goldberg et al., 2004; Hurwitz et al., 2004). Studies also showed that patients with cancer often resort to complementary and alternative medical means for treatment of cancer-related symptoms, and/or to reduce the adverse effects of chemotherapy (Shumay et al., 2001; Ott, 2002; Lee et al., 2006; Wu et al., 2007).

There is compelling evidence that patients in this country resort to supplements or substitute them for conventional pharmacotherapy. Several national surveys indicate that at least one-third of American adults take some form of dietary supplement, and botanicals comprise approximately 25% of the supplement market (Barnes et al., 2004). Botanicals have also been a major source of therapy in many traditional medical systems and have been used clinically for the treatment of a variety of diseases (Mashour et al., 1998; Xie et al., 2006; Wang et al. 2007c; Wicks et al., 2007). Botanical ingredients in natural products contain bioactive constituents with medical benefits (Akerele, 1993; Leung, 2007; Zhou et al., 2007; Li and Zhang, 2008). Furthermore, botanicals have contributed significantly to cancer therapy, and it is likely that extracts and active constituents from herbal medicine will continue to play an important role in cancer therapeutics (Liu and Jiang, 2006;

Ng et al., 2006; Shieh et al., 2006; Ozaslan et al., 2007). In this chapter, we will discuss the potential roles of ginseng herbs in the treatment of colorectal cancer.

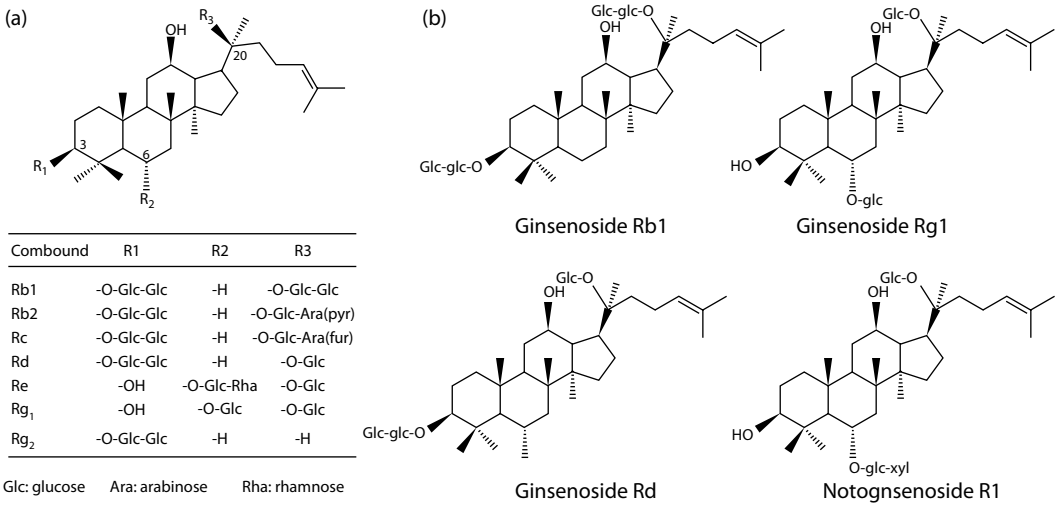
MEDICINAL USE OF BOTANICALS IN GINSENG FAMILY

Panax L. is a small genus of the family Araliaceae. Nearly all species in the genus *Panax*, such as *Panax ginseng* C.A. Meyer (Asian ginseng), *Panax quinquefolius* L. (American ginseng), and *Panax notoginseng* (Burk.) F.H. Chen (notoginseng), are important herbs used for different medical conditions (Chen et al., 2001; Wang et al., 2007c). Asian ginseng and notoginseng are considered as Chinese herbal medicines, and American ginseng is one of the most commonly used botanicals in the United States (Wang et al., 1999; Ng, 2006).

It is generally believed that the active compounds in Asian ginseng, American ginseng, and notoginseng are triterpene glycosides or dammarane saponins, commonly referred to as ginseng saponins (ginsenosides and notoginsenosides), and their levels can be used to develop quality controls for these herbs (Fuzzati, 2004; Chao et al., 2006; Wang et al., 2006a). There are over 50 different known ginseng saponins, and they are characterized by a four *trans*-ring rigid steroid aglycone skeleton and attached sugar moieties. Based on the aglycone skeleton, ginseng saponins can be divided into protopanaxadiol group and protopanaxatriol group, except for ginsenoside Ro, which is derived from oleanolic acid group (Figure 24.1).

Ginseng has many reported health benefits (Attele et al., 1999; Liu et al., 2006; Yamakage et al., 2006; Yoo et al., 2006). Regarding its anticancer effects, a case–control study on more than one thousand subjects in Korea showed that Asian or Korean ginseng intakers had a decreased risk for many different cancers compared with nontakers (Yun and Choi, 1995, 1998). It also suggested that ginseng has a nonorgan specific preventive effect against cancer (Yun, 2003).

Regarding the responsible anticancer constituents from Asian ginseng, published studies showed that some saponins could reduce proliferation of cancer cells and sensitize cancer cells to chemotherapeutic agents *in vitro* (Lee and Huemer, 1971; Kim et al., 2007; Koo et al., 2007). Several investigators found antitumor properties and other pharmacological activities of ginseng, and ginsenosides Rg3 and Rh2 are recognized as active anticancer saponins (Helms, 2004). Jia et al. (2004) noted that ginsenoside Rh2 inhibited proliferation and induced apoptosis in cancer cell lines, and sensitized drug-resistant breast cancer cells to paclitaxel. Kim et al. (2004) studied 11 ginsenosides and determined that Rg3 and Rh2 inhibited proliferation of prostate cancer cells.



Combound	R1	R2	R3
Rb1	-O-Glc-Glc	-H	-O-Glc-Glc
Rb2	-O-Glc-Glc	-H	-O-Glc-Ara(pyr)
Rc	-O-Glc-Glc	-H	-O-Glc-Ara(fur)
Rd	-O-Glc-Glc	-H	-O-Glc
Re	-OH	-O-Glc-Rha	-O-Glc
Rg_1	-OH	-O-Glc	-O-Glc
Rg_2	-O-Glc-Glc	-H	-H

Glc: glucose Ara: arabinose Rha: rhamnose

FIGURE 24.1 (a) Ginsenosides in American ginseng. (b) Saponins in notoginseng.

Iishi et al. (1997) used a rat model to determine the effects of ginsenoside Rg3 in inhibiting colon cancer cell proliferation.

AMERICAN GINSENG

Ginseng root has been used for centuries in Oriental medicine as a panacea that promotes longevity (Yun, 2003; Fuzzati, 2004). However, relatively few studies focus on American ginseng, which is a popular herbal supplement in U.S. consumers and patients (Attele et al., 1999; Helms, 2004).

American ginseng is an obligate shade perennial plant native to eastern North America. The commonly used part of the plant is the root, which is harvested after several years of cultivation. The largest growing area in the United States is in Wisconsin. The bioactive constituents of American ginseng are ginsenosides, which are present in the root, leaf, stem, and berry of the plant. More than 60 ginsenosides such as Rb1, Rb2, Rc, Rd, Re, Rg1, and Rg3 have been identified (Wang et al., 1999; Assinewe et al., 2003; Wang et al., 2006b) in American ginseng (Figure 24.1a). Previous studies of American ginseng were focused on its activities on the cardiovascular system, such as anti-ischemic, antiarrhythmic and antihypertensive effects (Yuan and Dey, 2001; Kim and Park, 2003). These pharmacological effects are, to a significant extent, considered to be linked to the antioxidant properties of the herb (Kitts et al., 2000; Wang et al., 2007c).

American ginseng extracts were found to inhibit the growth of breast cancer cells (Corbit et al., 2006). We previously investigated the effects of several herbal extracts on reducing chemotherapeutic side effects and found that American ginseng can attenuate cisplatin-induced nausea and vomiting in a rat model, while not affecting its anticancer properties in human cancer cells (Aung et al., 2007). In addition, the extract from American ginseng enhanced the antiproliferation effect of cisplatin on human breast cancer cells, suggesting that it possesses its own anticancer activity (Aung et al., 2007). Our group also showed that after steaming treatment of American ginseng, antiproliferative effects were improved significantly, possibly due to the altered ginsenoside profile (Wang et al., 2006c; Wang et al., 2007a; Wang et al., 2008).

To explore the mechanisms involved in cancer cell inhibition, we observed the effects of American ginseng on gene expression and apoptotic pathways. From the analysis of microarray hybridization, we found that the anticancer mechanism of American ginseng extract and its representative compound, ginsenoside Rg3, has many of the same characteristics, and alterations of gene expression level imply the important information for exploring this mechanism. The two recognized genes regulated by the extract and Rg3 (AKAPA8L and PITPNA), suggest that American ginseng takes effect through the regulation of cell mitosis and some intracellular singling pathways (Luo et al., 2008). In a separate study, the observed expression profiling on selected pathways revealed various apoptotic related genes that inhibited growth in human colorectal cancer cells by American ginseng. The mitochondrial apoptotic pathway was found to play a key role in cancer chemoprevention by steamed American ginseng extract (Wang et al., 2009a). These expression analyses may lead to the identification of markers that predict the responsiveness of colorectal cancer cells to American ginseng treatment.

NOTOGINSENG

Notoginseng is a Chinese herbal medicine that has a long history of use in China and other Asian countries. This herb is distributed in southwest China, Burma, and Nepal. Notoginseng is cultivated commercially in southwest China, especially in the Yunnan Province. The portion of the plant commonly used in remedies is the root, which is dug up after the fruit has ripened.

The earliest scientific description of notoginseng was in *Materia Medica*, a dictionary of Chinese herbs, written by Li Shi Zhen (AD 1518–1593). In *Materia Medica*, notoginseng was also called "more valuable than gold," indicating the significance of this herb in traditional Chinese medicines. Notoginseng is regarded as the emperor herb in the treatment of different types of

wounds because it is favored for the treatment of both internal and external hemorrhage (Ng, 2006; Wang et al., 2006a).

Modern pharmacological researches on notoginseng have found that notoginseng exerts various effects on the cardiovascular system, central nervous system, endocrine system, and inflammation response (Sun et al., 2005; Ng, 2006; Wang et al., 2006a). Consistent with the hemostatic effect of notoginseng reported in ancient China, recent studies showed that the alcohol extract of notoginseng resulted in reduction of the extent of bleeding and provided better hemostatic effects than no treatment, placebo treatment, or treatment with hydrophilic or lipophilic extracts (White et al., 2001). Notoginseng can also decrease blood pressure, improve blood supply, protect against shock, and protect the cardiovascular system and brain vasculature. Its protective mechanism could be partly due to protection against damage by oxygen free radicals, and also by binding to the estrogen receptor since ginsenosides share many of the protective actions of estrogen in various body systems. Pharmacokinetic and pharmacodynamic studies have shown that intranasal preparation of notoginseng saponins is a promising development and may be beneficial for the treatment of Alzheimer's disease. Notoginseng extracts were also found to possess the capacity to adjust energy metabolism and treat diabetes (Ng, 2006).

Notoginseng has a very distinct saponin profile compared with that of American ginseng (Chen et al., 2001; Sun et al., 2005). The main bioactive compounds in notoginseng are saponins, which are dammarane saponins. Oleanane-type saponin, present in Asian ginseng and American ginseng, is not found in notoginseng. To date, 56 saponins have been isolated from the notoginseng plant, out of which 35 belong to the protopanaxadiol group, while 21 belong to protopanaxatriol group (Sun et al., 2005). Ginsenosides Rb1, Rg1, Rd, and notoginsenoside R1 are the main saponins in notoginseng root (Figure 24.1b).

Some studies have shown that notoginseng has antitumor effects (Chen et al., 2001; Ng, 2006). Recently, we observed that notoginseng root extract and its constituents possess significant antiproliferative effects on human colorectal cancer cells (Wang et al., 2007d). Other plant parts of notoginseng also displayed potential antiproliferative effects on colorectal cancer cells (Wang et al., 2009b). The flower extract's most potent cancer cell growth inhibitory effects were shown within special chemical compositions (Wang et al., 2009b).

Our group also found that notoginseng extract can increase the effects of cancer chemotherapy. Using HCT-116 human colorectal cancer cell line, the antiproliferative effect of notoginseng extract combined with 5-FU was investigated. Compared with the control, when cells were treated with 5-FU or notoginseng separately, cell proliferation was reduced by 31% and 25%, respectively. The combination of 5-FU and notoginseng reduced cell proliferation by 59%, suggesting that combining notoginseng with 5-FU can reduce the dose of 5-FU, while significantly increasing the antiproliferation effect on the cancer cells. Since it is well known that 5-FU has cytotoxic effects on primary cells, this synergistic effect between notoginseng and 5-FU makes it possible to reduce the dose of 5-FU in combination with notoginseng and thereby further decrease dose-related toxicity (Wang et al., 2007b).

In another study, notoginseng's potential to enhance the effects of irinotecan without affecting irinotecan's activity was observed. It appears that irinotecan-induced toxicity can be reduced by using notoginseng as a chemo-adjuvant (Wang et al., 2007d). When notoginseng potentiates the tumoricidal effects of chemotherapeutic agents, smaller chemotherapy doses can be used. Data obtained from our studies will have the potential to advance treatment regimens and improve the quality of life for patients suffering from colorectal cancer.

SAPONIN STRUCTURE-ACTIVITY OBSERVATION AND HEAT TREATMENT OF GINSENGS

Ginseng saponins belong to a family of triterpene glycosides or triterpene saponins. Ginseng saponins (except ginsenoside Ro) possess the four *trans*-ring rigid steroid skeleton, with a modified side chain at C-20. Sugar residues are attached to the –OH of the aglycon. As mentioned above,

ginsenosides can be mainly classified as protopanaxadiol and protopanaxatriol groups. For the protopanaxadiol group, sugar residues are attached to the β-OH at C-3 and another –OH at C-20 of the aglycon, for example, ginsenosides Rb1, Rb2, Rc, Rd, Rg3 and Rh2. For the protopanaxatriol group, sugar residues are attached to the α-OH at C-6 and another –OH at C-20 of the aglycon, for example, ginsenosides Re, Rg1, Rh1, and notoginsenoside R1 (Figure 24.1).

Structure–activity relationship elucidates the relations between chemical structure and their pharmacological activity for a series of compounds (Ooi et al., 2006; Benjamin et al., 2008). The anticancer activities of ginseng saponins are related with the type of aglycons and sugar residues (Helms, 2004; Wang et al., 2007e). The main anticancer saponins so far identified are from the protopanaxadiol group. The three most potent compounds in this group are Rg3, Rh2 and their aglycon, protopanaxadiol, and the latter two may have stronger effects (Popovich and Kitts, 2002; Wang et al. 2007e). Other compounds in the protopanaxadiol group showed less or no anticancer activities, probably due to the fact that the sugar residues are attached to the –OH at C-20 (Wang et al., 2006c).

Ginsenoside Rg3 was isolated from Asian ginseng, American ginseng, and notoginseng (Xu et al., 1987; Chen et al., 2002). However, Rg3 is only a trace saponin in different species of genus *Panax* (Fuzzati, 2004). Rg3 can also be obtained from mild acidic hydrolysis of protopanaxadiol group saponins, such as Rb1, Rc, and Rd (Figure 24.2). Since Rg3 was found to effectively inhibit the growth of cancer cells (Mochizuki et al., 1995), studies of Rg3 sources were emphasized. In 2003, Rg3 was approved as a new anticancer drug in China (Lu et al., 2008). Although this saponin can be obtained by biological transformation and chemical synthesis, the process is complicated, the yield is limited and thus, the cost of the product is high. As shown in Figure 24.2, Rh2 and protopanaxadiol are also derived from the protopanaxadiol group saponins. In Asia, Asian ginseng root can be prepared as (1) air-dried to white ginseng or (2) steamed at approximately 100°C to red ginseng. Compared with white Asian ginseng, red ginseng has stronger anticancer activities (Yun et al., 2001) due to its relatively high Rg3 content. It seems likely that the steaming process or heat-treatment of ginseng is a good approach to transfer inactive ginsenosides to active anticancer compounds such as Rg3, Rh2, and protopanaxadiol.

Our laboratory treated American ginseng berry at various temperatures and heating times to observe the changes in ginsenoside content and anticancer activities on human colorectal cancer

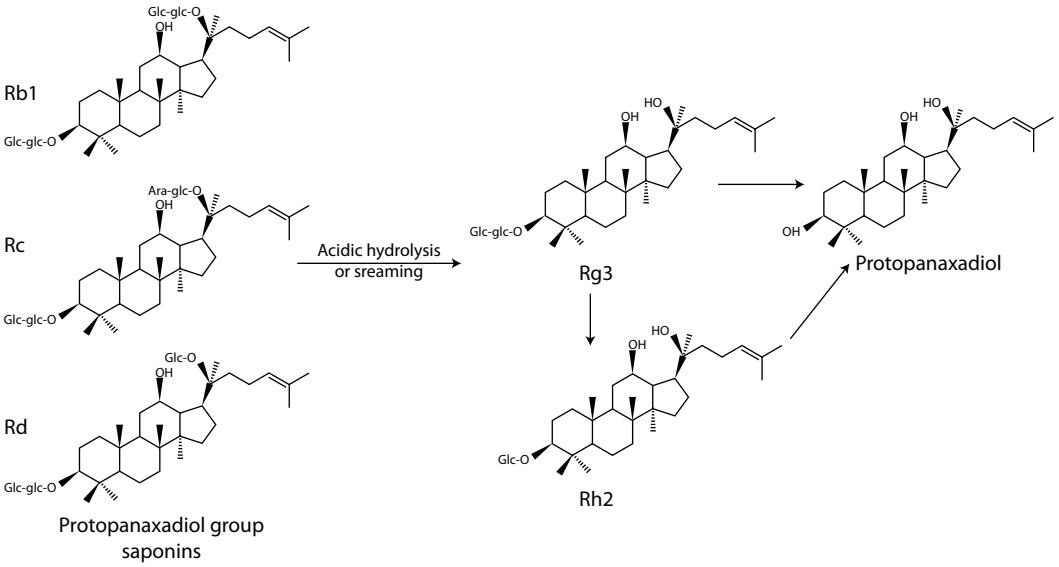


FIGURE 24.2 Chemical conversions starting from protopanaxadiol group saponins using acidic hydrolysis or steaming process.

cells. We found that steamed American ginseng berry extract very significantly augmented the content of Rg3. When human colorectal cancer cells were treated with steamed berry extract (120°C, two hours), the antiproliferation effects were 98% for HCT-116 and 99% for SW-480 cells. At the same treatment concentration, the effects of unsteamed extract were 34% for HCT-116 and 5% for SW-480 cells. This suggested that steamed American ginseng berry augmented Rg3 content and anticancer activity significantly (Wang et al., 2006c). We also steamed American ginseng root, with comparable change of the chemical constituent and antiproliferative activities to that of steamed berry extract (Wang et al., 2007a). Recent studies suggested that increasing the steaming time resulted in additional chemical changes and an increase in cancer cell growth inhibitory effects (Wang et al., 2009a).

Constituent changes of notoginseng after steaming treatment have also been reported (Lau et al., 2004). After the treatment, the content of Rb1, Rg1, Rd, and notoginsenoside R1 decreased, while Rg3 had some increase, and the trend is similar to what we observed after the steaming treatment of American ginseng. Recently, we performed steaming treatment on notoginseng root. After the treatment, the content of Rg3 was found to have increased remarkably, and antiproliferative effect on colorectal cancer cells were significantly increased (Sun et al., 2010).

SUMMARY

Previous studies suggested that American ginseng and notoginseng possess anticancer activities. We recently observed that using a special heat-preparation or steaming process, the content of Rg3, a previously identified anticancer ginsenoside, increased significantly and became the main constituent in the steamed American ginseng. As expected, using the steamed extract, anticancer activity increased significantly. Notoginseng has a very distinct saponin profile compared with that of American ginseng. Steaming treatment of notoginseng also significantly increased its anticancer effect.

The next logical step would be to characterize the effects of the two ginseng herbs (unsteamed and steamed) and their active constituents on colorectal cancer, and their mechanisms of action. Data obtained from future studies will help develop useful products for complementary and alternative therapies in oncology and expand our understanding about the biological mechanism behind the antitumor activity of ginseng and its active compounds.

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25 Effects of Pentacyclic Triterpenes from Olives on Colon Cancer

M. Emília Juan and Joana M. Planas

CONTENTS

INTRODUCTION

Colorectal cancer is one of the leading causes of death in both men and women in Western countries, being usually lethal when diagnosed at later stages of progression (Ferlay et al., 2007). Genetic predisposition is thought to account for about 15% of all colorectal cancer, notably hereditary nonpolyposis colorectal cancer and familial adenomatosis polyposis. Apart from these genetic factors, environmental aspects are thought to be involved in colon carcinogenesis, among which, dietary habits play a pivotal role. Mediterranean countries have lower rates of colorectal cancer compared with other Western countries (Trichopoulou et al., 2000). For example, colorectal cancer mortality in Greece is about 40% lower than in the United Kingdom (Ferlay et al., 2007). There is a 25-fold variation in the incidence rates of colorectal cancer between different countries in the world, which is highly suggestive of underlying environmental influences in the etiology of this disease (Parkin et al., 1999). It has been suggested that these underlying environmental factors are predominantly dietary and that up to 80% of sporadic colorectal cancers are therefore potentially preventable (Cummings and Bingham, 1998).

Diet and lifestyle are most likely related to colon cancer etiology through overconsumption of energy, coupled with inadequate intakes of protective substances, including micronutrients, dietary fiber, and a variety of phytochemicals (Levi et al., 1999; Watson, 2006). A diet high in red and processed meats can increase the risk by 12–20%, while high fish intake can lower the risk by up to 40%. It remains unclear whether these associations with meat are due to the constituents, such as cholesterol, heme and fatty acids, or products from preservation and cooking, including *N*-nitroso

compounds and heterocyclic amines. In contrast, a high fruit and vegetable intake decrease colorectal cancer risk as a result of the fiber and/or phytochemical components therein. Vegetable consumption is associated with having a greater effect on colon cancer while fruit has a greater effect on rectum cancer (Riboli and Norat, 2003). Other dietary products like milk, are suggested to have a protective effect (Cho et al., 2004).

AN OVERVIEW OF APOPTOSIS

In multicellular organisms, the total number of cells is a balance between the cell generating effects of mitosis and cell death that is induced through apoptosis. A disruption of this delicate balance can lead to various human diseases such as cancer (Kroemer et al., 2007). Therefore, dietary compounds that can restore the induction of apoptosis in precancerous cells may protect against cancer development and may have a chemopreventive or even a therapeutic potential (Watson, 2006).

Apoptosis is frequently defined mechanistically as a pathway of regulated cell death that involves the sequential activation of caspases, a family of cysteine proteases, and controlled both positively and negatively by BCL-2 family members (Degterev and Yuan, 2008). Moreover, programmed cell death is characterized by distinct biochemical and morphological changes that include cell rounding, membrane blebbing, cytoskeletal disassembly, chromatin condensation and DNA fragmentation (Kroemer et al., 2007). Inversion of the plasma membrane occurs so that the phosphatidyl serine that is usually restricted to the cytoplasmic face of the inner leaflet is exposed to the extracellular environment. *In vivo*, the externalized phosphatidyl serine and other induced signals allow recognition by phagocytic cells, which engulf apoptotic cells (Letai, 2008). As a result of the efficient mechanism for the removal of apoptotic cells an inflammatory response is not stimulated. *In vitro* apoptotic bodies swell and finally lyse (Degterev and Yuan, 2008).

The cellular alterations, which allow for packaging of the dying cell and its subsequent engulfment by neighboring cells or phagocytes, can be ascribed to the actions of caspases, a family of cysteinyl aspartate proteases that cleaved a wide range of cellular proteins (Kurokawa and Kornbluth, 2009). Caspases are synthesized as inactive zymogens and activated by proteolytic cleavage and can be classified into initiator and effector caspases. The initiator caspases comprise a subset of caspase family including caspases -8, -9, and -10, which are activated early in apoptotic signaling. Their cleavage targets are restricted and include effector caspases (Hengartner, 2000). The effector caspases, which include caspases -3, -6, and -7, are activated by initiator caspases after apoptotic signaling. The effector caspases cleave numerous target proteins, broadly distributed throughout the cell, resulting in the morphological changes that are characteristic of apoptosis (Hengartner, 2000).

Signalling Pathways in Apoptosis

Apoptosis is a genetically predetermined mechanism that may be elicited by several molecular pathways (Figure 25.1). The best characterized and the most prominent ones are called the extrinsic and intrinsic pathways (Kroemer et al., 2007). In the extrinsic pathway (also known as "death receptor pathway") apoptosis is triggered by the ligand-induced activation of death receptors at the cell surface. Such death receptors include the tumor necrosis factor (TNF) receptor-1, FAS (or CD95) and TNF-related apoptosis inducing ligand (TRAIL) receptors -1 and -2, which bind to specific ligands TNF, FASL, or TRAIL (Hengartner, 2000). This association leads to the receptor aggregation and recruitment of the adaptor molecules such as FADD or TRADD and caspase-8 to form the specific intracellular death-induced signaling complexes (DISC). Caspase-8 becomes activated and propagates apoptosis by direct cleavage of executioner caspases -3, -6, and -7, culminating in apoptosis (Degterev and Yuan, 2008).

Most cell death proceeds via the intrinsic or mitochondrial pathway, wherein proapoptic stimulus results in a net increase of free cytosolic cytochrome *c*, which is a particularly important event in the induction of apoptosis (Kroemer et al., 2007). Once cytochrome *c* has been released into the

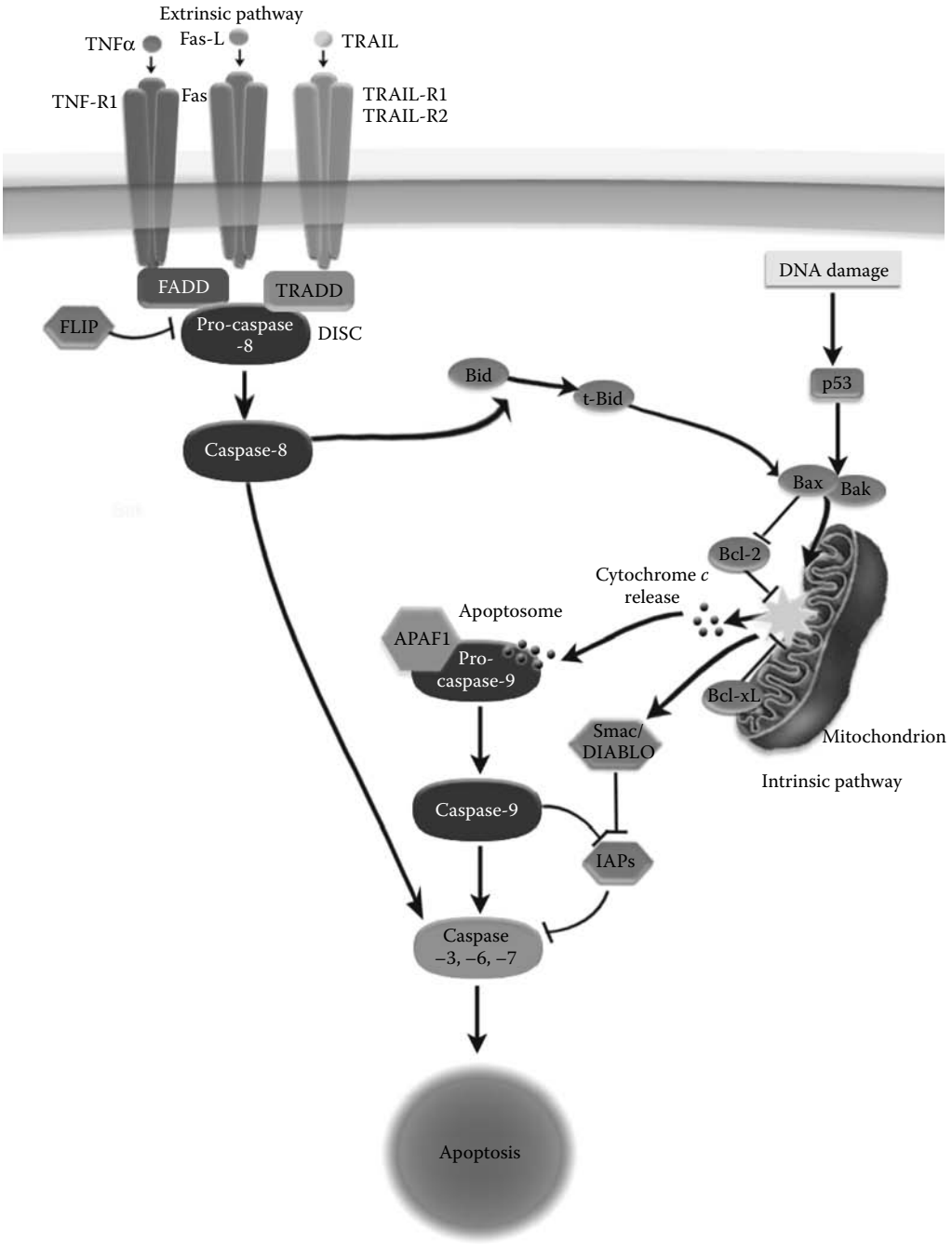

FIGURE 25.1 Signaling pathways in apoptosis. The extrinsic cell death pathway is initiated by recruitment of adaptor proteins, which binds to pro-caspases to generate a death-inducing signaling complex (DISC) that leads to activation of caspase-8. Caspase-8 directly cleaves and activates caspase-3, the executioner enzyme of apoptosis. The mitochondrial pathway is initiated by the release of apoptogenic factors such as cytochrome *c*. The release of cytochrome *c* into the cytosol triggers caspase-3 activation through the formation of the cytochrome *c*/APAF1/pro-caspase-9-containing apoptosome. The receptor and the mitochondrial pathway can be interconnected at different levels. Effector caspases cause cell-wide specific proteolysis and dysfunction, including the labeling of the cell with "eat me" signals, thus allowing the apoptotic cell to be recognized and engulfed by phagocytic cells.

cytosol it interacts with a protein called apoptosis activating factor-1 (Apaf-1). This leads to the recruitment of pro-caspase 9 into a multiprotein complex with cytochrome *c* and Apaf-1 called the apoptosome. Formation of the apoptosome leads to the activation of caspase-9 which in turn cleaves and activates downstream caspases, including caspase-3, caspase-6, and caspase-7, which carry out the execution phase of apoptosis (Letai, 2008). The ratio of pro-apoptotic and pro-survival meditors determines the relative permeability of the mitochondrial membrane to cytochrome *c*. When proapoptotic molecules Bax and Bak are translocated from the mitochondrial intermembrane, they result in a net increase of cytochrome *c* available to interact with the apoptosome. The effect of these proapoptotic molecules is mediated by either altering mitochondrial membrane permeability; by coupling of proapoptotic molecules with antiapoptotic factors (i.e., Bcl-2, bcl-xL, and Mcl-1), thereby neutralizing their antiapoptotic actions; or a combination of these (Vogelstein and Kinzler, 2004).

Release of second mitochondrial-derived activators (Smac/DIABLO) and Omi/HTRA-2 from the mitochondrial intermembrane neutralizes the actions of inhibitors of apoptosis (IAPs) such as cIAP1, CIAP2, and X chromosome-linked inhibitor of apoptosis (XIAP), thus causing a net stimulus of downstream caspases (Vogelstein and Kinzler, 2004). The mitochondrial and death receptor pathways communicate with each other at various levels. Activation of caspase-8 may led to cleavage of Bid, a protein of the Bcl-2 family, which in turn amplifies the cell death signal triggering cytochrome *c* release from mitochondria, thereby initiating a mitochondrial amplification loop (Letai, 2008).

OLIVES AND COLORECTAL CANCER

Different Mediterranean countries and regions have their own dietary traditions, but in all of them, table olives and olive oil occupy a central position and have been associated with a lower incidence of certain cancers, including colorectal (Levi et al., 1999). The ability of olive oil to modulate the risk of neoplastic diseases has been extensively studied in various animal models, human intervention and *in vitro* studies (Alarcon de la Lastra et al., 2001). This hypothesis has been supported by animal studies that showed a protective effect of olive oil against the UV induced damage of the skin (Budiyanto et al., 2000) and its ability in preventing the colon crypts aberrant foci growth and colon carcinoma in rats (Bartoli et al., 2000). Most of the health-promoting properties of olive oil have been attributed mainly to its high content of the monounsaturated fatty acid oleic acid, since diets rich in n-3 PUFAs and n-9 monounsaturated fatty acids (MUFAs) have been reported to inhibit colon carcinogenesis in both initiation and postinitiation phases (Bartoli et al., 2000; Rao et al., 2001; Reddy et al., 2005). However, recent evidence suggests that oleic acid on its own may not exclusively account for this beneficial activity (Llor et al., 2003) and other minor compounds are also implicated in the cancer chemopreventive activity. Thus elucidating the molecular mechanisms by which olive oil and its components impart their protective effects is crucial.

Although numerous data supports the protective activity of olive oil against colon carcinogenesis, few can be found regarding the role of olives, which also occupy a central position in the Mediterranean diet. The colon cancer chemopreventive activity of olives has been recently reported (Juan et al., 2006). An extract of the pentacyclic triterpenic acids present in the waxy coating of the olive fruit was obtained after successive extraction of olives with chloroform and methanol. The extract was analyzed by gas chromatography following the method described previously (Perez-Camino and Cert, 1999) and it mainly contained 1073 ± 122 mg/kg of maslinic acid, 377 ± 43 mg/kg of oleanolic acid, with traces of erythrodiol (Figure 25.2). The percentage of these compounds corresponded to 73.25% of maslinic acid and 26.75% of oleanolic acid (Juan et al., 2006). The antiproliferative activity of this compound was investigated in HT-29 human adenocarcinoma cells. The cells were incubated with increasing concentrations of this extract during 72 hours and displayed antiproliferative activity (Figure 25.3) with an EC_{50} value of approximately 74 μM of maslinic acid and 27 μM of olanolic acid (Juan et al., 2006). The inhibition of cell growth showed by the olive

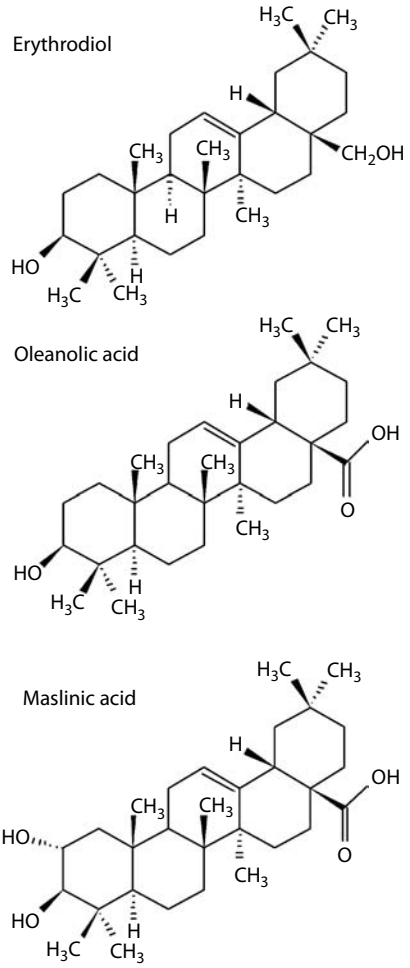

FIGURE 25.2 Chemical structures of maslinic acid, oleanolic acid, and erythrodiol.

fruit extract was not a consequence of cytotoxic effects, but appeared to result from either inhibition of cell growth or the induction apoptosis, or both. Treatment of HT-29 cells for 24 hours with different concentrations of the olive fruit extract induced the activation of caspase-3, which is generally accepted to be the major downstream effector caspase that cleaves major cell components during apoptosis (Hengartner, 2000). The activation of capase-3 was dose-dependent (Figure 25.4) and the minimal concentration with pro-apoptotic activity was 50 μM of olive fruit extract which caused a 200% activation compared with control cells (Juan et al., 2006). Execution of apoptosis beyond activation of caspase-3 by the olive fruit extract led to the characteristic hallmarks of programmed cell death, such as disintegration of the plasma membrane and inducement of nuclear fragmentation, as was evidenced by cell-staining with Hoechst 33342 dye and Hoechst 33258 staining (Figure 25.5). Confocal microscopic analysis revealed that the induction of apoptosis was preceded by an early increase in superoxide anion production in mitochondria of HT-29 cells. These cells were treated for four hours with 150 μM of olive fruit extracts (Figure 25.5). Although the mechanism is not yet fully elucidated, the release of cytochrome *c* and an increase in the production of superoxide anion in the mitochondria are considered to be crucial events in the induction of apoptosis induced by the olive fruit extract in HT-29 human adenocarcinoma cells (Juan et al., 2006).

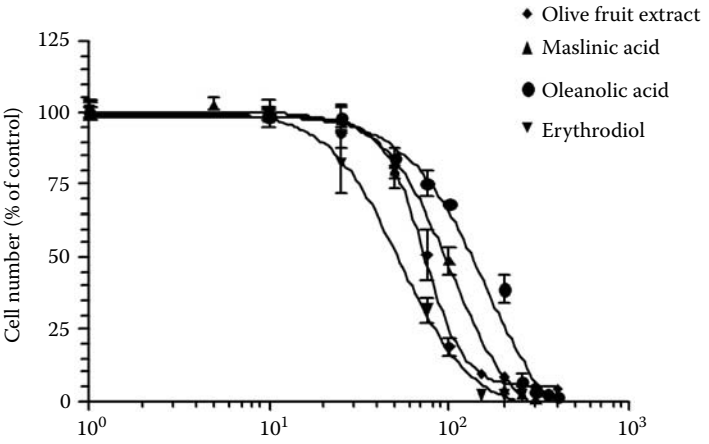

FIGURE 25.3 Effects of olive fruit extract and pentacyclic triterpenes on cell proliferation in HT-29 cells. Proliferation was measured over 72 hours in the absence (control) or presence of compounds at different concentrations, and cell numbers were determined subsequently using SYTOX-Green nucleic acid stain. EC_{50} values were derived from the dose–response relationship for three experiments and are given as mean ± SE. Olive fruit extracts, $EC_{50} = 74.0 \pm 3.2$ μM; maslinic acid, $EC_{50} = 101.2 \pm 7.8$ μM; oleanolic acid, $EC_{50} = 160.6 \pm 10.6$ μM; erythrodiol, $EC_{50} = 48.8 \pm 3.7$ μM.

BIOACTIVE TRITERPENOIDS FROM *OLEA EUROPAEA*

Pentacyclic Triterpenes in Olives

The olive fruit and leaf have long been known to contain a variety of sterols and triterpenoids (Vioque and Maza, 1963). Among the pentacyclic triterpenes stands out erythrodiol, oleanolic acid, and maslinic acid (Figure 25.2). These compounds are biosynthesized by squalene cycling, which gave erythrodiol as the first oxygenated derivative of β-amyrin in the pathway of nonsteroidal triterpenoids (Figure 25.6). Erythrodiol is the intermediate from which oleanolic acid and its isomer

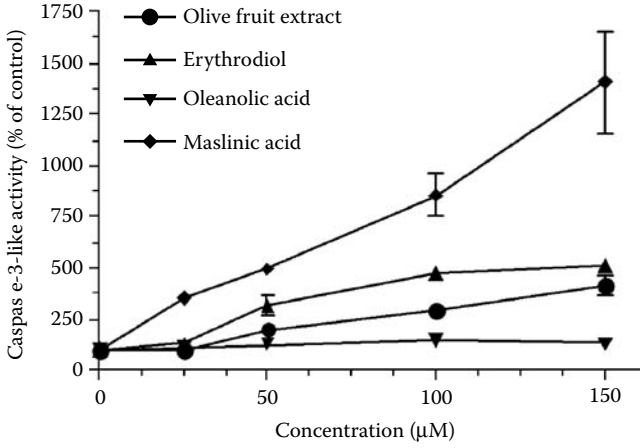

FIGURE 25.4 Effects of olive fruit extract and pentacyclic triterpenes on caspase-3 activity in HT-29 cells. Induction of caspase-3-like activity was determined after incubations with increasing concentrations of tests compounds for 24 hours. Thereafter, the cytosol of the cells was isolated and the activity of caspase-3 was evaluated based on the cleavage of the fluorogenic Ac-DEVD-AMC. Values are mean ± SE.

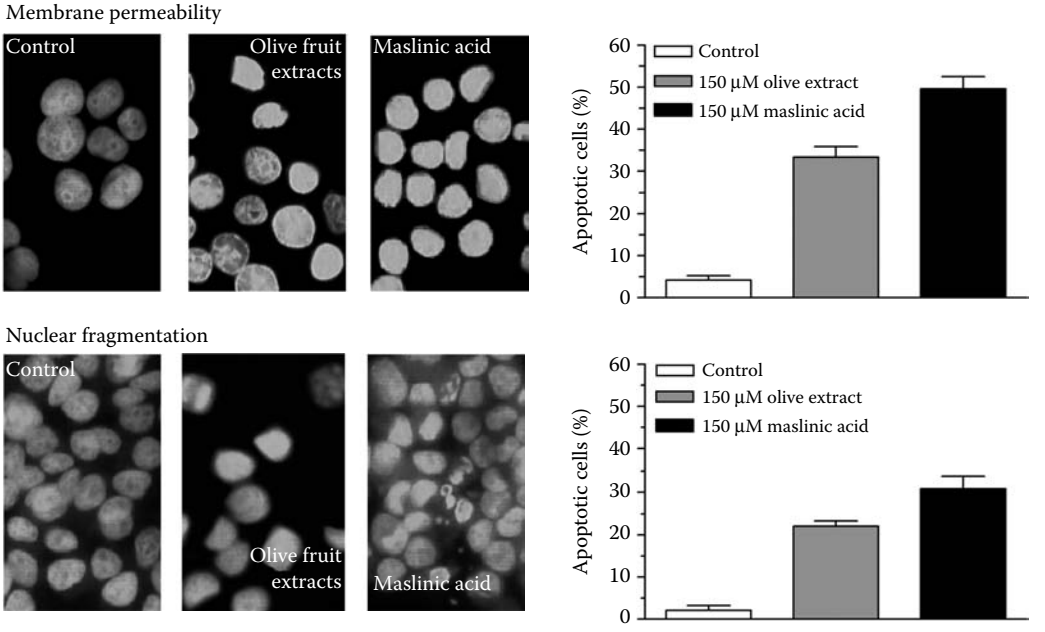

FIGURE 25.5 Effects of olive fruit extract and maslinic acid on early and late apoptotic events. Changes in membrane permeability, an early apoptosis marker, were determined by Hoechst 33342 staining in HT-29 cells incubated with 150 µM of olive fruit extract or 150 µM of maslinic acid for 24 hours. Nuclear fragmentation, as a late marker of apoptosis, was determined by staining DNA with Hoechst 33258. Cells that accumulated dye due to membrane disintegration or that displayed nuclear fragmentation, were counted and expressed as the percentage of apoptotic cells under control conditions or after the treatment. Values are mean ± SE.

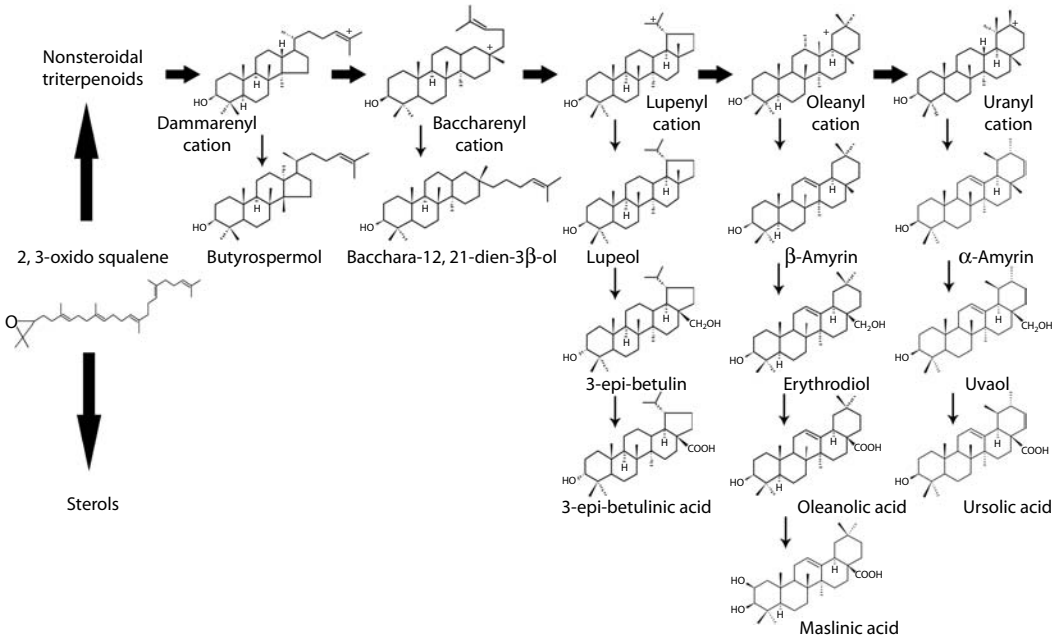

FIGURE 25.6 Biosynthetic pathway of nonsteroidal triterpenoids in *Olea europaea* fruit. The cyclation of 2,3-oxido squalene leads to the pathways of formation of sterols or nonsteroidal triterpenes.

maslinic acid are formed (Siti et al., 2007). In *Olea europaea*, these compounds protect the integrity of the olive fruit, as they act as insect antifeedants and antimicrobial agents (Vioque and Maza, 1963). Moreover, these non-nutritive dietary microconstituents are present in the skin of the fruits and absent either in the mesocarp or in the endocarp of the fruit (Vioque and Maza, 1963). Bianchi and coworkers (1994) characterized the cuticular lipid layer from olives indicating that maslinic acid was the main compound with amounts ranging from approximately 681 ± 63 mg/kg depending on the cultivar, followed by oleanolic acid with values of 420 ± 20 mg/kg, and erythrodiol with concentrations of 16 ± 1 mg/kg.

Pentacyclic Triterpenes in Olive Oil

Virgin olive oil is obtained in a process that involves pressing, which may dissolve the surface waxes of the fruit; consequently, part of oleanolic and maslinic acids may be transferred to the oil. However, although the presence of these compounds in olive fruits has been long known (Vioque and Maza, 1963) the data corresponding to olive oil is scarce. The amount of these compounds in the oil is much lower than in the fruit, and depends on the oil quality. Extra virgin olive oil with acidity less than 1%, contains 64.2 ± 8.1 mg/kg of maslinic acid and 57.2 ± 7.4 mg/kg of oleanolic acid, depending on the fruit variety (Perez-Camino and Cert, 1999). The hydrolytic processes that take place in the fruit during extraction facilitate the release of these triterpenes from the skin. Consequently, these values increased to 193.9 ± 14.0 mg/kg for maslinic and 244.0 ± 28.1 mg/kg for oleanolic acid in virgin olive oil (Perez-Camino and Cert, 1999). Erythrodiol is present in olive oil in concentrations of around 90 mg/kg (Blanch et al., 1998). A special source of this compound could be "orujo" olive oil which is obtained from the waste of olives, cuticle and leaves after cold-press extraction of virgin olive oil. This byproduct is richer in pentacyclic triterpenoids and contains erythrodiol in concentrations of up to 690 mg/kg (Perez-Camino and Cert, 1999; Albi et al., 1986). Actually, the total amount of the triterpenic alcohols, erythrodiol plus uvaol, is a quality parameter to detect adulterations with "orujo" olive oil in virgin olive oils.

MASLINIC ACID

Defects in the regulation of cell cycle progression are the most common feature of transformed cells (Watson, 2006) for that reason compounds that exert antiproliferative activities may serve as cancer chemopreventive agents. In HT-29 colon cancer cells, maslinic acid showed antiproliferative activity (Figure 25.3) with half-maximal effect for growth inhibition of around 100 μM (Juan et al., 2008a).

The effect of maslinic acid on apoptosis was evaluated in HT-29 cells (Juan et al., 2008a). This compound increased the activity of caspase-3 in a time- and a dose-dependent manner. The lowest concentration that induced an activation of this protease was 25 μM (Figure 25.4). At this concentration, caspase-3 activity was 350% compared with control cells and at 250 μM increased to 7100%. Maslinic acid alone induced an activation of caspase-3 with an activity superior to that exerted by the same concentration in the olive fruit extract. Apoptosis provides a protective mechanism against neoplasia by removing genetically damaged stem cells from the epithelium before they can undergo clonal expansion. Consequently, maslinic acid stands as a promising new compound for the chemoprevention of colon cancer, since one of the major goals in cancer therapy is to restore the sensitivity of transformed cells towards apoptotic signals and to allow the execution of apoptotic cell death (Watson, 2006). Execution of apoptosis beyond activation of caspase-3 led to the characteristic hallmarks of programmed cell death, such as disintegration of the plasma membrane that can be characterized by cell-staining with Hoechst 33342 dye (Figure 25.5). Maslinic acid increased the accumulation of the Hoechst dye 33342 in HT-29 cells over time. After exposing HT-29 cells to 150 μM of maslinic acid for eight hours, 14.8 ± 0.7% of cells showed accumulation of Hoechst 33342. This accumulation increased to 49.4 ± 2.9% following 24 hours of incubation (Juan et al.,

2008a). Activation of caspase-3 was followed by full execution of apoptosis with increased fragmentation of DNA and chromatin condensation, which was evidenced by Hoechst 33258 staining (Juan et al., 2008a). Apoptotic bodies were detected after 8 hours of incubation of HT-29 cells with 150 μmol/L maslinic acid in 9.7 ± 0.9% of cells, and after 24 hours nuclear fragmentation was detected in 30.8 ± 2.9% of HT-29 cells (Figure 25.5).

In cancer cells, there is a crucial role of reactive oxygen species (ROS) in cell growth and apoptosis. However, this role might be that of a double-edged sword. ROS can initiate cell transformation by causing alterations leading to mutations during DNA replication (Gackowski et al., 2002) whereas in already transformed cells ROS plays an important role in the initiation and execution of apoptosis (Wenzel et al., 2005; Kroemer et al., 2007). The balance of ROS and antioxidant levels therefore critically determines apoptosis in cancer cells and overcoming the antioxidative defense systems by accelerating ROS production could promote apoptosis (Wenzel et al., 2005). HT-29 cells exposed to 150 μM of maslinic acid showed markedly increased levels of superoxide anion radicals in mitochondria after four hours of incubation. Reactive oxygen species production occurs in an early phase suggesting that the compound triggers a rapid release of cytochrome *c* from mitochondria into the cytosol that in turn activates procaspase-9 and the downstream effectors, including the pro-caspases -3, -6, and -7, followed finally by the cleavage of proteins and DNA that characterize the final phase of apoptosis. Reactive oxygen species can affect divergent cellular functions depending on the cellular level and their compartmentation. Mitochondria are the primary cellular site of reactive oxygen species production and, under certain conditions, elevated mitochondrial ROS levels can serve as pro-apoptotic signals (Wenzel et al., 2005). As enzymatic and/or nonenzymatic antioxidant systems control ROS levels, the balance of ROS production and their removal in a given cell type is most critical also for the elimination of transformed cells that when allowed to bypass apoptotic elimination can lead to solid tumors. Therefore, dietary constituents promoting mitochondrial ROS-production could be as important in cancer prevention as dietary antioxidants.

OLEANOLIC ACID

Oleanolic acid also inhibited cell growth in a dose-dependent manner but in a lesser extent, causing complete inhibition at 320 μM (Figure 25.3). This growth inhibition may be attributed, at least in part, to cell cycle arrest, since oleanolic acid is involved in the G0/G1 checkpoint control and the inhibition of DNA replication in the human colon carcinoma cell line HCT15 (Li et al., 2002).

On the other hand, oleanolic acid did not activate caspase-3 even at the highest concentrations tested (Figure 25.4). The inability of this compound to induce apoptosis was also reported for the human colon carcinoma cell line HCT15 (Li et al., 2002). Although oleanolic acid has been described to inhibit tumor initiation and promotion steps its overall antitumor activity is relatively weak (Liu, 2005). For this reason, new synthetic analogs of this compound have been synthesized in order to enhance its potency (Suh et al., 1999). The failure to generate superoxide anions in mitochondria of oleanolic acid treated cells may explain the lack of apoptotic activity, shown by this triterpene (Juan et al., 2008a).

ERYTHRODIOL

Erythrodiol inhibited cell growth with a half-maximal effect at a concentration of around 50 μM. These results are particularly interesting since it is well known that defects in the regulation of cell cycle progression are the most common feature of transformed cells (Watson, 2006). Consequently, the antiproliferative actions of this pentacyclic triterpene indicate that it may serve as cancer-protective agent. It is important to mention that the growth inhibition was not a consequence of cytotoxic effects, since even at concentrations of 100 μM which caused complete inhibition of proliferation, less than 5% of cells were found to be nonviable (Figure 25.3) (Juan et al., 2008b).

Erythrodiol, which is a precursor of oleanolic and maslinic acid, also displayed pro-apoptotic activity in HT-29 cells (Figure 25.4). Caspases are specific proteases and it is generally accepted that caspase-3 is the major downstream effector caspase which cleaves major cell components during apoptosis (Hengartner, 2000). The pro-apoptotic activity displayed by erythrodiol was associated with a reduction in adherent cell number, and a dose-dependent increase in caspase-3 activity in adherent cells that started at 50 μM of erythrodiol with a maximal activation of 320% as compared with control cells (Juan et al., 2008b).

CONSUMPTION OF PENTACYCLIC TRITERPENES IN THE MEDITERRANEAN DIET

According to data from the literature, the mean daily consumption of table olives in the Mediterranean countries corresponds to approximately 40 g or 10 medium size olives. If the mean concentration of maslinic and oleanolic acids is 681 and 420 mg/kg, respectively (Bianchi, 1994), then the estimated intake of maslinic acid is 28 mg/day, and the intake of oleanolic acid is about 17 mg/day. Moreover, the contribution of virgin olive oil cannot be underestimated. The mean concentration of maslinic acid in virgin olive oil is 172 mg/kg and 231 mg/kg for oleanolic acid (Perez-Camino and Cert, 1999). The mean daily consumption of virgin olive oil is 33 g. Therefore, under these conditions, the total mean daily intake provided by habitual consumption of olives and virgin olive oil is 34 mg of maslinic and 25 mg of oleanolic acid. If the bioavailability of these compounds remains at 2.3% (Yang et al., 2005) as reported for triterpenoid 23-hydroxybethylinic acid, then the intestinal epithelium is exposed to high concentrations of these compounds. Whether it is estimated that 30% of these compounds are not absorbed and remained unaltered in the intestinal tract and reach the colon in 250 mL, the concentration of maslinic and oleanolic acids would be approximately 86 μM and 66 μM, respectively. These concentrations induced a 50% inhibition of cell proliferation and led to a threefold activation of caspase-3. Thus, the daily consumption of table olives and virgin olive oil could provide enough of these compounds to attain the described health-protecting properties.

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26 Fruit Phenolics in Colon Cancer Prevention and Treatment

Michael A. Lea

CONTENTS

INTRODUCTION

On a Western diet, colon cancer is the major type of gastrointestinal cancer. Epidemiological studies have indicated that a decreased incidence of colon cancer may be associated with a number of factors. These include increased consumption of fruits and vegetables, increased dietary fiber, increased exercise, increased intake of folic acid, calcium and vitamin D, and decreased fat consumption. Not all studies have been consistent and the relative importance of these factors is the subject of ongoing debate. This chapter will focus on a group of molecules found widely in fruits and vegetables that can be characterized as polyphenolic molecules. Evidence that they may have potential as either chemopreventive or therapeutic agents will be reviewed.

POLYPHENOLIC MOLECULES: THEIR NATURE AND DISTRIBUTION

Polyphenols constitute one of several groups of dietary phytochemicals that may act against cancer development (Waladkhani and Clemens, 2003). Polyphenols are widely distributed in fruits (Manach et al., 2004). The simplest dietary polyphenolic molecules are hydroxylated derivatives of benzoic acid such as protocatechuic acid and gallic acid. Other polyphenolic molecules with a single aromatic group include hydroxycinnamic acids such as caffeic and ferulic acids. There are types of polyphenols with two linked aromatic groups such as curcumin and resveratrol. A large group of dietary significant polyphenols have a structure with three rings including anthocyanins, flavones, and isoflavones. Polymers of these molecules exist and many occur as a variety of glycosides.

Estimates of daily consumption differ greatly but the total dietary intake could be as high as 1 g per day (Scalbert et al., 2005). The polyphenolic content of fruits vary and for a given type there are differences between cultivars and duration of development. Geographical and seasonal variation also occur. When considering the impact of dietary fruit polyphenolics, rates of consumption must be considered in addition to polyphenolic content. Thus, in studies in Poland (Cieslik et al., 2006) and France (Brat et al., 2006), it was found that apples may not be the fruits with the highest concentration of polyphenolics but apples can be the greatest dietary source of fruit polyphenolic compounds.

MAJOR FRUIT POLYPHENOLS

Phenolic Acids and Derivatives

Caffeic, chlorogenic, and ferulic acids have been examined for chemopreventive action against gastrointestinal tumors in rats. In a review of the literature on this topic, Nichenametla et al. (2006) noted the conflicting results. They concluded that at this stage phenolic acids should not be considered as anticarcinogenic compounds and that more organ specific studies were needed to establish the parameters of their cancer causation or prevention.

Ellagic acid and ellagitannins are derived from gallic acid. Pomegranate juice has the highest content of ellagitannins of commonly consumed fruit juices and as reviewed by Heber (2008) has exhibited some anticancer properties. While the evidence is most substantial against prostate cancer, a suppression of inflammatory cell signaling in colon cancer cells has been reported. Standardization of foodstuffs or dietary supplements always presents a problem. Zhang et al. (2009) tested 27 pomegranate supplements and found that only five had the typical pomegranate profile by HPLC, 17 had ellagic acid as the predominant constituent with minor or no detectable pomegranate tannins, and five had no detectable tannins or ellagic acid. The authors concluded that standardization of pomegranate extract supplements based on their ellagic acid content does not guarantee pomegranate supplement authenticity.

A pro-anthocyanidin fraction from apples was found to inhibit the proliferation of SW620 human colon cancer cells and to decrease the number of azoxymethane-induced aberrant crypt foci in the colons of rats (Gosse et al., 2005). The proanthocyanidin fraction decreased the activity of enzymes involved in polyamine synthesis and triggered apoptosis in SW620 cells.

Anthocyanins

Anthocyanins and their aglycones, the anthocyanidins, are the most abundant flavonoid molecules in fruits and berries. There is epidemiological evidence to suggest that anthocyanins may contribute health benefits and experimental data indicating a potential to contribute to chemopreventive efficacy against cancer (Hou, 2003; Cooke et al., 2005). Actions of anthocyanins include antioxidant, antimutagenic, antiinflammatory and proapoptotic activities. Structure–activity studies suggest that the numbers of sugar units and hydroxyl groups on the anthocyanidins are positively associated with the biological activities of anthocyanins (Hou, 2003).

Stoner and colleagues (Wang and Stoner, 2008; Stoner, 2009) have been prominent advocates of the development of berry preparations with high anthocyanin content as chemopreventive agents.

A feature of anthocyanins are their blue and red colors that give an immediate visual indication of their presence and an approximate measure of their concentration. Anthocyanin-containing fruit extracts have inhibited tumor development in the Apc^{Min} mouse model and have inhibited multiple biomarkers of colon cancer in rats (Wang and Stoner, 2008).

In a study of the action of extracts of anthocyanin-enriched plums and peaches, growth inhibitory effects were seen in several human colon cancer lines (Lea et al., 2008). In one of the cell lines there was evidence for increased differentiation as judged by increased activity of the enzymes alkaline phosphatase and dipeptidyl peptidase. The action of the fruit extracts was additive with the action of butyrate, an inhibitor of histone deacetylase, and with the action of U0126, a MEK1/2 inhibitor. Further fractionation of the extracts suggested that most of the growth inhibitory activity was in a fraction containing polyphenols other than anthocyanins. Similarly, McDougall et al. (2008) found that in lingonberry extracts the anthocyanin-rich fraction had considerably less antiproliferative activity against colon cancer cells than the tannin-rich fraction that was almost entirely composed of procyanidins. On the other hand, Jing et al. (2008) concluded that anthocyanins are the primary antiproliferative components against colon cancer cells present in anthocyanin-rich fruit extracts so there is conflicting evidence on the relative effectiveness of anthocyanins and other polyphenols.

Flavanols

The main flavanols are catechins and proanthocyanidins. The latter are polymeric flavanols. Grapes are a notable fruit source. Work on the anticarcinogenic action of catechins has been mainly performed with compounds in tea rather than molecules that are commonly found in fruits. Dietary grape seed proanthocyanidins decreased the incidence of azoxymethane-induced distal colon crypt foci in rats (Singletary and Meline, 2001).

Flavonols

The major flavonol in the human diet is quercetin, which is widely distributed in fruits and vegetables. Quercetin usually occurs in the form of glycosides including rutin. Rodent studies have shown that dietary administration of quercetin inhibits chemically induced carcinogenesis, especially in the colon (Murakami et al., 2008). Bischoff (2008) concluded that despite earlier concerns regarding mutagenicity quercetin has an excellent safety profile and its availability in highly purified form lends it to clinical trials. Some combination studies with quercetin and other agents have been performed and more can be anticipated.

Structure–activity studies with polyphenolics indicate different relationships depending on the end-point examined. With respect to the effects of flavonoid molecules on cell proliferation, the studies of Depeint et al. (2002) with HT29 human colon cancer cells pointed to a crucial role for the C ring of flavonols in which structural changes such as saturation, ring opening, loss of the carbonyl group or position of attachment of the B ring resulted in decreased activity.

Many human intervention studies have been performed with quercetin but these have usually been short-term in nature and without biomarkers that would indicate a preventive effect against colon cancer (Williamson and Manach, 2005).

Lignans

Lignans are most notably associated with flaxseed but they are commonly found in a variety of plant materials including fruits and berries. The plant lignans matairesinol and secoisolariciresinol did not protect against intestinal tumor formation when administered to Min mice (Pajari et al., 2006). This observation contrasted with some earlier work that suggested that lignans might have a protective role against colon carcinogenesis.

Resveratrol

Resveratrol is not a major fruit polyphenol in quantity but its presence in the skin of grapes and in red wine and early indications of a cancer-preventive action have encouraged much research

(Francy-Guilford and Pezzuto, 2008; Pezzuto, 2008). An impressive number of cellular effects have been reported for resveratrol and many of these are on recognized molecular targets in the control of cancer (Kundu and Surh, 2008).

Prolonged daily administration of resveratrol decreased the number of aberrant crypt foci in colorectal mucosa of rats treated with azoxymethane and a mechanism involving changes in *bax* and *p21* expression was suggested (Tessitore et al., 2000). Anticarcinogenic action of resveratrol in other experimental models for colon cancer especially the $Apc^{Min/+}$ mouse has been reviewed by Bishayee (2009). Decreases in cyclin D1 levels and cyclooxygenase activity together with changes in the ratio of pro-apoptotic and antiapoptotic members of the Bcl-2 family have been documented. Resveratrol is notable for the great variety of its effects (Harikumar and Aggarwal, 2008; Kundu and Surh, 2008; Pirola and Frojdo, 2008) and in addition to effects on signal transduction pathways, cell cycle regulation, and apoptosis it has an action that mimics caloric restriction. This has implications not only for decreasing cancer but also for increasing longevity. We can anticipate a continuing high level of research activity on resveratrol and resveratrol analogs.

BIOAVAILABILITY OF POLYPHENOLIC MOLECULES

The polyphenol content of many commonly consumed fruits were reviewed by Manach et al. (2004). One of the concerns in considering the chemopreventive or therapeutic potential of polyphenolic molecules is the limited uptake from the gastrointestinal tract and the low circulating concentrations that have been observed. Many polyphenolic molecules occur in fruits as glycosides that are typically thought to require conversion to the aglycone before absorption. However, there is evidence for transporters of quercetin glucosides. Inhibition of colon cancer cell proliferation was greater with quercetin 3-beta-glucoside than with quercetin or its rutinoside (Lea et al., 2009). In the case of colonocytes there will be direct exposure to polyphenolic molecules in the diet so that concentrations used in studies with cells in culture may be more relevant to the situation in the organism than for other cell types.

A review of 97 bioavailability studies in humans indicated that plasma concentrations of polyphenolic metabolites range from 0–4 μM (Manach et al., 2005). The polyphenols that were found to be most well absorbed in humans were isoflavones and gallic acid, followed by catechins, flavanones, and quercetin glucosides. The least absorbed dietary polyphenols were proanthocyanidins, galloylated catechins and anthocyanins.

Polyphenolic molecules are subject to metabolic modification by methylation, glucuronidation, and sulfation. There has been relatively little study of the action of these metabolites relative to the parent compounds.

EPIDEMIOLOGICAL STUDIES

Arts and Hollman (2004) concluded that data obtained at that time suggested beneficial effects of both flavonoids and lignans on cardiovascular disease but not on cancer with the possible exception of lung cancer. In a review by Ramos (2007), some epidemiologic studies suggested an inverse relationship between flavonoid consumption and the incidence of cancer at different sites including gastrointestinal cancer. The WCRF/AICR (2007) report noted that there is a substantial amount of evidence but it is inconsistent. The report concluded that there is limited evidence suggesting that fruits protect against colorectal cancer.

INHIBITION OF CARCINOGENESIS

Earlier work on dietary factors that may be associated with a lower risk of colon cancer were reviewed by Lipkin et al. (1999). These studies included human epidemiological work and investigations with laboratory animals. Among the factors considered were a variety of polyphenolic

molecules including some—such as quercetin and its derivatives—that are widely distributed in fruits and vegetables. Although quercetin inhibited colonic neoplasia induced by azoxymethanol in mice (Deschner et al., 1991) it did not decrease the number of tumors in mice with a mutation in the Apc gene (Mahmoud et al., 2000). In rats, Dihal et al. (2006) found that quercetin but not the glycoside, rutin, inhibited azoxymethane-induced colon cancer.

POSSIBLE MOLECULAR MECHANISMS FOR THE ACTION OF POLYPHENOLIC MOLECULES ON COLONOCYTES

The possible mechanisms for the anticarcinogenic actions of polyphenols presented in Table 26.1 are intended to be representative of the major effects that have been postulated but are not exhaustive. References are more to reviews than the primary literature.

There is some overlap in the above parameters and some actions may be secondary effects. However, rather than there being one primary effect it seems likely that there are multiple sites of action which may in combination contribute to a regulatory effect on cancer cells.

SELECTIVE OR NOT FOR TUMOR CELLS?

A selective action of polyphenolic molecules on transformed vs. untransformed cells would be an important feature for the use of these molecules in cancer chemoprevention or treatment. Selectivity is hard to establish in culture because untransformed cells generally do not grow well in culture and those that do grow well are likely to have acquired some transformed properties. Greater resistance to the growth inhibitory effects of anthocyanin-containing extracts have been recorded for the reportedly untransformed NCM460 human colon cells than for colon cancer cells (Malik et al., 2003; Zhao et al., 2004). However, in other studies, inhibition of proliferation in NCM460 cells by

TABLE 26.1
Direct or Indirect Actions of Polyphenols

Effects	Reference
Activation of sirtuins	Pezzuto (2008)
Antiangiogenic	Bhat and Singh (2008)
Antimutagenic	Nichenametla et al. (2006)
Antioxidant	Loo (2003)
Antiproliferative	Wolter et al. (2004)
Chelation of metals	Cilla et al. (2009)
DNA demethylation	Fini et al. (2007); Hauser and Jung (2008)
Down regulation of NF-κB	Nandakumar et al. (2008)
Inhibition of inflammatory targets including cyclooxygenases and lipoxygenases	Kim et al. (2009); Lee et al. (2007); Zykova et al. (2008)
Inhibition of matrix metalloproteinases	Ko et al. (2005)
Inhibition of metastasis	Kampa et al. (2007)
Inhibition of polyamine synthesis	Wolter et al. (2000)
Inhibition of protein kinases and growth signal transduction	Lopez-Lazaro (2002); Walker et al. (2000)
Inhibition of telomerase	Kampa et al. (2007)
Modulation of carcinogen metabolism	Nichenametla et al. (2006); Yang et al. (2001)
Modulation of steroid and growth factor signaling	Kampa et al. (2007)
Pro-apoptotic	D'Archivio et al. (2008); Martin (2006); Ramos (2007)
Pro-oxidant	Loo (2003)

anthocyanin-containing fruit extracts was similar to that seen in colon cancer cells (Lea et al., 2008). Preferential uptake of polyphenols in tumor tissue would be advantageous for chemoprevention or treatment. After administration of an anthocyanin-rich preparation to human subjects for seven days the concentration of anthocyanins in colon tumor and normal colorectal tissue taken either proximal or distal to the tumor was 0.40, 0.22 and 0.28 nmol/g, respectively (Thomasset et al., 2009).

PRO-APOPTOTIC EFFECTS OF POLYPHENOLIC MOLECULES

In reviewing the targeting of apoptosis with dietary agents including polyphenols, Martin (2006) voiced an opinion that has been presented by several authors that combinations of dietary agents may be more effective than single agents. Effects of dietary flavonoids on apoptosis were reviewed by Ramos (2007) who concluded that common effects involved release of cytochrome *c* from mitochondria, activation of caspases, and regulation of Bcl-2 family members. There was also inhibition of survival/proliferation signals. There is evidence that polyphenols can exert effects on both the extrinsic death receptor pathway and on the intrinsic mitochondrial pathway.

ANTIOXIDANTS OR PROOXIDANTS?

The antioxidant potential of polyphenols has been widely recognized and this has prompted advertising claims that fruit juices with high polyphenol content will destroy free radicals. While dietary polyphenols are generally characterized as antioxidants, they can under appropriate conditions have a pro-oxidant action. Both of these actions have been invoked as potentially being of value against cancer cells. Reactive radicals that can cause mutations in DNA may be important in carcinogenesis and polyphenols that can scavenge free radicals have an obvious attraction as possible chemopreventive agents. Reactive oxygen species may be important not only in the initiation of cancer but also in its progression. It has been suggested that in highly invasive or metastatic cancer large but tolerable amounts of hydrogen peroxide may function as signaling molecules to activate redox-sensitive transcription factors that are involved in the survival and proliferation of cancer cells (Loo, 2003). On the other hand, when polyphenols cause the production of excessive levels of hydrogen peroxide the antioxidant defenses of the cancer cell may be overwhelmed resulting in an inhibition of proliferation and in cell death. The challenge would be to recruit the antioxidant properties in the early stages of carcinogenesis and to use the pro-oxidant action against established cancer cells. We know that metal ions can enhance the pro-oxidant action of polyphenolic molecules but our ability to exert a fine control seems at present to be very limited.

Structure–activity studies of flavonoids indicate that structural determinants can differ for antioxidant and prooxidant activities. Cao et al. (1997) found that the conjugation between rings A and B did not affect the antioxidant activity but was very important for the copper-initiated prooxidant action while *O*-methylation decreased both antioxidant and prooxidant activities of the flavonoids.

There has been some debate on whether polyphenolic-induced hydrogen peroxide formation is a factor in the antiproliferative activity of fruit extracts and polyphenolic molecules and whether that should be regarded as an artifact (Liu and Sun, 2003; Lee et al., 2005). In part this discussion rests on methodology with respect to the assay of hydrogen peroxide and appropriate levels of catalase that may prevent the effect. It appears that hydrogen peroxide formation may at least contribute to apparent antiproliferative effects of polyphenols and should be considered in evaluating effects on growth.

If polyphenolic molecules do exert an antioxidant action then there is some question as to where it is exerted. Halliwell et al. (2005) have suggested that greater attention should be directed to effects within the gastrointestinal tract rather than after absorption and that more attention should be directed to the role of flavonoid metabolites.

HUMAN INTERVENTION STUDIES FOR COLON CANCER PREVENTION

Clinical trials on cancer chemoprevention by polyphenols are more advanced for polyphenols from tea and soy than for those polyphenols that are most prevalent in fruits (Thomasset et al., 2006). The suitability of anthocyanins for development as cancer chemopreventive agents was reviewed by Thomasset et al. (2009) who noted that a variety of fruit extracts have been shown in animal models to reduce the formation of aberrant crypt foci.

Combinations of cancer chemopreventive agents have been advocated (Francy-Guilford and Pezzuto, 2008) and there is evidence that combinations of polyphenolic molecules may have additive or synergistic effects.

In addition to studies on colon cancer incidence, the influence of dietary flavonoids on colon cancer recurrence has been examined. In contrast to some earlier studies that had found no association, Bobe et al. (2008) obtained evidence that a flavonol-rich diet may decrease the risk of advanced colorectal adenoma recurrence. Their results suggested that the protective effect might be achieved only at flavonol levels that are higher than what is commonly consumed by Western populations.

A frequently voiced concern regarding polyphenolic molecules is the achievement of human tissue concentrations that would approximate those frequently used in experimental models. A pilot study by Thomasset et al. (2009) examined uptake in colorectal tumors of anthocyanins over a seven-day period in patients receiving an anthocyanin-rich preparation from bilberries (Mirtocyan). The authors concluded that repeated administration of bilberry anthocyanins exerted pharmacodynamic effects and generated concentrations of anthocyanins in humans resembling those seen in studies with Apc^{Min} mice. It might be noted that the Mirtocyan preparation contained 36% (w/w) of anthocyanins but it also contained about 18% of other polyphenolic molecules so that evidence for a small decrease in proliferation might be attributable to other polyphenolic molecules. In a thoughtful commentary on the study by Thomasset and colleagues, Meyskens (2009) welcomed their work but considered the many difficulties inherent to chemoprevention trials. He and the authors noted that similar plasma anthocyanin concentrations have been achieved in mice and humans but the concentrations in human tissue were 1/10 to 1/20 of those in mice. This raises a concern on whether mice are a good model for the processing of anthocyanins in humans. There is clearly a need for more extended studies of this type. Meyskens (2009) outlined the background information and design elements that will be important in future chemoprevention trials. These included strong epidemiologic and preclinical experimental data. The trials should be well characterized and use reproducible agents at reasonable doses for an adequate duration. Ideally, normal and tumor tissue should be compared and markers of biological effects should be well established with adequate numbers of subjects and appropriate controls.

CHEMOTHERAPEUTIC POTENTIAL

The flavonol, quercetin, was the lead compound for the development of some kinase inhibitors (Ferry et al., 1996; Walker et al., 2000). Several kinases may be affected including phosphoinositide 3-kinase and some protein kinases. In combination with chemoradiotherapy, quercetin facilitated the elimination of HT-29 colorectal cancer xenografts in mice at dose levels that appear tolerable in humans (Priego et al., 2008).

On the basis of their cancer chemopreventive efficacy and their superior stability as compared to that of the aglycones, Thomasset et al. (2009) suggested that anthocyanins seem much more suitable for further drug development than their anthocyanidin counterparts. The animal model studies that supported this conclusion included anthocyanin preparations from cherry, bilberry, raspberry, chokeberry and grape and the parameters followed in mice and rats included aberrant crypt foci, adenoma number, and colon tumor number and burden.

ADDITIVE OR SYNERGISTIC EFFECTS MEDIATED THROUGH INHIBITION OF PROTEIN KINASES AND HISTONE DEACETYLASES

There is evidence that additive or synergistic effects on colon cancer cell proliferation and differentiation can be obtained with combinations of inhibitors of protein kinases and histone deacetylases (Lea et al., 2007). Inhibitors of MAP kinase and phosphatidyl 3-kinase signaling pathways may be particularly relevant. Quercetin and related flavonoid molecules can serve as inhibitors of critical protein kinases and in combination with histone deacetylase inhibitors such as butyrate or valproate could act on colon cancer cells to inhibit cell proliferation and induce differentiation. This is illustrated in Figure 26.1 for the combined action of valproate and quercetin 3-glucoside for

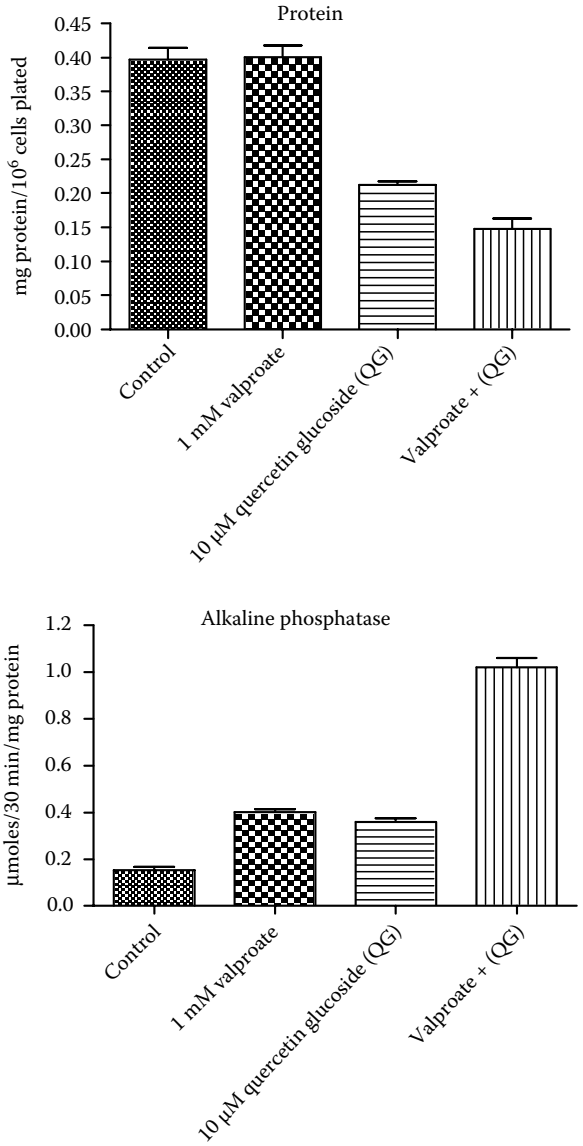

FIGURE 26.1 Effects of incubation of Caco-2 cells for 72 hours with valproate and/or quercetin 3-glucoside on protein synthesis and alkaline phosphatase activity.

72 hours on Caco-2 cell proliferation as judged by protein amount and differentiation as monitored by alkaline phosphatase activity (M.A. Lea and C. Ibeh, unpublished observation).

Butyrate is the prototype for a large group of carboxylic acids that have inhibitory activity for most histone deacetylases. It is notable that that this group includes some phenolic acids such as chlorogenic acid (Bora-Tatar et al., 2009). Combined actions on protein kinases and histone deacetylases could contribute to enhanced effects that have sometimes been seen with polyphenolic mixtures relative to single polyphenolic molecules.

TOXICITY OF POLYPHENOLS

Since there are circumstances in which polyphenolic molecules enhance the formation of reactive oxygen species, it may be anticipated that there will be circumstances in which polyphenols are potentially mutagenic or increase carcinogenesis. Pereira et al. (1996) noted previous reports of genotoxic activity of quercetin and they observed an increase in the incidence and multiplicity of azoxymethane-induced adenocarcinomas in rats. This observation is contrary to some other studies considered above and below in the present review but supports the view of Pereira et al., that until the activity of quercetin is better understood, its further evaluation as a chemopreventive agent should proceed with caution.

For all substances there will be a toxic dose limit. The consensus seems to be that normal dietary levels of polyphenols have a good safety record but supplements with the potential to greatly increase consumption should be used cautiously and with adequate testing (Mennen et al., 2005).

CONCLUSIONS

The epidemiological evidence for a beneficial effect of fruit consumption on general health is quite good even if support for a specific benefit of a decrease in colon cancer is not strong. In addition to traditional nutrients, fruits provide a good source of polyphenolic molecules. Whole fruits or dried preparations are a better source of polyphenolic molecules than fruit juices but the latter may be a useful and pleasant contribution to the diet. An advantage of taking plant polyphenolics in the form of whole fruit or juices is the combination with sugar. Polyphenolic compounds can impart an astringent or bitter taste that in excess can be unpleasant. In the development of plant cultivars with high concentrations of polyphenolic molecules, palatability can be a limiting factor. The history of dietary supplements in the prevention of cancer has had a number of setbacks. Until we have stronger evidence for the beneficial effects of specific polyphenolics, it seems too soon to make strong recommendations to the public. The traditional wisdom of an apple a day keeping the doctor away comes with no guarantees but apples and other fruits do offer good sources of a variety of polyphenolics. These are provided at a dose level that is unlikely to present a toxic hazard and may well contribute to good health.

ACKNOWLEDGMENTS

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27 Diet, Epigenetics, and Colonic Fermentation and Their Role in Colorectal Cancer

Ramesh P. Arasaradnam and K.D. Bardhan

CONTENTS

INTRODUCTION

Diet and its relation to cancer is a fascinating story of discovery, opportunity, blind alleys, and tantalizing clues—but with many gaps.

Colorectal cancer (CRC) occurs far more frequently in Western populations than in other races suggesting genetic differences play a crucial role in determining susceptibility. However, study of disease prevalence in migrants from the East to the West demonstrated the major role of "environment"—for such migrants were found to develop CRC at rates approaching those of the native population. We commonly think of the environment as the physical world around us. In gastrointestinal disease however, the "environment" is the gut lumen with its changing dietary contents, its resident bacteria (a generally stable population) and the interaction between these which

leads to the creation of new chemicals (as opposed to those which have been ingested). Such chemicals in turn can affect the DNA of the colonic epithelial cells (colonocytes). As a result the genetic message can be subtly changed.

Genetic predisposition plays a key role in the development of CRC but only in a small proportion of the population: for the majority the single most important factor aging. This is intuitively understandable for with increasing age, more time has elapsed for changes of the genetic message to occur. With age there is also progressively diminishing efficiency in correcting these transcription errors. Consequently "hits" accumulate and may subtly change the genetic message. Once a "threshold" has been crossed, the altered message leads to excessive colonocyte proliferation which manifests as polyps. In some, such proliferation becomes uncontrolled, that is, it is now a cancer.

The message can also be altered from "epigenetic" effects, when simple chemicals created in the gut lumen as a result of fermentation attached to the genome. For example, the insertion of a methyl group can halt the genetic message without actually altering the basic genetic sequence.

While recognizing the importance of genetic influences and of the huge strides made in our knowledge in this area, we would like to elaborate more on the gut "environment" and its consequences; an area where there is now accumulating information. We also draw attention to different approaches to examining gut fermentation, which we have reason to believe is the key factor which influences such environment.

COLORECTAL CANCER—EPIDEMIOLOGY

Colorectal carcinoma (CRC) is the most common gastrointestinal tract malignant lesion and is responsible for 20,000 deaths per year in the United Kingdom (Cancer Research UK, 2009). Almost 30,000 new cases are diagnosed per annum with an average 40% five-year survival. Most do not have symptoms until the cancer is advanced and in 95% of patients with CRC in the United Kingdom, the disease is usually advanced at presentation (Duke's stages B–D) (DOH, 1998) Globally, the majority of CRC cases occur in developed countries, particularly North America and Western Europe. Since the adoption of Western dietary habits in Japan, the incidence and mortality rates of CRC have increased markedly. Observational studies lend support to the influence of environmental factors which demonstrate an increase in colorectal cancer incidence among migrants from low- to high-risk countries, compared with age and sex matched controls (McMichael et al., 1988). This observation has been further confirmed among Japanese descendants living in the United States who experience risks of developing colorectal cancer similar to those of the U.S. population. In effect, this equates to a fivefold population-based difference in risk over half a century, purely due to a change in environment (Willett, 2002).

Effect of Age and Genes on CRC

The risk of developing colorectal cancer increases with age and has been suggested to be the most important risk factor (DePinho, 2000). Trends for age-specific incidence rates for colon cancer in the United Kingdom are shown in Figure 27.1. Of note, newly diagnosed cancer rates for CRC increase exponentially after the age of 40. This steep increase continues as aging advances. This may reflect the time needed for the adenoma-carcinoma sequence to be completed as first hypothesized by Morson (1974). In the aged, a critical threshold of random accumulation of somatic mutations is thought to be reached leading to the development of cancer but it is more likely due to a combination of effects such as cumulative mutational load, epigenetic gene silencing, telomere dysfunction and even altered stromal environment (DePinho, 2000). The perceived key genes regulating this process have been studied but with inconsistent results. Part of the reason for this has only recently been made apparent in a report identifying novel genes (at least 200) which are mutated in colorectal carcinogenesis (Sjoblom et al., 2006).

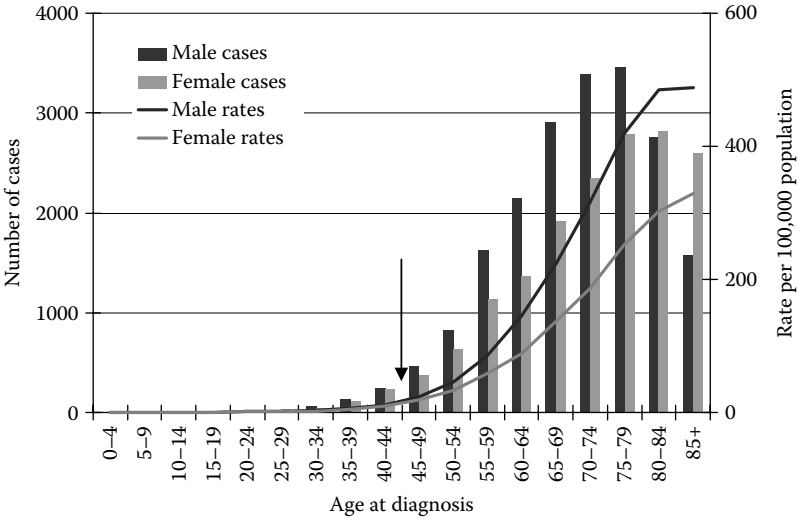

FIGURE 27.1 A number of new CRC cases and age-specific incidence rates by sex in the United Kingdom (2004). Black arrow denotes exponential increase in age-specific incidence of CRC after the age of 40 (http://www.cancerresearch.co.uk).

INFLUENCE OF DIET AND COLORECTAL CANCER RISK

The importance of environmental exposure (especially diet) is highlighted by the fact that only a small proportion of cancers can be attributed to germ line mutations. With economic development, dietary patterns have altered globally with more dietary energy available from animal sources. Following Burkitt's seminal observations, major studies were carried out by Doll and Peto (1981) from which they concluded that diet caused cancer in up to 30% of the population in the United States and other industrialized countries. This figure was subsequently confirmed by the WCRF/AICR report in 1997 stating that up to a third in the variance of cancer incidence between populations can be attributed to habitual variation in diet. This has been further reaffirmed with data describing changes in rates of different cancers including colon cancer within genetically identical populations that emigrate from their native country to settle in other countries (WCRF/AICR, 2007).

Dietary Fiber

The role of dietary fiber and its protective effect against colorectal adenomas and CRC has been the subject of much debate in the last decade. Current epidemiological evidence is conflicting with some investigators finding dietary fiber reduces risk (Bingham et al., 2003) while others failed to find any protection (Park et al., 2005). This may be explained by the differing analytical methods, particularly in the types of questionnaires used to recollect details of food consumed from periods ranging from the previous 24 hours to one month earlier. Another reason is that dietary fiber/nonstarch polysaccharide (NSP) is a heterogeneous group. Depending on its makeup such fiber is fermented by resident gut microflora to form either propionic acid, butyric acid and acetic acid, or a varying mixture of all three (Arasaradnam et al., 2004). Butyric acid being the main short chain fatty acid (SCFA) is produced in millimolar quantities in the colonic lumen and has multiple roles in gut health. Some of the differences between two key studies with conflicting findings are tabulated in Table 27.1.

A similar problem arises in the intervention studies of dietary fiber using adenomas as a surrogate end point. Earlier studies in 2000 showed no benefit with dietary fiber supplementation (Alberts et al., 2000; Bonithon-Kopp et al., 2000) while a later study in 2003 did (Peters et al., 2003). The total quantity of fiber (g/day) consumed was low and types of fiber studied were different. The

TABLE 27.1
Comparison between the Two Largest Studies Studying the Effect of Fiber and CRC

Study Characteristics	Bingham, Day et al., 2003—EPIC Study	Park, Hunter et al., 2005
Study design	Prospective study	Pooled analysis of prospective studies
Number in study	519,978	720, 628
CRC cases	1065	8081
Mean (energy adjusted) fiber intake	Lowest quartile: 12–25 g/day Highest quartile: 31–91 g/day	Lowest quartile: 17–20 g/day Highest quartile: 20–41 g/day
Source of fiber	Cereals	Fruit and Vegetables
Adjusted covariates	Physical activity, smoking, alcohol, red meat, folate	Age, multivitamin use, red meat, alcohol, physical activity, smoking and folate
Findings	40% reduction in risk (9% reduction in risk after adjustment for folate)	Reduction in CRC risk—lost after adjustment for folate intake; inverse relationship lost after 5 year latency period (period between dietary assessment and outcome)
Limitations	Small number of CRC cases? Underestimate of true CRC risk; short follow-up period	Use of variable food frequency questionnaires

Note: European Prevention in Cancer.

studies in 2000 used ispaghula husk, wheat-bran, and fruit and vegetables while the later positive study used grains, cereals, and fruits. These compounds have a range of properties and functions according to their "fermentability." Fibers that are totally nonfermentable (for humans, unlike animals, lack the necessary microflora) have poor antitumor potential in *in vivo* models (Roediger, 1982). In contrast, it is the fibers which are fermented but only to a limited extent that yield products along the entire length of the colon and afford protection. Thus, it is evident that analyzing specific short chain fatty acids (SCFAs) is likely to give more consistent observations. Dietary fiber is believed to protect against CRC development through physical (nonfermentation) actions as well as from the products of their fermentation. The former include binding of bile acids, dilution of faecal carcinogens, and accelerating colonic transit of faeces. Fermentation by microbes results in SCFAs which we increasingly recognize have a powerful protective effect.

Meat and Fish

There is good evidence that consumption of red meat increases the risk of CRC, but the degree of risk identified varies across the studies. The Nurses Health Study in 1990 suggested a link between red meat intake and increased risk of colorectal cancer (Willet et al., 1990). Others have shown a 50% higher risk of distal colon cancer in those with high long-term consumption of meat. Similarly, the EPIC study (Norat et al., 2005), which is the largest prospective nutritional study to date, has shown a 35% greater risk of developing colon cancer in those who consumed red and processed meat (~180 g/day). This association was particularly strong in individuals with a low dietary fiber intake. In addition, the EPIC study also confirmed the previously held view of an inverse relationship between oily fish intake and risk of colorectal cancer. In the Health Professionals Study (Wu et al., 2006), higher levels (although not statistically significant) of meat-derived mutagens were shown to be associated with increased risk of distal colonic adenoma.

Some of the proposed mechanisms for the observed risk between red meat consumption and CRC risk is through increase in colonic genotoxic load due to exposure of carcinogens such as

heterocyclicamines and benzo-α-pyrenes as well as production of endogenous intestinal nitrosamines from interaction with microbes. Heterocyclicamines and benzo-α-pyrenes are formed mainly during certain methods of food preparation (exposure to naked flame). Additionally, single and double strand DNA breaks have also been shown to be associated with red meat diets, but the mechanism is unknown.

Fruits and Vegetables

Although fruits and vegetables are beneficial for general health there is conflicting evidence that they afford any protection against developing CRC. Epidemiological evidence from Block et al. (1992) showed a doubling in risk of cancers in those with a low intake of vegetables (after controlling for confounding factors). Similar protective effects against colorectal cancer were also noted in the WCRF/AICR (1997) report. However, subsequent prospective studies failed to find any protective effect of fruit and vegetable consumption against colorectal cancer. As such, the International Agency for Research on Cancer (IARC, 2003) concluded that there was limited evidence for cancer-preventive effects of consumption of fruit and vegetables against CRC. This view is upheld to some extent by the recent WCRF/AICR (2007) report although the panel acknowledges that there is a clear dose–response relationship with dietary fiber providing the strongest (relative) protective effect against CRC.

There are several reasons for this null effect of vegetable consumption and colon cancer risk. Consumption of fruit and vegetables lead to the production of secondary plant metabolites. These phytochemicals are complex compounds with several anticarcinogenic properties. At the molecular level, phytochemicals show promise in cancer prevention as they exert important biological actions including alteration in gene expression, modulation of DNA repair, inhibition of carcinogen formation, and stimulation of apoptosis (Johnson, 2007). The principle limitation is their poor absorption in humans. As a result their bioavailability is compromised.

Calcium and Vitamin D

Dietary calcium has been known to exert a protective effect on colorectal adenoma formation as well as CRC. Most prospective studies (Wu et al., 2002), have shown an inverse relationship between calcium intake and colorectal cancer. The Women's Health Initiative (Wactawski-Wende et al., 2006) showed an additional feature, namely, a threshold effect. Women were assigned to receive 1000 mg of calcium carbonate and 400 IU of vitamin D3 daily. A protective effect was found up to 600 mg, but none beyond. In colorectal adenoma recurrence trials, higher intakes of dietary calcium were associated with reduced risk of adenoma recurrence compared to levels associated with colorectal cancer. In the Calcium Polyp Prevention Trial (Baron et al., 1999), a 15–19% reduction in adenoma recurrence was observed with supplementation of 1200 mg of calcium. Interestingly, this protective effect held for as long as 5 years after cessation of supplementation. Thus it would appear that the protective effect of calcium is dose-dependent in the prevention of colorectal adenomas and CRC. *In vivo* and *in vitro* studies have shown that calcium has (a) anticarcinogenic properties due to its ability to bind to potentially carcinogenic bile acids, (b) ability to reduce cellular proliferation, and (c) increase cellular apoptosis.

Data on the protective role of vitamin D is growing. Earlier epidemiological studies were insufficient to conclude on the protective effects of vitamin D on colon cancer. However, a large study of patients with skin cancer has shown a lower incidence of secondary solid organ cancer, for example, colon cancer in those with high sun exposure. A meta-analysis of case–control studies has revealed a 50% lower incidence of CRC in individuals with >82 nmol/L of vitamin D (Gorham et al., 2005). Epidemiological studies on vitamin D are difficult as they rely on status measurements (dietary intake) and cannot account for vitamin D from sun exposure. In postmenopausal women, calcium and vitamin D supplementation was shown to result in a 20% reduction in cancer risk (including

colon cancer) and improved survival. Vitamin D3 (activated following hydroxylation in the liver to form 1,25-dihydroxyvitamin D3) has been shown *in vitro* to regulate cellular differentiation and induce apoptosis *in vivo*. These protective cellular effects of vitamin D thus pave the way for a potential therapeutic role as anticancer agents.

EPIGENETICS—DNA METHYLATION AND COLORECTAL CANCER

Epigenetic alterations are heritable alterations in gene expression not mediated by alteration in DNA sequence, that is, the message is altered but not the gene itself (Jaenisch et al., 2003). Such changes include DNA methylation and are among the most common molecular alterations in human cancers including colorectal cancer. The addition of a methyl group to the carbon-5 position of cytosine residues is the only common covalent modification of human DNA. It occurs almost exclusively at cytosines that are followed immediately by guanine (CpG dinucleotides). The majority of the genome displays a depletion of CpG dinucleotides and those that are present are nearly always methylated. Conversely, small stretches of DNA known as *CpG islands* (usually located within the promoter regions of human genes), while rich in CpG dinucleotides, are nearly always *free* of methylation. Methylation of CpGs within these *islands* are associated with transcriptional inactivation of the corresponding gene, which appears to be tissue specific. In CRC for example, aberrant methylation resulting in gene silencing can occur in important tumor suppressor genes such as $p16^{INK4a}$ (Merlo et al., 1995). Both genomic DNA hypomethylation as well as gene-specific (promoter) hypermethylation have been observed in CRC. DNA methylation is vital in controlling gene transcription through histone modification. Such conformational changes can induce either activation through acetylation for example or repression due to methylation of a histone residue—see Figure 27.2.

Although the methylation profile within the human genome is as yet undetermined, it is estimated that 70% of CpG dinucleotides are methylated in mammals. Hypermethylation in gene promoter regions are associated with transcriptional silencing, which is at least as common as inactivation of tumor suppressor genes through DNA mutations (Jones et al., 2002). Aberrant hypermethylation is thought to be an early event in CRC as it is detectable in early precursor lesions, for example, aberrant crypt foci (ACF). Virtually all pathways in colorectal carcinogenesis, for example, loss of control of cell cycle regulation ($p16^{INK4a}$, $p14^{ARF}$), silencing of DNA mismatch repair genes (*hMLH1,* O^6*-MGMT*), possible loss of function of apoptosis genes (*DAPK, APAF-1*), and altered carcinogen metabolism (*GSTP1*) involves *hyper*methylation (Esteller, 2002). Both genomic hypomethylation and CpG island aberrant methylation are known to occur simultaneously in CRC.

Age-Related DNA Methylation

Genomic loss of methylation has been known to occur with aging. This phenomenon has yet to be shown in the *normal* aging colon until recently (Arasaradnam, 2007). Most of the attention surrounding DNA methylation has been centered on gene-specific methylation. In the colon, age-related methylation accounts for 70% of aberrant gene-specific methylation events. This process is thought to be a nonstochastic, that is, predictable event and is tissue specific. For example, methylation of the *ESR1* (tumor suppressor gene) promoter has been shown to increase with age in normal human colon and is present also in adenomas thus supporting the idea that epigenetic gene silencing is an early event (Issa et al., 1994).

DNA Methylation and Dietary Factors

Epigenetic changes such as DNA methylation are potentially reversible and gene expression can be reestablished. The active ingredient in green tea [(–)epigallocatechin-3-gallate (EGCG)] provides a good example of reversal of aberrant methylation with a dietary food component reaffirming the

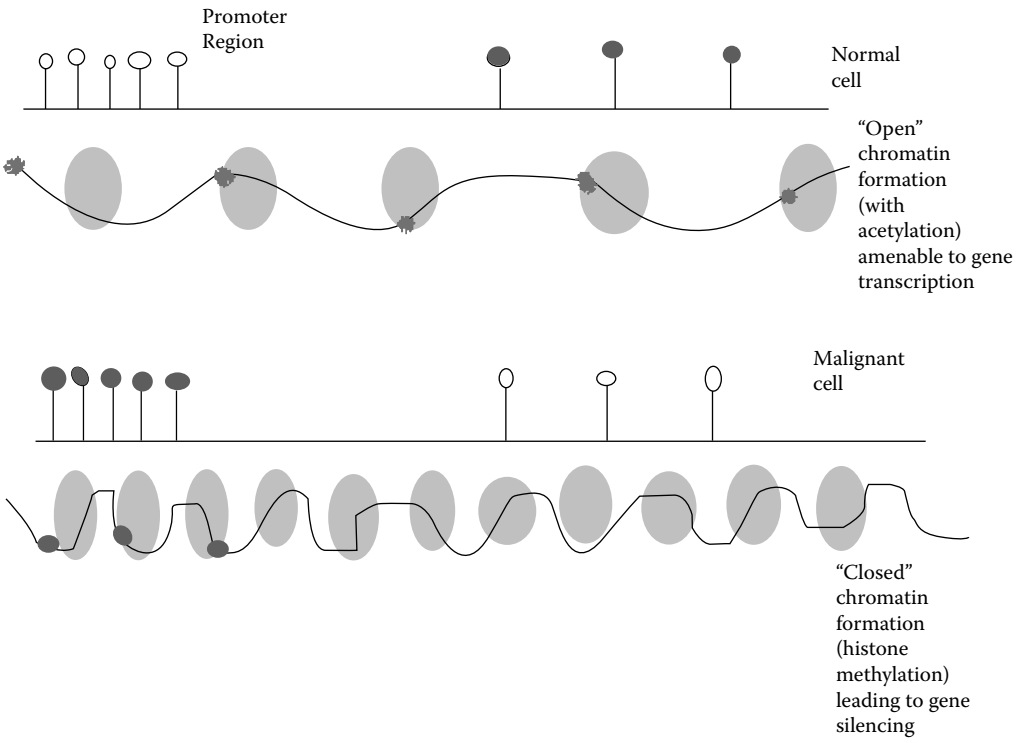

FIGURE 27.2 A stretch of genomic DNA in normal and malignant cell. The white pins denote unmethylated CpG sites within the promoter region of normal cells which become methylated (dark pins) during malignant transformation. Conversely, globally the remaining CpG dinucleotides are methylated in the normal cell (dark pins) and unmethylated in malignancy (white pin). The nucleosome (DNA and histones) shown as gray ovals depicts the open and closed formation which affects gene transcription either through acetylation (irregular dots) which results in activation or repression due to methylation of certain histone residues (smooth dots).

role of diet in cancer prevention. The concept of dietary intervention gained further momentum following evidence in animal models and epidemiological studies in humans showing that folate status can modulate risk of development of colorectal adenoma and cancer. For detailed review on mechanisms of diet influencing epigenetics in colorectal carcinogenesis, please see Arasaradnam et al. (2008). A few examples are discussed below in brief.

Folic Acid

Folate through its role in the one carbon metabolism is crucial for both DNA synthesis as well as methylation. Until recently, it was one of the nutrients most strongly implicated in terms of protection against CRC. Epidemiological studies on relationship between folate intake and risk of colorectal adenoma formation remain inconclusive. Issues surrounding folic acid and its effect on methylation and colorectal carcinogenesis remain complex. Folate seems to act as a double-edged sword; protective against initiation of carcinogenesis in a normal colon but also promoting carcinogenesis when dysplasia is established (Kim, 2004). Long-term follow-up studies (both in individuals who are folate depleted and repleted) are required to determine the true effect folate status on colon carcinogenesis.

Alcohol

An association between higher alcohol intake and colorectal cancer particularly for distal rather than more proximal disease has been noted by Pederson et al. (2003). The carcinogenic effect of

alcohol within the colon is mediated by a number of pathways but usually with a net result of low folate levels and hence reduction of methionine pool. In the Netherlands Cohort Study (van Engeland et al., 2003), higher frequency of promoter methylation of specific genes involved in colorectal cancer carcinogenesis (*APC-1A, p14^{ARF}*, *p16^{INK4a}*, *hMLH1*, *O^6-MGMT*, and *RASSF1A*) was observed in those with low folate/high alcohol compared with high folate/low alcohol. This finding would suggest that the mechanistic link between high alcohol intake and increased CRC risk seems to be mediated through low folate status.

Selenium

Selenium (Se) is an essential trace element with both antioxidant and pro-apoptotic properties. Interest in selenium and cancer prevention stemmed from early population based studies noting an inverse relationship between selenium status and carcinogenesis in particular colon cancer in geographical areas where selenium was low in soil. The large Nutritional Prevention of Cancer Trial (Clark et al., 1996), which was a randomized double blind placebo controlled interventional trial provides the strongest evidence for the protective effect of selenium against colorectal cancer. *In vitro* studies using caco-2 cells exposed to selenite as well as rodents supplemented with selenite/selenomethionine showed evidence of colonic global *hypo*methylation and promoter *hyper*methylation of *p53* and *p16* genes in the presence of selenium deficiency, that is, opposite of the normal. In human colon cancer, selenium (sodium selenite) has been shown to play a role in chemoprevention by inhibiting DNA methyltransferase (DNMT), which may reduce gene promoter hypermethylation. The resultant effect is an increase in transcript levels of tumor suppressor proteins, for example.

Phytoestrogens

Phytoestrogens which include coumestans, isoflavones, and lignans are plant derived oestrogen-like compounds. They are naturally occurring in many foods including fruits, legumes such as soy and rice as well as whole grain products which contain lignans. Both isoflavonoids and lignans are detected in plasma and urine, with high levels detected in individuals living in areas of low cancer incidence (Adlercreutz, 1998). Phytoestrogens have several purported biological actions including antioestrogenic, anti-inflammatory and anticarcinogenic effects (cell cycle arrest and induction of apoptosis) but the evidence for the protection of soy and isoflavonoids against CRC remains weak.

Lignans conversely, do not have inherent oestrogeneic activity but rather are converted to oestrogenic compounds in the colon. Although animal studies have shown promising results in protection not only against CRC but also colonic polyps (Mutanen et al., 2000) human epidemiological studies are lacking.

Green Tea

Green tea, which is a popular beverage particularly in the Far East, has been shown in murine models to possess anticarcinogenic properties. The major (polyphenol) ingredient, (–)epigallocatechin-3-gallate (EGCG) is a potent inhibitor of catechol-*O*-methyltransferase (COMT) activity. Both COMT and DNMT belong to the same family of s-adenosyl methionine (SAM)-dependent methyltransferases. In an *in vitro* experiment using HT 29 cells, (where molecular modelling was used to show that EGCG fits into the catalytic pocket of DNMT1) prevention of carcinogenesis is through competitive inhibition of DNMT1. The authors also showed resultant reversal of methylation in *p16^{INK4a}* (tumor suppressor gene), retinoic acid receptor (*RARB*), O^6-methylguanine methyltransferase (*MGMT*) and *hMLH1* genes (Fang et al., 2003). Thus green tea (EGCG) provides a good example of a food component with the capacity for reversal of aberrant methylation.

Effects of Dietary Factors on DNA Methylation in the Normal Colon

In a case–control cohort of 248 individuals with a normal colon, a wide range in genomic DNA methylation profile was observed in colonic tissue and in particular, evidence of genomic

hypomethylation in those over 40 years of age (Arasaradnam, 2007). This observation offers support to the phenomenon of age-related methylation as well as the observation of doubling of colonic cancer rates in the United Kingdom in those over 40 years of age (Cancer Research UK, 2009). No association was noted with anthropometric measures, smoking habit, alcohol consumption, or consumption of specific diets.

Gene promoter methylation of *ESR-1* (tumor suppressor gene) but not *N-33* (putative tumor suppressor gene) within colonic tissue was found to be significantly inversely associated with several food groups namely Mediterranean and whole grain/cereal diet and positively with red meat diet. This finding suggests that perhaps promoter methylation of *ESR-1* in particular is more responsive to dietary changes, at least in the normal colon. Furthermore, promoter gene methylation of *ESR-1* was shown to be positively associated with BMI. Hypermethylation of *hMLH1* (involved in DNA repair) has also been shown to be positively associated with BMI in individuals with colonic adenomas (Ye et al., 2006).

Urinary entero-lignans concentrations (phytoestrogen metabolites derived from plant foods including whole grain wheat) as well as fruit and vegetable diets were also significantly inversely associated with detectable gene methylation of *N-33*. Taken together, the existing evidence as to possible beneficial effects of lignan (particularly whole grain) consumption against CRC is mounting—through alternative pathways such as epigenetic marking. Additionally those over 40 years of age who consumed lower Mediterranean as well as fruit and vegetable diets had significantly lower detectable levels of promoter gene methylation of *ESR-1*, and *N-33*, respectively. These findings reaffirm the influence on methylation by these types of diets, at least in relation to gene-specific CpG methylation in the normal colon.

Colonic Fermentation

The ancient Hebrews capitalized on the concept of fermentation where the rudimentary use of yeast in flour made all the difference in the end product, that is, leavened bread. Since then, its use has evolved in other areas namely in the wine and beer industry. Yet, only recently has there been renewed interest in this biochemical reaction occurring in both prokaryotic and eukaryotic cells. Fermentation is defined as the process of deriving energy through oxidation of organic compounds such as sugars (carbohydrates). Emerging evidence suggests that fermentation of undigested foods in the colon by its resident bacteria (for which there are an order of magnitude greater than human cells collectively) influence colonic health through protection against inflammation and tumor formation. Colonic fermentation radiates within the intrinsic triangle encompassing colonocytes (constant), colonic bacteria (slightly variable), and diet (variable). As such, variation in diet is likely to have a significant impact on colonic fermentation and to a lesser extent, resident colonic bacteria.

Gut Microflora

The human gastrointestinal tract contains all three domains of life—bacteria, archaea, and eukaryocytes. Bacteria living in the gut achieve the highest cell densities recorded for any ecosystem. The gut flora does not exist independent of the human body, rather interactions between the gut flora and human physiology occur at several levels (Backhed et al., 2005). In addition to influence on intestinal peristalsis, the gut flora also affects the expression of various host genes that regulate nutrient uptake, metabolism, angiogenesis, mucosal barrier function, the development of the enteric nervous system and maturation of mucosal immunity (O'Hara and Shanahan, 2006). Furthermore, the anatomical structure of the colon lends itself perfectly to act as a fermenting chamber with the gaseous molecules emitted having direct effects on the colonocyte. These products of fermentation, also known as the "fermentome," can be perturbed through dietary modification thereby having direct impact on colonic as well as metabolic health and disease (Arasaradnam et al., 2009).

Most symbionts in humans can be divided broadly into two groups—bacteroides and firmicutes species (Xu et al., 2007). Observations of reduced incidence of inflammatory bowel disease (IBD) in Asian subjects but an increase in the disease incidence in second generation Asians who have adapted a "Western lifestyle," that is, similar to native population have been noted in the United Kingdom (Carr and Mayberry, 1999). The resident symbiont population is largely unchanged as is the individuals genomic profiling. Hence the increase in disease provides support for environmental pressures, namely, diet which perhaps alters fermentation "unsuited" for the individual (malfermentation) and resultant colonic disease. In fact Marchesi et al. (2007) have been able to demonstrate depletion of certain micro-biota related metabolites in the faeces of patients with IBD suggesting disruption of gut bacterial ecology and perhaps fermentation. As detailed before, up to a third in the variance of cancer incidence between populations can be attributed to habitual variation in diet, as highlighted in the recent World Cancer Research Fund report (WCRF/AICR, 2007). Little is known if fermentation patterns are altered in colorectal cancer or its precursor—colorectal adenoma. As there is now good evidence to support a link with diet it is therefore reasonable to postulate an alteration in the "fermentome" which may be quantifiable. In the obese (an excess of firmicutes species is observed) are observed compared with lean individuals suggesting that obesity has a microbial component) (Ley et al., 2006). All of the above conditions have an increased propensity to develop CRC hence understanding the "fermentome" and applying "fermentonomics" to gut microflora will help in stratification of CRC risk.

Volatile Organic Compounds (VOCs)

Fermentation results in the release of volatile organic compounds. A proportion is absorbed hence can be detectable in blood and urine samples. The majority is excreted in the faeces which also can be sampled to obtain a VOC profile. A representative faecal VOC profile from two healthy volunteer is shown in Figure 27.3.

The common approach in past decades has been to study the microbes themselves, a difficult task for almost half cannot be cultured to enable further characterization. Studying the more readily accessible VOC profile (surrogate marker of colonic bacterial activity) provides a new insight on their activity as well as how this in turn bio-regulates bowel health.

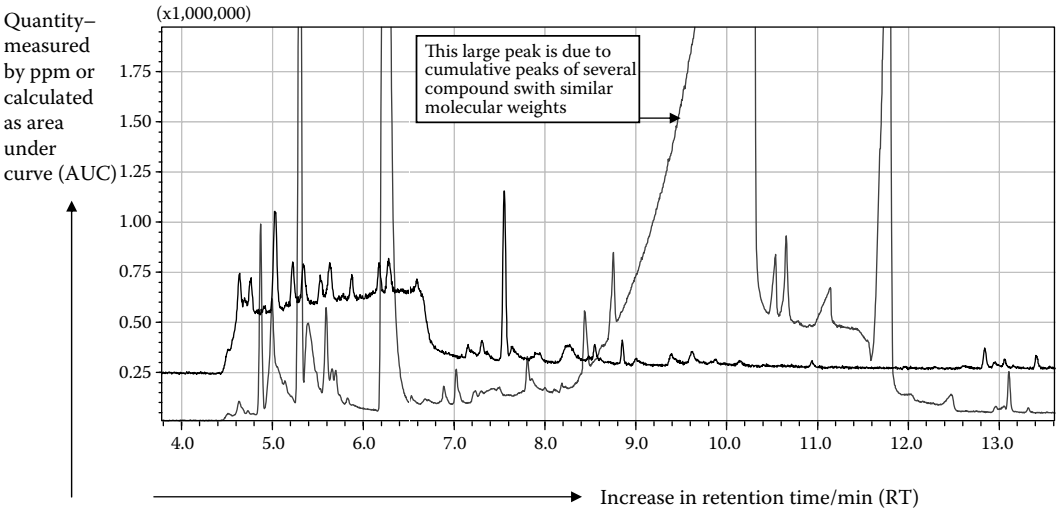

FIGURE 27.3 Composite human chromatogram profile of faeces from two individuals: vegetarian (dark tracing) and omnivore (light tracing).

SUMMARY POINTS

- One-third of all human cancers can be attributed to diet.
- Gene mutation plays a major role in CRC in only a minority; in the majority a stronger risk factor is aging.
- The occurrence of colorectal cancer in the United Kingdom rises exponentially from age 40 onwards.
- Dietary fiber characterized by degree of fermentability confers greater protection than nonfermentable fiber.
- Fermentation results in production of short-chain fatty acids (SCFAs) which are protective.
- The genetic message changes subtly over time as "hits" from the colonic environment accumulate; hence the relationship with age.
- An equally important mechanism and now a growing field is epigenetic changes whereby the DNA sequence is unaltered but the "message" altered through chemical change (methylation).
- This process can be potentially reversed through dietary means of which green tea is one example.
- The colon has the highest concentration of microbes and which are essential for health.
- They ferment undigested fiber to produce SCFAs, which have important regulatory effects.
- The traditional approach to study such microbes is by culturing them; the limitation is that at least half of the species cannot by cultured.
- We have taken a different approach, by investigating the product profile of this "fermentome" through analysis of volatile organic compounds released.

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28 Alcohol Consumption and Risk of Colorectal Cancer

Brenda W.C. Bongaerts and Matty P. Weijenberg

CONTENTS

INTRODUCTION

Worldwide, an estimated two billion people drink alcohol (in this chapter the more popular term "alcohol" is being used to address ethanol, the member of the family of alcohols used to produce alcoholic drinks). Alcoholic drinks include beers, wines and spirits, or liquors. Their consumption is culturally bound and consumption levels vary across and within countries and populations. Also, the proportion of abstainers varies considerably across countries and a common explanation for the fluctuant rates of use and abstinence include availability, price, culture, and religion. Consumption levels in industrialized countries is on average four times higher than in nonindustrialized countries; levels tend to be highest in Europe, North America, and Australia (WHO, 2004).

Since long, it is known that alcohol contributes to an increased risk of illnesses and death. Alcohol has been related to more than 60 different medical conditions and diseases, both acute and chronic. Many chronic diseases, for example several forms of cancer, are associated with overall past tissue

exposure to alcohol and with regularity of drinking and heavy drinking occasions. Acute conditions on the other hand, such as intoxication, road accidents and injuries, are frequently related to the amount of immediate alcohol intake. Alcohol use is estimated to represent, on average, 4% of the disease burden worldwide. The burden varies from country to country, however, due to variations in average consumption levels and patterns of drinking (Room et al., 2005).

When reviewing the literature on alcohol-related conditions it is important to realize that most studies have focused on high-level drinkers and alcoholics, while, in fact, the gross majority of drinkers are classified as light or moderate drinkers. Great interest into the health effects of moderate drinking has only arisen after studies had indicated that moderate drinking was involved in reducing the risk of certain forms of cardiovascular disease and osteoporosis in postmenopausal women. Still, no universal definition of moderate drinking exists, hampered by international differences in defining a "standard drink" and by variations in assessing population consumption levels and drinking patterns. Throughout this chapter the following definitions of light, moderate, and heavy alcohol consumption are used: light alcohol consumption = approximately one standard drink per day (about 10 g of alcohol per day), moderate alcohol consumption = more than one to less than three standard alcoholic drinks per day (10 g to <30 g of alcohol per day), and heavy alcohol consumption = three and more standard drinks per day (≥30 g of alcohol per day).

Among the main alcohol-associated conditions—either positively or inversely associated with alcohol intake—are several forms of cardiovascular disease, liver disease, diabetes mellitus type 2 and bone and brain disorders. In addition, alcohol has been linked to a number of cancers (WCRF/AICR, 2007). Epidemiological studies have shown a linear dose–response relationship between both duration and amount of heavy alcohol consumption and the risk of squamous-cell carcinoma of the mouth, pharynx, larynx, and esophagus. This implies that the higher the consumption levels are, the higher the risk of the mentioned cancers. Moreover, a high risk is suggested for especially those anatomical sites which are in closest or direct contact with alcohol upon ingestion, for example the mouth and the hypopharynx. Alcohol is further known to cause liver fibrosis and hepatitis which are linked to liver tumorigenesis. Indeed, heavy alcohol consumption has been shown to increase cancer risk of the liver, the main organ that breaks down alcohol. The risk of liver cancer increases with increasing amounts of alcohol ingested. In recent years, evidence has piled up for chronic alcohol consumption as a risk factor of both premenopausal and postmenopausal breast cancer. Alcohol is suspected to interfere with estrogen pathways in various manners, thus influencing hormone levels and estrogen receptors as mechanisms of disease. Finally, high intakes of alcohol have been reported to increase the risk of colorectal carcinomas, which is the focus of this chapter.

In this chapter, the current knowledge in the field of alcohol consumption and colorectal cancer is being reviewed. But before looking in detail into the actual relationship through discussing the present epidemiological studies on the topic, additional background information is provided on the epidemiology of colorectal cancer and on the alcohol metabolism.

COLORECTAL CANCER

Worldwide, colorectal cancer accounts for about one million new cancers diagnosed each year, representing approximately 9% of the total cancer incidence (Curado et al., 2007). Colorectal cancer is a very common disease in industrialized parts of the world such as Northern America, Western Europe, Australia and New Zealand, and is relatively uncommon in Africa and large parts of Asia. Both the typical geographical spread of colorectal cancer and findings from migrant studies suggest that the development of colorectal cancer can be ascribed to environmental factors, of which diet is a plausible candidate (WCRF/AIRC, 2007). Indeed, risk factors for colorectal cancer include a high consumption of red and processed meat, a high intake of alcohol, a high body mass index—more specifically, an increased amount of abdominal fat—and smoking. High intakes of dietary fiber and calcium, and a high level of physical activity have been shown to decrease the risk of colorectal cancer. But besides environmental risk factors, the disease also has a hereditary component and a

family history of colorectal cancer contributes to an excess risk of developing the condition (de la Chapelle 2004). However, less than 10% of all colorectal cancers are attributable to familial cancer syndromes. As for each type of malignancies, age is the main risk factor. Among individuals of 50 years and older, the incidence rapidly increases. Lifetime risk of developing a colorectal tumor before the age of 75 is slightly higher for men (4–6%) than for women (2–4%). Patient prognosis is heavily dependent on disease stage at the time of diagnosis, despite advances in diagnostic and therapeutic methods. Almost 50% of all colorectal cancer patients die of their disease. After lung and stomach cancer, colorectal cancer is the third most common cause of cancer death worldwide.

The process of colorectal tumor development involves the accumulation of several molecular aberrations that alter the normal behavior of a healthy cell, providing it with a certain growth advantage. As such, normal growth may progressively convert to malignant behavior (Vogelstein et al., 1988). In 1990, the "classical" molecular model for the development of colorectal cancer was proposed by Fearon and Vogelstein, known as the adenoma-carcinoma sequence or the Vogelstein model (Fearon and Vogelstein, 1990). This model describes the pathway of events leading from normal mucosa via a premalignant polyp, or adenoma, to a carcinoma (Figure 28.1). It was assumed that an accumulation of genetic and molecular aberrations would exert their effect on the cell through a loss of control over cell growth and differentiation. Research over the last 25 years, however, has shown that the natural history of adenomas and carcinomas is extremely variable and that tumorigenesis is a much more complex and heterogeneous process than described by the Vogelstein model. By examining the molecular and genetic characteristics of colorectal tumors, it has become clear that colorectal cancer is not a single disease at all and that several tumor subtypes exist based on distinct molecular features (Ogino and Goel, 2008).

At least three broad subgroups have been identified so far (Jass, 2007). In this paragraph these three classifications will be introduced only briefly in order to aid the understanding in the sections that follow.

Chromosomal Instability

The first molecularly distinct subgroup of colorectal tumors is called chromosomal instability (CIN). Approximately 85% of all nonhereditary colorectal cancers display this typical form of genetic instability which is characterized by an abnormal amount of chromosomes (aneuploidy). Aneuploidy results from accelerated gains and losses of either entire chromosomes or large portions of chromosomes during cell division. The underlying cause of aneuploidy still remains to be

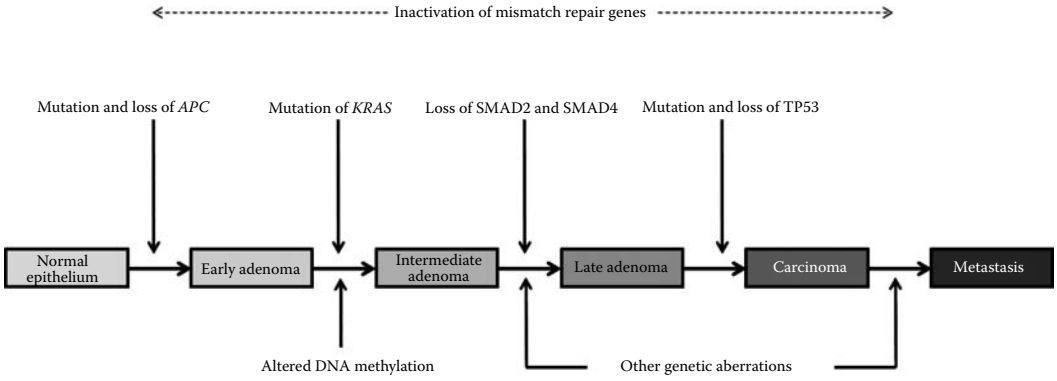

FIGURE 28.1 A multistep model for colorectal cancer development. The normal epithelium of the clorectal tract progresses trough the stage of adenoma to a colorectal carcinoma. Progression is characterized by the accumulation of particular genetic aberrations and inactivation of DNA mismatch repair genes that can occur at any point in the carcinogenic process.

clarified. Chromosomally instable tumors are usually nonmucinous tumors located in the right side of the colorectal tract and they often carry mutations in tumor suppressor genes *APC* and *TP53*, and oncogene *KRAS*.

Microsatellite Instability

The second identified molecular colorectal cancer subgroup established is called microsatellite instability (MSI). This form of genetic instability affects about 10–15% of all nonhereditary colorectal tumors and is characterized by a defective DNA mismatch repair system which is most commonly caused by the functional loss of the *MLH1* mismatch repair gene. The result is an accumulation of unrepaired gene mutations that are largely found in specific DNA regions called microsatellites. A distinction is made into a high and a low degree of instability. Microsatellite instable tumors are associated with a mucinous appearance, localization in the right-sided colon, and a mutated *KRAS* oncogene yet a normal *TP53* tumor suppressor gene.

CpG Island Methylator Phenotype

The third molecular classification concerns colorectal tumors with the CpG island methylator phenotype (CIMP). These tumors are characterized by an excessive methylation of areas in the DNA that are rich in clusters of CpG dinucleotides (CpG islands). The term methylation refers to the attachment of a methyl group to the DNA. In virtually each type of human cancer, excessive CpG island methylation prohibits the transcription of genes, thus leading to a loss of function of these genes. Similar to microsatellite instability, a distinction is made into a high and low grade of CpG island mehtylation. CIMP-high (CIMP-H) tumors are linked to a right-sided localization in the colorectal tract, female sex, microsatellite instability and mutations in the *KRAS* ongene, yet an unmutated *TP53* tumor suppressor gene. CIMP-low (CIMP-L) tumors are associated with male sex and mutations in *KRAS*.

The fact that these three colorectal tumor subgroups have such distinct molecular features is reason to suspect that they have different etiologic pathways as well. And different etiologic pathways may point to different influences of colorectal cancer risk factors, among which is alcohol consumption.

THE ALCOHOL METABOLISM

After alcohol is ingested, it is absorbed from the small intestine and taken up into the blood. Next, the alcohol is rapidly distributed over all organs and body fluids, reaching levels that equal those in the blood and the liver. Whereas only a minor percentage of the ingested alcohol (<5%) will leave the body through breath and urine most of it will be broken down in the liver, the main organ for alcohol oxidation. Of the pathways of alcohol metabolism in the liver as depicted in Figure 28.2, the most common route involves the two enzymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) (Seitz and Stickel, 2007). ADH first oxidizes alcohol to acetaldehyde, a highly reactive, toxic and carcinogenic metabolite. In a second step, acetaldehyde is oxidized by ALDH into acetate, a less active metabolite that is further degraded into water and carbon dioxide for easy elimination. In addition to the ADH pathway, cytochrome P450 2E1 (CYP 2E1) plays a role in the conversion of alcohol to acetaldehyde. CYP 2E1 is the key component of the microsomal ethanol oxidizing system (MEOS), an enzymatic system located in microsomes that metabolizes drugs and other substances (Lieber, 2004). The MEOS accounts for only a small proportion of the alcohol metabolism as the activity of CYP 2E1 is induced after heavy chronic alcohol ingestion (more than 40 g (four glasses) of alcohol per day for at least one week) and returns to normal levels after a few days of abstinence. The induction of CYP 2E1 is associated with the activation of procarcinogens to

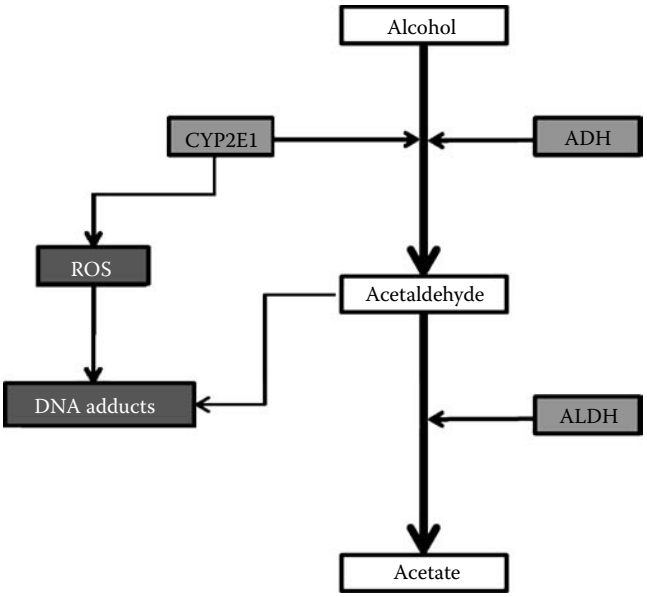

FIGURE 28.2 Alcohol metabolism. Most of the alcohol is oxidized to acetaldehyde by alcohol dehydrogenase (ADH) enzymes. A small part is converted by the microsomal enzyme cytochrome P450 2E1 (CYP2E1). Then acetaldehyde is oxidized to acetate by aldehyde dehydrogenase (ALDH) enzymes. Carcinogenic agents are generated during the alcohol metabolism including acetaldehyde, reactive oxygen species (ROS) formed through CYP2E1, and adducts formed through interactions of acetaldehyde or ROS with DNA.

their ultimate carcinogen form and with the generation of free radicals, or reactive oxygen species (ROS), which can interfere with the integrity of the DNA.

Whereas the liver is the main organ for metabolizing alcohol, microbes in the saliva and lumen of the large intestine are capable of producing significant levels of acetaldehyde from alcohol (Salaspuro, 2003). In the saliva, oral bacteria convert alcohol to acetaldehyde and increasing alcohol intake leads to increasing acetaldehyde concentrations. Likewise, the colonic microflora is able to increase the intestinal acetaldehyde levels owing to ADH activity. Further metabolism of acetaldehyde to acetate is limited, however, because of low ALDH activity in both salivary and colonic microbes. Therefore, oral and colonic acetaldehyde concentrations are higher than those in the blood.

Genetic Variations in Alcohol Metabolism

In the previous section, the enzymes ADH and ALDH are the main regulators of the levels of acetaldehyde present in the body after alcohol ingestion. Both constitute a complex family of different enzyme classes based on kinetic and structural properties. The *ADH1A*, *ADH1B*, and *ADH1C* genes encode the three classes of ADH enzymes that account for the largest part of the alcohol oxidation process, whereas the class of ALDH2 enzymes is largely responsible for the conversion of acetaldehyde into acetate. Genetic variants of the genes coding for the ADH and ALDH enzymes result in enzymes that differ in the rate at which they oxidize alcohol and acetaldehyde respectively (Bosron and Li, 1986). Because of single nucleotide polymorphisms, three variant alleles exist for both *ADH1B* (*ADH1B*1*, *ADH1B*2*, and *ADH1B*3*) and *ADH1C* (*ADH1C*1*, *ADH1C*2* and *ADH1C*3*). With regard to *ADH1B*, the *ADH1B*1* variant is the predominant allele in most populations whereas the *ADH1B*2* variant is common in Asian populations. ADH1B enzymes encoded by the *ADH1B*2* allele are 40 times more active than those encoded by the *ADH1B*1* allele. The presence of these

alleles is associated with a protective effect against alcoholism; due to their rapid production of large amounts of acetaldehyde, various unpleasant side effects arise such as a flushing syndrome with sweating, nausea, vomiting, and an accelerated heart rate. Consequently, individuals carrying the allele will most often be alcohol abstainers. To date, not much is known about the *ADH1B*3* allele yet, as it has been identified in individuals of African origin only. Regarding *ADH1C*, both the *ADH1C*1* and *ADH1C*2* allele variants are present at equal frequencies in Caucasian populations. In Africans and Asians the *ADH1C*1* allele predominates with frequencies of 75–90% and 85–100%, respectively. ADH1C enzymes encoded by the *ADH1C*1* variant convert alcohol approximately 2.5 times faster than enzymes encoded by the *ADH1C*2* allele. The *ADH1C*3* allele has only recently been described in native American populations and has not been studied in relation to alcohol yet. For *ALDH2*, a single nucleotide polymorphism is known that converts the normally functioning *ALDH2*1* allele into the practically inactive *ALDH2*2*. Homozygotes for the *ALDH2*2* variant are unable to oxidize acetaldehyde. Moreover, the presence of even a single *ALDH2*2* allele is a strong protective factor against alcoholism; the produced acetaldehyde is removed from the body only slowly and leads to toxic side effects, such as the previously mentioned flushing syndrome. The allele is prevalent in Asian populations with a frequency of approximately 40% compared with a frequency of below 5% in European and African populations. Asian populations are therefore most suitable for studying the effects of the polymorphism in relation to alcohol-associated colorectal cancer.

Mechanisms of Alcohol-Induced Colorectal Cancer

The exact mechanisms by which alcohol invokes colorectal carcinogenesis have not been clarified yet. A widely accepted idea is that alcohol-related tumor development is closely related to the alcohol metabolism. Whereas the alcohol molecule itself is not carcinogenic, evidence has piled up for acetaldehyde as the important cancer-causing agent. Acetaldehyde is a known carcinogen in animals (IARC, 1999). In rats, for example, acetaldehyde is capable of inducing inflammation and transforming the cells lining the trachea. Also, acetaldehyde enhances cell injury of the gastrointestinal mucosa, that will respond with excessive cell growth (Seitz et al., 1990). Another process through which acetaldehyde may promote tumor formation is by interfering with DNA-replication during cell division. *In vitro* studies have shown that acetaldehyde causes point mutations in genes and induces sister-chromatid exchanges and gross chromosomal aberrations in human cells (Helander and Lindahl-Kiessling, 1991; Maffei et al., 2002). Acetaldehyde can react with DNA leading to (cancer-causing) chemicals being bonded to certain parts of the DNA, called DNA adducts, which have been found in chronic alcohol consumers (Wang et al., 2000). These adducts may trigger replication errors and/or mutations in oncogenes or tumor suppressor genes, putting the cell at risk for malignant transformation. The level of DNA-adducts is influenced by several factors that are either directly or indirectly influenced by chronic alcohol consumption, for example, activity of the antioxidative defense system, the DNA repair system, and apoptosis. In addition, acetaldehyde impairs the process through which naturally occurring damage to the DNA is repaired by inhibiting DNA repair enzymes and important antioxidative peptides, such as O^6 methyl guanine methyltransferase and glutathione, respectively. Inevitably, in 2007, the International Agency for Research on Cancer (IARC) confirmed that alcoholic beverages are carcinogenic to humans (Baan et al., 2007).

With regard to (alcohol-related) colorectal cancer, folate is another often-studied substance. Folate is a B-vitamin and a natural constituent of foods such as green leafy vegetables and legumes. It has attracted considerable attention as a potential colorectal cancer chemoprotective agent. Folate is important for overall cell regeneration and protection of the DNA through production of *S*-adenosyl-methionine, the universal methyl donor. A growing body of epidemiologic, clinical, and animal studies have suggested that folate deficiency is an important factor in colorectal carcinogenesis (Kim 1999). In drinkers of more than 10 alcoholic drinks per day, an inadequate dietary

intake of folate is very common together with deficiencies of many other micronutrients. In addition, alcohol abusers have been shown to malabsorp folate (Halsted et al., 2002), thereby passing over its beneficial health effects. Whereas insufficient intakes and malabsorption do not per definition apply to low and moderate drinkers, a diminished bioavailability of folate may occur in relatively low level drinkers as well. Both acute and chronic consumption may increase the loss of folate in the urine through a reduced tubular re-absorption (Mason and Choi, 2005). A final concept of how alcohol may further reduce the availability of folate in the body is through high acetaldehyde levels in the colorectum that are capable of cleaving the folate molecule into metabolic inactive components. Yet, direct evidence for this concept is only provided by *in vitro* experiments (Shaw et al., 1989).

In addition to its harmful effects on a molecular level, acetaldehyde has been shown to exert direct toxic effects to the mucosal epithelium of the colorectal tract. Experimental studies in rats showed that chronic alcohol intake increased mucosal cell regeneration in the rectum (Seitz et al., 1990). The regenerative process was suggested to be induced by acute mucosal cell injury as a principal result of the toxicity of acetaldehyde. These toxic effects of acetaldehyde on the colorectal mucosa have been confirmed in humans as well (Simanowski et al., 2001). As known from studies in both experimental animals and humans, mucosal cell hyperregeneration, regardless of its underlying cause, predisposes to colorectal cancer development.

ALCOHOL CONSUMPTION AND COLORECTAL CANCER

To benefit a structured discussion of the current epidemiologic literature on the relationship between alcohol intake and colorectal cancer the following sections are divided into six parts. In the immediately following section, epidemiologic studies on the overall relationship between alcohol consumption and colorectal cancer are discussed, followed by further three sections on findings for the different types of alcoholic beverages, the anatomical subsites of the colorectal tract and sex-specificity of the relationship, respectively. The section thereafter addresses the literature considering genetic susceptibility of alcohol-related colorectal cancer according to genetic variations in the rate of the alcohol metabolism. Finally, studies on alcohol intake and the risk of colorectal tumors harboring specific genetic and molecular defects are reviewed in the last section.

Alcohol Consumption and Risk of Overall Colorectal Cancer

The relationship between alcohol consumption and the risk of colorectal cancer has been investigated in numerous case–control and cohort studies. In general, these epidemiological studies reported positive associations whereas occasionally null-associations and even inverse associations were found. Several methodological aspects of these studies, including study design, size of the study population, and methods used to assess alcohol consumption levels, may explain the inconsistent findings. However, more clarity on the relationship between alcohol intake and colorectal cancer is provided by large meta-analyses (Longnecker et al., 1990; Bagnardi et al., 2001; Moskal et al., 2006), a pooled analysis of data of eight large prospective studies on alcohol and colorectal cancer risk (from here on named "The Pooling Project") (Cho et al., 2004) and the European Prospective Investigation into Cancer (EPIC) multicenter study (Ferrari et al., 2007). The findings of these large studies are summarized in Figure 28.3. Overall, a positive association between alcohol consumption and risk of colorectal cancer is observed. The Pooling Project and the EPIC multicenter study observed a threshold in alcohol consumption of 30 g (approximately three alcoholic drinks) per day above which the risk of colorectal cancer increased significantly, that is, compared with alcohol abstainers, daily drinkers of 30 g of alcohol and more were associated with a 16–64% higher risk of colorectal cancer (Cho et al., 2004; Ferrari et al., 2007). Results from the meta-analyses by Longnecker et al. (1990), Bagnardi et al. (2001), and Moskal et al. (2006) showed statistically sig-

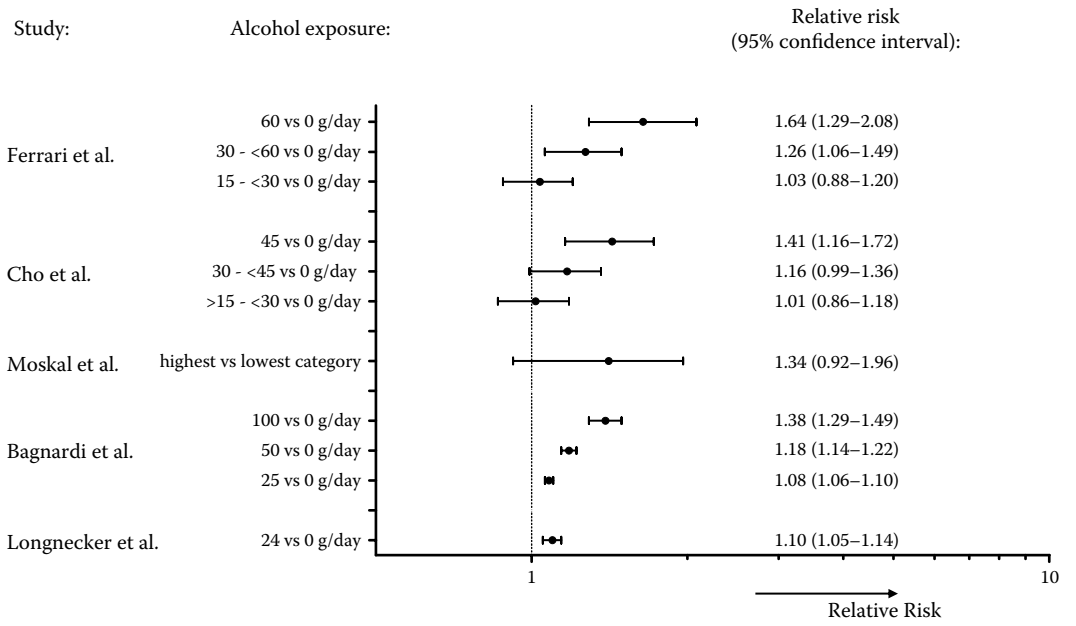

FIGURE 28.3 Results for the association between alcohol consumption and the risk of colorectal cancer, according to large meta-analyses, the Pooling Project of Prospective Studies on Diet and Cancer and the European Prospective Investigation into Cancer. (Adapted from Longnecker, et al. 1990. *Cancer Causes Control* 1:59–68; Cho, et al. 2004. *Ann Intern Med* 140 (8):603–13; Ferrari, et al. 2007. *Int J Cancer* 121 (9):2065–72.)

nificantly elevated colorectal cancer risk estimates for consumption levels below 30 g per day, that is, levels from 3–25 g of alcohol (approximately one-half to two and a half glasses of alcohol) per day were associated with 4–15% excess in risk. All studies showed that risk estimates increased with increasing alcohol consumption levels.

Alcohol Consumption and Risk of Colorectal Cancer According to Beverage Type

Next to total alcohol consumption, different beverage types have often been studied in relation to colorectal cancer risk. Epidemiologic studies have reported various positive and null-associations, whereas even inverse associations were found occasionally. Apart from methodological aspects, an often-proposed explanation for the differential associations between the various beverage types and disease involves differences in sociological and psychological characteristics of drinkers (Gronbaek et al., 1999). Studies have shown that wine drinkers eat fruit and vegetables more often, eat fat less often and in general, report a better subjective health than beer and liquor drinkers. Additionally, drinking patterns such as drinking during the meal and binge-drinking, are generally closely linked to a specific type of alcoholic beverage and may imply a distinct risk of disease. Until today, however, the above-mentioned aspects have not been studied with regard to alcohol-related colorectal cancer. But the inconsistent results for alcoholic beverage types that have been found by the individual epidemiologic studies may also be explained by specific substances present in the beverages. Wine for example, contains flavonoids and antioxidants that may reduce cancer risk. Beer on the other hand, used to contain nitrosamines (before the early 1980s), which have been shown to enhance cancer risk. Yet, the large epidemiologic studies mentioned earlier, have not been able to provide evidence for these speculations. Different types of alcoholic beverages were studied for their relationship with colorectal cancer, but the estimated risk outcomes did not

differ statistically significantly from one another (Longnecker et al., 1990; Cho et al., 2004; Ferrari et al., 2007; Moskal et al., 2006). It is therefore likely that the positive relationship between alcohol and colorectal cancer is explained by the alcohol content of the beverages, rather than other beverage-specific substances. Within the field of cardiovascular research, similar conclusions are drawn: associations between alcohol consumption and cardiovascular disease are explained by the total amount of alcohol intake, not by the type of beverage consumed. And not only for colorectal cancer, but for other cancer types as well, concluding evidence for beverage-specific carcinogenicity is lacking. The observed effects are therefore ascribed to the total amount of alcohol ingested instead (Boffetta and Hashibe, 2006).

Alcohol Consumption and Risk of Colorectal Cancer According to Anatomical Tumor Localization

When examining alcohol consumption in relation to the risk of colorectal cancer, the question that arises is whether associations are different for the various anatomical subsites of the colorectal tract. After all, men and women differ with respect to anatomical localization of colorectal cancer. Women more often have tumors in the right-sided colon (the proximal colon), whereas tumors in men are predominantly located in the left-sided colon (the distal colon) and the rectum. Furthermore, differences between the proximal and distal colon and the rectum exist with regard to physiologic function and vascular supplies (Konishi et al., 1999; Nawa et al., 2008). And finally, higher levels of the toxic acetaldehyde following alcohol ingestion have been shown in the distal colon and rectum as opposed to the proximal colon (Seitz and Simanowski, 1988), which may consequently imply a higher cancer risk for these sites. Thus considering these differences, it is plausible that alcohol consumption, and other risk factors as well, may be differentially associated with the risk of colorectal cancer according to anatomical subsite. However, individual epidemiologic studies have reported rather inconsistent and sometimes contradictory risk estimates for colon and rectal cancer with alcohol intake. Still, alcohol has relatively more often been associated with the risk of rectal cancer than colon cancer. The large meta-analyses (Longnecker et al., 1990; Moskal et al., 2006), the Pooling Project (Cho et al., 2004) and the EPIC multicenter study (Ferrari et al., 2007) have studied the distinct tumor sublocalizations with regard to alcohol intake as well. Whereas some (Cho et al., 2004; Ferrari et al., 2007), but not others (Longnecker et al., 1990; Moskal et al., 2006), have observed higher risk estimates for the distal part of the colon and the rectum than for the proximal part of the colon, the differences were not statistically significant. Thus, despite the fact that plausible theories have been opted to expect differential associations between risk factor and colorectal subsites, the current literature has not yet been able to confirm this for alcohol consumption.

Alcohol Consumption and Risk of Colorectal Cancer in Men and Women

Most studies that have examined the association between intake of alcohol and risk of colorectal cancer, have also looked into sex-specificity of the relationship. There are several reasons to expect different findings for men and women. First, men and women have different drinking habits and patterns. Men on average drink more than women. They largely represent the beer and liquor drinkers, whereas the majority of drinking women consume mainly wine. Second, as mentioned before there are differences in tumor incidence among men and women, that is, proximal colon tumors are present more often in women, whereas tumors of the distal colon and rectum are observed more frequently in men. And finally, gender differences have been observed with respect to alcohol pharmacokinetics. Evidence suggests that although men and women eliminate approximately the same total amount of alcohol per unit body weight per hour, women eliminate significantly more alcohol per unit of lean body mass per hour than men. As such, blood alcohol concentrations in women decrease faster than those in men, leading to a different alcohol exposure (Mumenthaler et al., 1999). In spite of all the above-mentioned differences, the bulk of case–control and cohort studies have not been able to

consistently show that the relationship between alcohol intake and risk of colorectal cancer is different for men and women. Again the lack of consistent findings may largely be attributable to methodological flaws and small study samples, more specifically, samples without a considerable number of alcohol consuming women. It was the large meta-analyses (Longnecker et al., 1990; Moskal et al., 2006), The Pooling Project (Cho et al., 2004) and the EPIC multicenter study (Ferrari et al., 2007) mentioned previously, that brought more clarity to the issue of sex-specificity. None of these large studies found strong evidence pointing toward a differential association between alcohol intake and risk of colorectal cancer for men and women, and the relationship is considered not to be sex-specific.

Alcohol Consumption and Risk of Colorectal Cancer According to Genetic Variations of Alcohol-Metabolizing Enzymes ADH and ALDH

As described in the preceding sections, colorectal carcinogenesis is believed to be closely related to the alcohol metabolism in which acetaldehyde is the culprit metabolite. Levels of acetaldehyde in the body are mainly determined by ADH and ALDH enzyme activities. Thus, the existing polymorphic genes that result in functional differences with regard to ADH and ALDH enzyme activity will lead to differences in acetaldehyde levels. Consequently, different levels of acetaldehyde imply a different exposure to its carcinogenic actions resulting in a different cancer risk. Next, an overview is given of the findings regarding the risk of alcohol-related colorectal cancer according to the variants of the *ADH* and *ALDH* genes.

ADH1B

The genetic epidemiological studies that examined alcohol consumption, polymorphisms in *ADH1B*, and cancer risk, have mostly focused on esophageal cancer and Asian populations. Colorectal cancer risk has been studied only rarely and in populations none other than the Japanese. In a Japanese case–control study, Yin et al. (2007) observed that alcohol drinkers in the highest consumption category with the *ADH1B*2/*2* genotype were associated with a 50-fold increase in colorectal cancer risk compared with abstainers with this fast metabolizing genotype (*ADH1B*2/*2*). The group of drinkers with *ADH1B*1/*1* and *ADH1B*1/*2* genotypes combined was associated with an even higher risk of developing colorectal cancer compared with the previously mentioned reference group. Nevertheless, the test for interaction between alcohol consumption and *ADH1B* genotype was not statistically significant and thus the authors concluded that the relationship between alcohol intake and colorectal cancer risk was not affected by *ADH1B* genotype.

ADH1C

The modifying effect of the *ADH1C* polymorphism on alcohol-related colorectal tumors is studied somewhat more often. Chen et al. (2001) and Giovannucci et al. (2003) reported an increased risk of colorectal cancer and adenomas, respectively, for drinkers in the highest consumption category with the *ADH1C*2/*2* genotype (slow alcohol metabolizers), compared with the reference group of drinkers in the lowest consumption category with the *ADH1C*1/*1* genotype (fast alcohol metabolizers). Contradictory to these two studies, Tiemersma et al. (2003) considered low level drinkers with the *ADH1C*1/*2* and *ADH1C*2/*2* genotypes combined as reference group. Compared to this combined reference group, the authors observed an increased risk of colorectal adenomas for high level drinkers with the *ADH1C*1/*1* genotype. These three studies were performed in Caucasian populations. In a Japanese case–control setting, Yin et al. (2007) observed results similar to those reported by Tiemersma et al. The highest risk of colorectal cancer was seen for drinkers in the highest consumption category with the *ADH1C*1/*1* genotype. In none of these four studies the interaction term between alcohol consumption and *ADH1C* genotype was statistically significant. Considering the inconsistent results across studies and the absence of statistically significant interaction terms, it therefore seems unlikely that the *ADH1C* genotype is able to modify the relationship between alcohol consumption and risk of colorectal cancer.

ALDH2

Since polymorphisms in *ALDH2* are very rare in populations other than the Asian, all studies on alcohol consumption, *ALDH2* genotype and risk of colorectal cancer have been performed in Japanese populations only (Murata et al., 1999; Matsuo et al., 2002; Yin et al., 2007). None of these three studies, however, observed statistically significantly elevated risks of colorectal cancer for alcohol drinkers with either the normally functioning *ALDH2*1/*1* genotype or the partially inactive *ALDH2*1/*2* variant compared with abstainers with the *ALDH2*1/*1* genotype. Again, no evidence is provided for a modifying effect of the *ALDH2* genotype on alcohol-associated colorectal cancer.

Alcohol Consumption and Risk of Colorectal Cancer Harboring Molecular and Genetic Aberrations

Years of research on the molecular and genetic characteristics of colorectal tumors have made it clear that colorectal cancer is not a single disease but a complex and heterogeneous disease. There exist distinct molecular tumor subgroups and their specific molecular and genetic abnormalities point to different underlying etiologies. Studying these distinct subgroups may thus increase current ideas and knowledge, for example, of colorectal tumorigenesis and alcohol-related pathways to disease. Thus, epidemiologic research has embarked upon a molecular level. Until today, studies that have examined associations between alcohol consumption and molecular subgroups of colorectal cancer are limited to the molecular subclassifications of MSI and CIMP (Slattery et al., 2001; Satia et al., 2005; Slattery et al., 2007; de Vogel et al., 2008). Whereas alcohol consumers in the highest category of intake compared with those in the lowest, were associated with a higher risk of CIMP-H colon cancers (Slattery et al., 2007), no consistent findings were reported on alcohol intake and MSI tumors (Slattery et al., 2001; Satia et al., 2005; de Vogel et al., 2008). Somewhat more numerous are the publications describing the relationship between alcohol intake and single genetic and molecular aberrations in colorectal cancer. Positive associations for high versus low alcohol intakes have been observed for colorectal tumors harboring a wild type *APC* tumor suppressor gene (Diergaarde et al., 2003), for tumors with TP53 protein overexpression (Fredrikson et al., 1996) and for a subgroup of microsatellite instable tumors that harbored the wild type *BRAF* oncogene (Slattery et al., 2007). In contrast to these positive findings are the null-findings and the negative associations that have been found between alcohol intake and colorectal tumors with and without single aberrations, such as *APC*, *KRAS*, *TP53*, *MLH1,* and *BRAF* mutations and TP53 protein overexpression. Because of the limited number of studies and their small study samples, no clear conclusions can be drawn on the relationship between alcohol consumption and specific subgroups of colorectal tumors or tumors harboring single genetic and molecular defects. Possibly, meta-analyses and pooled analyses could provide more insight into the relationship between alcohol intake and colorectal cancer on the molecular level. Yet, molecular studies are scarce and the initiation of new and larger studies is a difficult and costly undertaking. Moreover, for pooling of data it is important that there is consensus on molecular definitions. For example, definitions that constitute chromosomally instable tumors are not yet available and still require extensive additional research.

CONCLUDING REMARKS

The current existing epidemiologic literature on alcohol and risk of colorectal cancer can be summarized as follows. Alcohol intake has consistently been shown to increase the risk of colorectal cancer. More specifically, consumption of three alcoholic drinks per day and more is associated with a significantly elevated risk of the disease. The risk is likely to be solely attributable to the total amount of alcohol ingested, rather than beverage-specific substances. Furthermore, it is unlikely that associations with alcohol differ greatly for the different anatomical localizations of the colorectal tract and the relationship between alcohol intake and colorectal cancer is not different for men and women. There is no clear evidence that single nucleotide polymorphisms in the alcohol-metabolizing

genes *ADH1B*, *ADH1C,* and *ALDH2* modify the relationship between alcohol consumption and colorectal cancer risk. With regard to the mechanism of alcohol-related colorectal cancer, there is no substantial evidence that alcohol intake is associated with distinct molecular subgroups of colorectal cancer or with colorectal tumors harboring single molecular and genetic aberrations.

Although quite a lot is known about alcohol consumption as a risk factor for colorectal cancer nowadays, an equal lot still remains uncertain, including associations between alcohol intake and molecular subgroups of colorectal cancer to unravel the mechanisms behind colorectal cancer development. Addressing this issue in future research is complex and costly, still, an increase in the number of molecular studies would offer the opportunity for large meta-analyses and pooled analyses to be performed. Only then may study samples be significantly large enough to provide insight into the role of alcohol consumption in colorectal cancer development. In addition, current research has also shown that a major role seems to be granted to genetic cancer-susceptibility in unraveling cancer mechanisms. In view of the rising rates of both alcohol consumption (especially among young people) and colorectal cancer, there is a growing need for a more thorough understanding of alcohol-related colorectal cancer in order to develop promising prevention strategies, to identify potential risk groups and possibly, to even improve colorectal cancer treatment in future.

SUMMARY POINTS

- Of the two billion people in the world that drink alcohol, the gross majority is classified as moderate drinkers.
- Colorectal cancer is among the top three of the most common forms of incident cancers worldwide.
- Alcohol is not a carcinogenic agent in contrast to its first metabolite acetaldehyde, which is closely linked to cancer development in general.
- Alcohol consumption is associated with an increased risk of colorectal cancer when compared with abstaining.
- The risk of colorectal cancer increases with increasing alcohol intake.
- The relationship between alcohol consumption and colorectal cancer risk does not depend on the type of alcoholic beverage, but on the total amount of alcohol ingested.
- The relationship between alcohol consumption and colorectal cancer risk is most likely similar for the different anatomical localizations of the colorectal tract.
- The relationship between alcohol consumption and colorectal cancer risk is similar for men and women.
- There is no clear evidence that genetic variations in the rate of alcohol break down are likely to underlie a genetic susceptibility to alcohol-related colorectal cancer.
- There is no clear evidence that alcohol consumption is associated with specific molecular subgroups of colorectal tumors, or with tumors harboring specific single molecular aberrations and mutations.
- To gain further insight into the mechanisms of alcohol-induced colorectal cancer, it is important to create large study samples through the pooling of individual epidemiologic studies.

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29 Plants Antioxidants and Lung Cancer Risk

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CONTENTS

INTRODUCTION

Lung cancer has a high incidence and mortality, being the most common cause of cancer death worldwide and the first among men in industrialized countries. It is estimated to account for 960,000 new cases and 850,000 deaths each year among men, and 390,000 cases and 330,000 deaths among women (Boyle and Levin, 2008). In 1987, lung cancer mortality surpassed breast cancer mortality among North American women and its incidence is still rising throughout the world. Furthermore, it has a low survival, with only 36% surviving one year after the diagnosis and a 12% surviving 5 years, according to the recently published EUROCARE IV study (Sant et al., 2009). This survival has not improved in the last 30 years. The factor with the highest influence on survival is the stage at diagnosis, with stage I individuals having a relatively good survival record. Nevertheless, more than 50% of all cases are diagnosed at stage IV, when surgery cannot be performed, and the disease has spread into the body. Currently, there are no effective screening strategies for lung cancer.

Tobacco is the main risk factor for lung cancer. Around 85–90% of all cases are caused by tobacco consumption (Ruano-Raviña et al., 2009). If tobacco did not exist, lung cancer incidence would fall to the eighth or ninth position with respect to overall cancer incidence. Lung cancer is influenced by the number of cigarettes smoked per day, duration of smoking, age at initiation, and the risk decreasing with the number of years since cessation of smoking (Boyle and Levin, 2008; Ruano-Raviña et al., 2003). Some researchers postulate that lung cancer in smokers and in never smokers might have completely different carcinogenic mechanisms and therefore should be studied as different entities.

There are many other risk factors for lung cancer besides tobacco. Residential radon exposure is the second risk factor (Committee on Health Risks of Exposure to Radon [BEIR VI] 1999), with pooled studies indicating a linear relationship between residential radon concentration and risk of

lung cancer (Darby et al., 2005). Other risk factors are occupational exposure to carcinogenic substances, family history of cancer, previous respiratory diseases, and race.

Lung cancer has four major histological types: squamous, adenocarcinoma, large cell, and small cell lung cancer (SCLC). The first three are generally grouped in the so-called nonsmall-cell lung cancer (NSCLC), with the fourth being considered separately. The reason is the different nature and prognosis of both types of cancer, where SCLC has a poorer expectancy and a different therapeutic approach. It is not within the scope of the present chapter to analyze the effect of antioxidants on the different histological types of lung cancer.

Dietary factors participate in around 30% of cancers in Western countries (Key et al., 2002). Lung cancer is not an exception, although given the importance of tobacco consumption; it is possible that in smokers diet plays a role modifying the carcinogenic effect of tobacco, rather than entangling a protective or risk effect by itself. Very few studies have been performed investigating the relationship between lung cancer and diet in never smokers.

The first evidence of diet influencing lung cancer was published in 1975, pointing to a protective effect of vitamin A against lung cancer appearance (Bjelke, 1975). Since then, several papers have been published analyzing the role of many different foods and nutrients on lung cancer. Some of them have been postulated as risk factors, some as protective, and others as controversial. Among those elements possibly posing a risk for lung cancer are red meat, high fat content foods, and also foods cooked at high temperatures (meat or fish), allowing for the formation of polycyclic aromatic hydrocarbons (PAH) which have a high carcinogenic potential (Dosil-Díaz et al., 2007; WCRF/AICR, 2007).

When studying the relationship among diet and cancer, we should not forget that fruit and vegetable intake are associated with a healthier lifestyle, making individuals with the highest consumption frequently leaner, more physically active, more likely to be never smokers, have higher education, and consume less energy and alcohol (Wright et al., 2008). These associations among diet, social behaviour, and lifestyle make us aware of the difficulty to assess the influence of diet on lung cancer risk, where tobacco consumption is definitively the major cause of the disease.

VEGETABLE AND FRUIT INTAKE AND LUNG CANCER

Fruit and vegetables are thought to have a protective effect against lung cancer. A recent key report of the World Cancer Research Fund and the American Institute for Cancer Research concludes that evidence is "limited suggestive" that vegetable intake is inversely associated with lung cancer (WCRF/AICR, 2007, p. 157) and that the evidence that fruits protect against lung cancer is convincing. Several studies have been published assessing the effect of fruit and vegetables on lung cancer risk. These studies include epidemiological research in humans, laboratory research in animals, and also in *in vitro* research. The translation to humans of this type of research is very difficult, since humans cannot be treated as laboratory animals in the sense that their diet cannot be manipulated. Humans and animals have also biologically different systems. There are also many risk or protective factors for lung cancer that should be studied simultaneously with diet in humans.

Fruits and vegetables contain many different classes of nutrients, with different effects on biological systems. Some of these classes have been studied extensively while others have not. One of them are carotenoids, a class of phytochemicals with proven antioxidant activity *in vitro* and *in vivo* in animal models (Rock, 1997). Overall carotenoid consumption and dietary beta-cryptoxanthin have been found to be protective against lung cancer in the World Cancer Research Fund Report, concluding that fruits containing carotenoids probably protect against lung cancer (WCRF/AICR, 2007). The report also concludes that high-dose beta-carotene (which is a carotenoid) supplements are a cause of lung cancer in tobacco smokers and that there is limited evidence that high-dose retinol (another carotenoid) supplements are a cause of lung cancer in tobacco smokers. A recently published systematic review and meta-analysis by Gallicchio et al. (2008) has found a protective effect for the total carotenoid consumption against lung cancer, but not for beta-carotene. Carotenoids

were also associated with lower lung cancer risk in the Missouri Women's Health Study, a case–control study. Those carotenoids included beta-carotene, beta-cryptoxanthin, lutein + zeaxanthin, and total carotenoids. Interestingly, the protective associations were found only for smokers but not for never or former smokers (Wright et al., 2003).

The results of recent research are intriguing because observational studies tend to ascribe a protective effect to some antioxidants while intervention studies have found a pernicious effect for the same antioxidants. There is an increasing interest in assessing the effect of vegetable and fruit intake and their effect on lung cancer, as is shown by reviews published recently in prestigious journals.

Some major cohort studies have found no clear protective effect for fruit, vegetables, or specific botanical groups on lung cancer. Interestingly, in these studies, addition of smoking to the adjustment greatly diminished the initial protective effect both in men and women. A protective effect has been observed for specific botanical groups such as for rosaceae (apples, peaches, nectarines, plums, pears, and strawberries), convolvulaceae (sweet potatoes and yams) and umbelliferae (carrots) in men but not in women (Wright et al., 2008).

A prospective study cohort with 10 years of follow-up showed that daily consumption of green tea delayed the onset of cancer in both smokers and nonsmokers (Nakachi et al., 2000).

Many systematic reviews, meta-analysis and pooling studies have been published synthesizing the results of experimental and observational studies assessing the effect of antioxidants (mainly vitamins A, C, and E) on lung cancer. It is important to highlight the different values of these review studies. Pooling studies give us more detailed explanations and allow more flexibility in the analysis for the effect of the different nutrients since they include individual data from the participants of all included studies, while meta-analysis only group the global estimations for each study, entailing a higher possibility of biasing the results.

Total vegetable consumption was protective in the World Cancer Research Fund Report, with an effect estimate of 0.95 (95%CI 0.92–0.98) per 80 g serving/day, showing a pooled analysis a nonsignificant reduced risk comparing high against low intake groups. The report concluded that there is limited evidence suggesting that nonstarchy vegetables protect against lung cancer. With respect to fruit consumption, a meta-analysis of 14 studies concluded that there is an effect of 0.94 (95%CI 0.90–97) per 80 g serving/day and the pooled analysis found a significant protective effect comparing the highest intake group vs. the lowest. The report concluded that the evidence showing a protective effect of fruits against lung cancer is convincing (WCRF/AICR, 2007).

Another recently published systematic review has shown that cruciferous (broccoli, cabbage, cauliflower, Brussels sprouts, kale) intake is inversely associated with lung cancer risk. Those consuming the highest quantities had a 22% lower risk in case–control studies and a 17% lower risk in cohort studies. Broccoli and cabbage had a strong inverse association against lung cancer. Cruciferous vegetables are a rich source of glucosinolates. Isothiocyanates are a specific class of glucosinolates which have shown an anticarcinogenic effect through different pathways, one of them being their antioxidant capability (Lam et al., 2009). The cruciferae family also contains other nutrients such as flavonoids and carotenoids with antioxidant activity in moderate quantities.

The European Prospective Investigation into Cancer and Nutrition (EPIC study) is focused to analyze the effect of diet on many cancer types including lung cancer. It is a large cohort study performed in several European countries recruiting hundreds of thousands of individuals. A recently published study derived from this cohort study with a median follow-up time of 6.4 years, observed a significant protective association between fruit consumption and lung cancer. The hazard ratio for fruits intake in the highest vs. the lowest quintile was 0.75 (95%CI 0.49–0.96). However, vegetable intake did not have such a clear protective association. The effect was very similar for the different smoking subgroups and gender. Apple consumption posed a significant protective effect. Interestingly, a high variation in fruit and vegetable consumption was observed across the participating countries, with higher intakes in southern Europe and with smokers tending to consume less fruit and vegetables (Linseisen et al., 2007).

NATURAL ANTIOXIDANTS PRESENT IN PLANTS

There are thousands of antioxidants present in plants. Figure 29.1 depicts two samples of the enormous diversity of fruits and vegetables and therefore the ample variety of antioxidants they contain. Some of them have been thoroughly studied but most have little or unknown properties. Many of them are classified as antioxidants because they belong to a determined chemical family. In addition to this great diversity, the diets consumed throughout the world are also very different, which means that people consume different vegetables or plants and therefore present a different risk of developing cancer. They may also consume the same fruits and vegetables, but due to different cooking styles, or even growth conditions, the total antioxidant bioavailability may be different.

Phytochemicals are bioactive constituents of plant foods not identified as nutrients because they are not essential to life by themselves. Various phytochemicals have been shown to have antioxidant, anticarcinogenic, anti-inflammatory, immunomodulatory, and antimicrobial effect in laboratory experiments. Since they have varying chemical structures, they can be classified accordingly into families. They include flavonoids, isoflavones (phytoestrogens), glucosinolates, terpenes, organosulphur compounds, saponins, capsaicinoids, phytosterols, and many others. Many fruits, vegetables, pulses, herbs, and teas are high in phytochemicals (WCRF/AICR, 2007).

Dietary antioxidants provide bioactive mechanisms to reduce free radical-induced oxidative stress. Oxidative stress results from either a decrease of natural cell antioxidant capacity or an increased amount of reactive oxygen species (ROS) in organisms. When the balance between oxidants and antioxidants is shifted by the overproduction of free radicals, it will lead to oxidative stress and DNA damage. If left unrepaired, it can cause base mutation, single and double strand breaks, DNA crosslinking, and chromosomal breakage and rearrangement (Chu et al., 2002).

Because antioxidants are a heterogeneous group of compounds, it is unclear whether individual compounds are more beneficial (or harmful) than others. Moreover, carcinogenesis is not uniform across all anatomic sites and therefore specific antioxidants could pose different effects depending on the target organ (Bardia et al., 2008). For this reason it is not very helpful to analyze the effect of antioxidants on overall cancer incidence or mortality since each cancer has its own and differential risk factors and it is difficult to translate a message for the general population with results for overall cancer appearance.

A good example of this diversity in antioxidants are flavonoids. Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Variations in the heterocyclic ring C give rise to flavonols, flavones, catechins, flavanones, anthocyanidins, and isoflavonoids (Hollman and Katan, 1999). Over 5000 different naturally occurring flavonoids have been described and several are common substances in the daily diet. Important sources of flavonoids are tea, onions,

FIGURE 29.1 The variety of fruits and vegetables in the human diet.

and apples. Nevertheless, the flavonoids subclasses are not uniformly distributed. For many plants, the skins of the fruit or the outer edge of the vegetable as well the leaves contain the most concentrated sources of flavonoids. Their content is also influenced by season, sunlight, climate, and food preparation and processing.

Flavonoids have a wide variety of biological effects, and those of particular interest for cancer prevention include their antimutagenic and antiproliferative capability, strong antioxidant capacity, and involvement in cell signalling, cell cycle regulation, and angiogenesis (Neuhouser, 2004). Some flavonoids have much stronger antioxidant activities against peroxyl radicals than vitamins C, E, and glutathione. Although storage conditions may affect their levels, flavonoids are heat stable and are subjected to relatively low loss during cooking and frying. Furthermore, dietary ingredient interactions may have little influence on its bioavailability.

In a case–control study assessing the effect of some flavonoids on lung cancer, Cui et al. (2008) observed little association for total flavonoids, thearubigins, naringenin, and myricetin. In contrast, lung cancer was inversely associated with the consumption of epicatechin, catechin, quercetin, and kaempferol in a dose–response manner in smokers and no effect was observed for nonsmokers. Total vegetable consumption protected against lung cancer in smokers while fruit consumption did not have an effect for smokers or nonsmokers. This study included subjects aged <65 years.

Polyphenols have shown a strong positive correlation between the total content and antioxidant activity. Polyphenols comprise flavonoids, phenolic acids, stilbenes, and lignans. Flavonoids are the most widely distributed and account for approximately two-thirds of plant polyphenols in human diet (Hollman and Katan, 1999).

Carotenoids are another important group of nutrients in the human diet. More than 600 carotenoids occur in nature, and 40 are commonly consumed in the American diet (Epstein, 2003). However, only six are found in appreciable concentrations in humans: beta-carotene, alpha-carotene, beta-cryptoxanthin, lycopene, lutein, and zeaxanthin and therefore they have been the most studied. Lutein and beta-carotene are the most widespread in vegetables and fruits. Beta-carotene levels are lower in smokers, and alcohol consumption can also decrease the conversion of beta-carotene to retinol. In the intervention studies, the doses of beta-carotene were over 20 to 30 times the average daily intake.

There are some research studies analyzing the total antioxidant effect of vegetables and fruits. These studies provide comprehensive results since they allow classifying different fruits or vegetables according to their total antioxidant content. To this end, Chu et al. (2002) analyzed the antioxidant content of broccoli, cabbage, carrot, celery, cucumber, lettuce, spinach, onion, potato, and red pepper. They found that red pepper, broccoli, carrot, and spinach had the highest antioxidant activities. There was a medium group comprising cabbage and yellow onion and the remaining had a lower antioxidant power. The total antioxidant activity of 100 g of red pepper was equivalent to that of 826 mg of vitamin C, followed by broccoli (775 mg of vitamin C), carrot (750 mg vitamin C) and spinach (737 mg vitamin C). When they measured the antiproliferative activity against $HepG_2$ human liver cancer cells *in vitro*, they observed that red pepper, spinach, cabbage, yellow onion, and broccoli exhibited relatively potent inhibitory activity on these cells growth in a dose–dependent manner. A similar study performed by the same authors in 11 common fruits (cranberry, apple, red grape, strawberry, pineapple, banana, peach, lemon, orange, pear, and grapefruit) found that cranberry had the highest total antioxidant activity, followed by apple, red grape, strawberry, peach, lemon, pear, banana, orange, grapefruit, and pineapple (Sun et al., 2002). The total antioxidant activity of 100 g of cranberry was equivalent to 3120 mg of vitamin C, followed by apple (1740 mg vitamin C), red grape (1140 mg vitamin C), and strawberry (1130 mg vitamin C), and the remaining fruits. Interestingly, there is no vitamin C in cranberry though it had the highest antioxidant activity. The highest antiproliferative activities against $HepG_2$ human liver cancer cells *in vitro* were observed again for cranberry, lemon, apple, strawberry, red grape, banana and grapefruit. Eight of the fruits tested showed the ability to inhibit human liver cancer cell growth *in vitro*. Nevertheless, they did not find a direct relationship between total antioxidant activity and antiproliferative activity of the fruits tested.

Halvorsen et al. (2002) used a different methodology to ascertain the antioxidant potential activity of dietary plants. They analyzed the antioxidant content of 123 different plants classifying them into cereals, roots and tubers, vegetables, fruits, berries, pulses, nuts and seeds and dried fruits, on the basis of the assumption that the total amount of electron-donating antioxidants (e.g., reductants) in the diet, derived from combinations of individual antioxidants that occur naturally in foods, may be a better concept than individual dietary antioxidants. For each plant species, they analyzed samples from three different geographical origins. They found more than 1000-fold differences between total antioxidants in dietary plants and also a high variation of antioxidant concentration for the same plant collected at different locations, probably related to the distinct cultivation methods, extraction rates, and storage conditions. An extract of their results is depicted in Table 29.1. Berries showed the highest antioxidant content followed remotely by pomegranate. Walnuts and sunflower seeds also posed a high antioxidant activity. Fruits (with the exception of pomegranate) and vegetables did not show a high antioxidant content compared with those mentioned previously.

Nevertheless, these studies provide a limited evidence for cancer protection, because the attenuation of reactive oxygen species through antioxidants is just one of the mechanisms to fight against cancer development and therefore, although some foods have high antioxidant capabilities, others can contain other substances that can diminish the probability of lung cancer through other pathways. Research using plant extracts on cell cultures could provide useful results to understand the possible role of phytochemicals in cancer prevention. In this sense, collaboration among nutritionists, pharmacologists, biologists, and epidemiologists is crucial in making a continuum of research on the effects of these elements and cancer prevention.

ANTIOXIDANTS AND LUNG CANCER RISK: EPIDEMIOLOGICAL STUDIES

Many randomized trials have been performed to test the potential protective effect of antioxidants, mainly vitamins A, C, and E, against lung cancer. Some of these trials were cumbersome and provided unexpected results to the scientific community. These results gave credibility to some hypotheses postulating that the supposed protective effects of these antioxidants were not as relevant as expected. The first of these trials were the The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group (ATBC, 1994), the CARET (Carotene and Retinol Efficacy Trial) (Omenn et al., 1996), and the Physicians' Health Study (Hennekens et al., 1996). While the ATBC study pointed to an increased risk of 18% for beta-carotene supplemented individuals, the CARET study pointed to an increased risk of 28% (combined with retinol). No effect was observed for alpha-tocopherol supplementation and the risk of lung cancer in the ATBC study and no effect was found for beta-carotene and aspirin in the Physician's Health Study. Many explanations have been proposed for these results, but it is very important to keep in mind that the participants were at a higher basal risk of lung cancer (they were smokers or occupationally exposed to carcinogens) and that administered doses were far above the physiological levels.

The initial cohorts of these intervention trials have been continued after the clinical trials finished and also many intervention studies and cohort studies have been published recently assessing the effect of different antioxidants. The results of some of these studies are summarized below.

Hemilä and Kaprio (2009), in an extended follow-up of the ATBC study on the overall mortality of smokers, found that vitamin C intake did not modify the effect of vitamin E supplementation. For participants with high vitamin C intake, the effect of vitamin E on overall mortality depended on age. There was an increased mortality in participants under 63 years of age and a decreased mortality in older participants.

The Women's Antioxidant Cardiovascular Study, a randomized factorial trial with nine years of supplementation and follow-up among women at high risk of cardiovascular disease, tested vitamin C, E, and beta-carotene (Lin et al., 2009). It recruited 7627 women. Vitamin E and beta-carotene supplementation showed no effect against lung cancer while vitamin C showed a higher incidence rate (RR 1.84; 95%CI 1.14–2.97), although the authors point out that this results could be due to chance.

TABLE 29.1
Plants Having an Antioxidant Content Higher than 1 mmol/100 g Classified by Their Type

Plant Type	Antioxidant Content (mmol/100 g)
Cereals	
Barley, wholemeal flour	1.09
Buck wheat, wholemeal flour	1.99
Buck wheat, white flour	1.23
Roots and Tubers	
Ginger	3.76
Red beet	1.98
Vegetables	
Chilipepper	2.46
Kale/curly kale	2.34
Red cabbage	1.88
Orange/yellow pepper	1.85
Parsley	1.70
Artichoke, leaves	1.67
Red/green pepper	1.64
Brussels sprout	1.14
Fruits	
Pomegranate	11.33
Grape	1.45
Orange	1.14
Plum	1.06
Pineapple	1.04
Lemon	1.02
Date	1.02
Berries	
Dog rose	39.46
Crowberry	9.17
Blueberry/bilberry, wild	8.23
Blackcurrant	7.35
Strawberry, wild	6.88
Blackberry, wild	6.13
Sour cherry	5.53
Blackberry, cultivated	5.07
Cowberry/cranberry	5.03
Elderberry	4.31
Raspberry, wild	3.97
Blueberry, cultivated	3.64
Raspberry	3.06
Cloudberry	2.83
Rowanberry	2.42
Strawberry, cultivated	2.17
Redcurrant	1.78
Gooseberries	1.45
Sweet cherry	1.02

continued

TABLE 29.1 (continued)
Plants Having an Antioxidant Content Higher than 1 mmol/100 g Classified by their Type

Plant Type	Antioxidant Content (mmol/100 g)
Pulses	
Broad bean/fava bean	1.86
Ground nut/peanut	1.08
Nuts and Seeds	
Walnut	20.97
Sunflower	5.39
Sesame seed	1.21
Dried Fruits	
Apricot, dried	3.24
Prune	2.60

Source: Adapted from Halvorsen, et al. 2002. *Journal of Nutrition* 132: 461–71.

Slatore et al. (2008) analyzed the effect of long-term use of supplemental multivitamins, vitamin C, vitamin E, and folate in the VITAL (VITamins And Lifestyle) study, a cohort study recruiting approximately 77,000 individuals. Vitamin C from supplements was not associated with lung cancer, while vitamin E produced a significantly elevated risk, with a dose–response effect. This effect was unchanged after adjusting for vitamin E consumption from food sources. An even higher effect was observed for current smokers, showing an interaction between smoking and supplemental vitamin E consumption. There was no association for former smokers. The effect for vitamin C or multivitamins use remained unchanged with smoking characteristics.

Another recently published investigation belonging to the VITAL study on beta-carotene, retinol, lycopene, and lutein supplements was focused on dietary supplements and lung cancer risk (Satia et al., 2009). It included 77,719 cohort members with a mean follow-up of four years. Five hundred and twenty-one lung cancer cases were diagnosed. A marginal positive statistical effect was found for those consuming the highest quantities of beta-carotene in the previous 10 years, a positive significant effect for those consuming retinol for more than four years (and marginally significant for those with the highest intake). Total vitamin A showed no effect, and luteine had a significant effect (HR 2.02; 95%CI 1.28–3.17) for individual supplement use. Lycopene had no effect on lung cancer appearance. These results did not differ greatly with gender or smoking status.

A pooled analysis of eight prospective studies on vitamins A, C, E, folate, and multivitamins including a total of 3206 incident cases showed a significant protective effect for vitamins C and E from food-only, but not for overall intake (Cho et al., 2006). Total vitamin C showed a protective effect only in men. Since the sources of vitamin C and beta-cryptoxanthin are very similar, when the authors adjusted for the latter, an attenuation of the effect of vitamin C was observed, while beta-cryptoxanthin was inversely related to lung cancer risk (RR 0.80; 95%CI 0.69–0.93). No effect modification was observed when the analysis was stratified by tobacco consumption. Women who were multivitamin users had a RR of 1.17 (95%CI 1.04–1.32) while the overall effect for both sexes was not significant. There was no effect for vitamin A, C, or E from supplemental sources.

Bardia et al. (2008) carried out a meta-analysis of intervention trials assessing the effect of beta-carotene and vitamin E (and selenium) and found an increased risk of lung cancer for beta-carotene (RR 1.12; 95%CI 1.02–1.22) and no effect for vitamin E supplementation. A meta-analysis performed by Tanvetyanon and Bepler (2008) to assess exclusively the effect of beta-carotene and lung cancer in

intervention trials showed and increased risk in the intervention groups (OR 1.21; 95%CI 1.09–1.34). For current smokers the effect was very similar but disappeared for former smokers and nonsmokers.

EFFECT OF ANTIOXIDANTS IN THE HUMAN BODY

Chemopreventive agents may have anticancer activity in certain tissues, but at the same time may induce carcinogenic activity in others (Lee and Park, 2003). Many antioxidants can donate or accept electrons, suggesting a dual potential as antioxidants. Many more possible physiological explanations have been proposed for the effect of the different antioxidants in the human body. These comprise both protective and harmful pathways, with the latter being thoroughly studied in the last years because of the lack of protective effect found for many antioxidants, mainly beta-carotene. A possible mechanism responsible for the harmful effect of beta-carotene supplementation in smokers involves the free radical-rich atmosphere in the lungs of cigarette smokers, which might enhance beta-carotene oxidation and the formation of oxidative metabolites. These metabolites may cause diminished retinoid signalling, by downregulating retinoic acid receptors beta expression and the retinoic acid level in lung tissue, and by upregulating the levels of AP-1. A highly significant increase was observed in the carcinogen metabolizing enzymes of CYP1A1/2, CYP3A, CYP2B1, and CYP2A in the lung of rats supplemented with high doses of beta-carotene.

Beta-carotene can act as a pro-oxidant as well as an antioxidant. Palozza et al. (2003) observed that there is an overproduction of free radicals with high concentrations of beta-carotene, whereas with low concentrations the carotenoid shows antioxidant activity. Furthermore, the effect can be modulated by the presence of other factors that are able to modulate the redox equilibrium, such as tobacco smoke. The same authors have demonstrated that beta-carotene, at pharmacological, but not at physiological concentrations, increased cell proliferation and induced detrimental histopathological changes in the lungs of cigarette smoke-exposed ferrets. It has also been shown to influence the heme-oxygenase-1 activity, a redox sensitive inducible isoform of heme-oxygenase.

Apoptosis, the programmed cellular death, is an important mechanism to protect from abnormal cellular proliferation, which is usually altered in cancer. Apoptosis of preinitiated and/or neoplasic processes represents a protective mechanism against neoplasic transformation and development of tumors by the elimination of genetically damaged cells or cells that may have been inappropriately induced to divide by mitogenic and proliferative stimuli. Carotenoids and beta carotene may also influence apoptosis through different pathways, such as activation of the caspase cascade or activation of transcription factors. Nevertheless, it is not clear if carotenoids modulate the apoptotic signalling through their intact molecule or through their products of oxidation (i.e., retinoids) (Palozza et al., 2004).

Vitamin C (ascorbic acid) is another antioxidant that has been shown to have both antioxidant and oxidant behaviour depending on the surrounding environment. It inhibits the formation of some nitrosamines, but accelerates the formation of others, which might generate carcinogens by transnitrosation. In 1998, Podmore et al. (1998) proposed that vitamin C exhibits both pro-oxidant and antioxidant properties when administered to healthy humans as a dietary supplement. In 30 volunteers supplemented with 500 mg of vitamin C, increased levels of 8-oxoadenine (a potentially mutagenic lesion) were observed. This is the usual vitamin C supplementation and they pointed out that lower supplementations could pose a higher antioxidant activity instead of the pro-oxidant one observed at high concentrations.

The presence of some antioxidant vitamins can alter the concentration of others. For example, Hemilä and Kaprio (2009) observed that smoking increases the plasma alpha-tocopherol disappearance rate, which is normalized by vitamin C supplementation.

The induction of detoxification enzymes, including those of the glutathione *S*-transferase family and NAD(P)H quinone reductase is other mechanism against chronic stress related diseases (Halvorsen et al., 2002). These enzymes, usually known as phase-2 enzymes, catalyze the conversion

of xenobiotics, mutagenic metabolites, or their precursors to compounds that are more readily excreted. Dietary plants enriched in compounds that induce phase 2 detoxification enzymes include members of several vegetable families, such as Cruciferae (broccoli, Brussels sprouts, cabbage, kale, cauliflower), Leguminosae (green beans), Umbelliferae (carrots, celery), Zingerberaceae (ginger), Liliaceae (asparagus, onions, leeks), Compositae (leaf lettuce), and Chenopodiaceae (spinach). Plants containing most antioxidants appear to belong to plant species other than those containing the best phase-2 enzyme inducers.

Catechins appear to have antimutagenic and anticarcinogenic activities against a wide variety of mutagens, including benzo[*a*]pyrene (B[*a*]P) and aflatoxin B1, and their activities are several times more powerful than those of vitamin C. Tea has high concentrations of these phytochemicals (Cui et al., 2008). It has been reported that epigallocatechin-3-gallate may be able to suppress inflammation, proliferation, and angiogenesis produced by cigarette smoke in human bronchial epithelial cells (Khan et al., 2008). Green tea administered to Kumming mice inhibited urethane induced lung neoplasia and also lung cancer induction with other carcinogens. Green tea and epigallocatechin-3-gallate also showed a decrease of NNK induced mouse tumorigenesis by 63% and 28%, respectively, and green tea also inhibited significantly benzo[*a*]pyrene lung tumorigenesis in A/J mice. Green tea showed some tumor reduction in p53 expressive tumors. Black tea showed some protection against tobacco-related carcinogens. Other compounds present in green tea showed some protection through different pathways against lung cancer tumorigenesis.

Other antioxidant substances that have been studied mostly *in vitro* or *in vivo* have also shown possible protective pathways against lung cancer appearance. Below is a summary of the effects of some of these substances.

Curcumin is the yellow pigment in turmeric that gives a yellow colour to food and is widely used as a spice. It is derived from the root of the plant *Curcuma longa*. Curcumin inhibits the growth of diverse lung cancer cells lines in a concentration-dependent manner and causes induction of apoptosis. It has also shown a decrease in expression of p53 and other genes involved in the regulation of cell division genes. A reduction in the number of lung tumor nodules has been observed in animal models (Khan et al., 2008).

Genistein is an isoflavone first isolated from soybeans. Genistein displays a moderate antimutagenicity in B[*a*]P 7,8-diol-9,10-epoxide induced mutagenesis *in vitro* and is the most potent inhibitor of P450 mediated activation of B[*a*]P of all tested isoflavones (Khan et al., 2008). Benzo[*a*]pyrene is one of the most potent carcinogens present in tobacco smoke. Genistein also induces apoptosis and inhibits cancer cell growth of various lung cancer cell lines. Cells treated with genistein show an increased expression of endogenous wild-type p53 while the mutant p53 protein remains unchanged.

Resveratrol is found in grape skins, peanuts, and red wine. It has shown to reduce tumor volume, tumor weight, and metastasis in mice and to inhibit tumor initiation, promotion, and progress (Jang et al., 1997). It also influences the activation of carcinogenic substances of tobacco through CYP activation and observational studies have shown a strong dose–response relationship with intake of red wine and a decreased risk of lung cancer (Ruano-Raviña et al., 2004). Resveratrol has also shown anticarcinogenic activity against other cancers different from lung cancer. Red wine also contains a high proportion of tannins, which have antioxidant properties.

Lycopene is a natural pigment synthesized by plants and microorganisms, but not by animals. It is an acyclic isomer of beta-carotene and therefore a carotenoid. When ingested in its natural form, found in tomatoes, it is poorly absorbed. Heat processing of tomato products increases bioavailability. The most common sources of lycopene are red fruits and vegetables such as tomatoes or watermelons. Lycopene is one of the most potent antioxidants and has been suggested to prevent carcinogenesis by protecting critical biomolecules such as DNA. This is due to its high number of conjugated double bonds, exhibiting higher singlet oxygen quenching ability compared with beta-carotene and alpha-tocopherol. Lycopene supplementation substantially inhibited smoke-induced squamous metaplasia in the lungs of ferrets (Khan et al., 2008). Lycopene participates in

regulating gene function, communications via gap junctions, modulation of hormone and immune activity and metabolism of carcinogens (Kavanaugh and Ellwood, 2007). Dietary lycopene intake is poorly correlated with serum lycopene levels and a single lycopene serum measurement may not accurately reflect a subject's usual lycopene intake over time. The FDA concluded that there is no credible evidence supporting a relationship between lycopene consumption, either as a food ingredient, a component of food, or as a dietary supplement, and any cancer. The same conclusions have been done for tomato consumption and lung cancer (Kavanaugh and Ellwood, 2007).

Pomegranate is another fruit with high antioxidant content. Pomegranate juice shows potent antioxidant properties attributed to its high content of polyphenols, including ellagic acid, galotannins, anthocyanins, and other flavonoids. It has been shown to reduce different cell lines proliferation and influence the carcinogenic activity of B[*a*]P (Khan et al., 2008).

GENERAL COMMENTS

There are many aspects of the effect of antioxidants in the human body that are unknown. These range from the real absorption of many of them to the biological mechanism through which they exert their anticarcinogenic action besides the scavenging of free radicals. Since their biological activity varies with their concentration and also with the formation of derived metabolites, it is important to know the real concentration they can achieve in the organism and the metabolites they can be transformed into, as well as knowing if these depend on other individual factors such as tobacco consumption or intake of other micronutrients. For example, carotenoid absorption in the small intestine is relatively inefficient (5–50%); the bioavailability of carotenes is increased by cooking and pureeing vegetables, particularly by adding oil because these compounds are fat soluble (WCRF/AICR, 2007). Something similar happens with lycopene in tomatoes, which is four times more bioavailable from tomato paste than from fresh tomatoes. The different chemical structures of polyphenols determine their selective gut absorption. Urine and plasma levels represent good markers to establish bioavailability of polyphenols and their metabolites (Khan et al., 2008). An issue of concern is if purified phytochemicals have the same protective effects as do the whole food or mixture of foods in which these compounds are present. The current knowledge shows that they are generally more effective when consumed as whole foods. This is very important, since one-sixth of Americans regularly consume multivitamin supplements (NIH State-of-the-Science Panel, 2006), and they consume much more than Europeans (Cho et al., 2006). The amount of nutrients and other substances contained in dietary supplements, in this context usually referred to as doses, may or may not be equivalent to normal diet levels. Lower amounts, similar to those present in normal diet are called physiological doses, while higher levels are named pharmacological doses or even mega-doses (WCRF/AICR, 2007). The evidence is consistent in that antioxidant supplements do not protect against lung cancer and therefore their consumption is not recommended at all. The evidence suggests that when antioxidants are consumed in foods, they seem to pose a protective effect against lung cancer and other cancers in general.

The effect of diet is very difficult to study in humans. This is especially true for nutrients or specific substances with antioxidant activity. When researchers try to ascertain the intake of a specific antioxidant on epidemiological studies, participants are asked about the foods they eat and this intake is later transformed into nutrients (i.e., antioxidants). For the same vegetable, its origin, quantity or portion, or cooking style may vary and also the foods that are consumed with it may affect its bioavailability. Furthermore, there is a great variability among individuals on absorption and metabolization of each substance and therefore the final effect of a specific nutrient is extremely difficult to assess (Ruano-Raviña et al., 2006). This issue is even more complicated when studying lung cancer, where smoking is the key determinant of the disease. Since tobacco carcinogenic substances may interact or modify the effect of antioxidants and individuals have different tobacco consumption and therefore different concentrations of carcinogens in their body, the effect of a same antioxidant with the same blood concentration may be different among two smokers with

different tobacco consumption. These are the reasons why research advances so slowly when trying to ascertain how diet and antioxidants influence the appearance of lung cancer, despite the great quantity of published studies.

CONCLUSIONS

There are many antioxidants present in plants. Some of them are nutrients such as vitamins A, C and E, and others are classified as phytochemicals, since they are not necessary for human life. In theory, a benefit is expected from antioxidant intake, which may contribute to lung cancer protection, but it is crucial to keep in mind that the best action to protect from lung cancer is not to smoke and for smokers, to quit the habit immediately.

As Lichtenstein and Russell (2005, p. 358) have pointed out when giving a message to the general population, it is better to "rely on food to get your nutrients" than "rely on supplements to get your nutrients." Many people could think that supplements provide an insurance policy against diet deficiencies and even protect them against diseases caused by their nonhealthy lifestyle. People with a healthy lifestyle consuming supplements could also get the wrong idea that increasing the consumption will offer them extra protection. The complex mixture of phytochemicals in fruits and vegetables provides a better protective effect on health than single phytochemicals (Lam et al., 2009). Many investigations tell us that the best way to prevent lung cancer is to consume moderate quantities of antioxidant plants.

Generally, no supplements should be recommended without solid evidence of safety and efficacy (Lee and Park, 2003). Chemopreventive supplements, if provided, should be administered at levels within the normal range of blood biochemical or molecular parameters of each individual, even though currently no antioxidant supplement has demonstrated a protective effect against lung cancer. Most studies are focused on the individual effect of one, two, or three antioxidants contained in them, although the great majority of these studies conclude that the effect found might be attributable to other substances present in the foods studied and that an individual antioxidant does not have an effect but in combination with others.

Research on the effect of diet on lung cancer occurrence should be focused on dietary patterns instead of individual nutrients or foods and also how this dietary pattern interacts with different smoking consumption. To disentangle the true effect of diet and antioxidants in lung cancer appearance, an interdisciplinary collaboration among different areas of knowledge is crucial. Intervention trials performed to date have used high doses of single nutrients or nutrient cocktails in an attempt to prevent, affect, or mitigate disease. These results have been mostly disappointing and this is especially true for lung cancer, which calls for in depth collaboration among researchers to have a comprehensive view on the real biological effect of antioxidants against this disease.

SUMMARY POINTS

- Lung cancer is the main cause of cancer death in the developed world.
- Tobacco is the main risk factor of the disease and accounts for around 85–90% of all lung cancer cases.
- Observational studies have found that fruit and vegetable intake protects against lung cancer appearance.
- Intervention trials in thousands of individuals supplemented with beta-carotene, alpha-tocopherol and retinol resulted in higher lung cancer mortality among those supplemented with beta-carotene.
- Some chemical families containing antioxidants such as flavonoids or carotenoids have provided evidence of protection for lung cancer in observational studies.

- There is biological plausibility to affirm that antioxidants may prevent lung cancer since *in vivo*, *in vitro*, and some observational studies have provided strong proofs for their ability to protect against lung cancer.
- Antioxidant supplements are not recommended to prevent lung cancer.
- The best way to prevent lung cancer is not to smoke and, if smoking, quit the habit immediately.
- If diet is to be used to diminish the risk of lung cancer, fruits and vegetables should be consumed frequently and different ones should be taken at each meal.

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30 Epidemiologic Review of Head and Neck Cancers, Oral Precancers, and Dietary Risk Factors in India

Mia Hashibe

CONTENTS

INTRODUCTION

Each year in India, it is estimated that 82,914 oral cavity cancer cases, 46,335 pharyngeal cancer cases and 26,373 laryngeal cancer cases occur (Ferlay et al., 2002). Thus, combined as head and neck cancers, it is the most common cancer (156,622 cases), surpassing cervical cancer (132,082 cases per year) and breast cancer (82,951 cases per year). The high incidence of oral cancer in India has been attributed to tobacco chewing, tobacco smoking, and alcohol drinking (IARC, 2004a,b; 2007, 2010).

The study of oral premalignant lesions is of importance for the prevention of oral cancer because premalignant lesions may be treated to prevent their progression to oral cancer or used as surrogate (intermediate) markers for oral cancer intervention. The risk factors for oral precancers are similar to those of oral cancer (Hashibe et al., 2000a,b).

Previous large-scale epidemiologic studies in Europe and the United States have shown that high fruit and vegetable intake is an important protective factor against head and neck cancers (Graham et al., 1977; Winn et al., 1984; Esteve et al., 1996; Kjaerheim et al., 1998). They have also reported, though less consistently, that meat intake and dairy intake may be risk factors for head and neck cancers. In this review, we will examine the findings from previous epidemiologic studies on head and neck cancers, oral precancers and diet, specifically in India.

EPIDEMIOLOGIC STUDIES

Six epidemiologic studies on head and neck cancer or oral precancers examining the association with dietary factors were identified. We focused on studies that had used food frequency questionnaires, excluding studies that had simple dietary assessments (i.e., low/medium/high fruit or vegetable intake or vegetarian/nonvegetarian diets). A summary of the characteristics of the six studies identified are shown in Table 30.1. The size of the case group varied from 158 to 591 cases. The food frequency questionnaire were heterogenous in the number of food items included, ranging from 21 to 92.

TABLE 30.1
Epidemiologic Studies on the Head and Neck Cancers, Oral Precancers and Diet in India

Reference	Location	Study Period	Cases	Controls	Dietary Assessment and Food Frequency
			Head and Neck Cancer		
Rajkumar et al. (2003)	Bangalore, Madras, Trivandrum	1996–1999	591 oral cavity cancer cases	582 hospital based controls, frequency matched on age, sex and center	21 common items
Heck et al. (2008)	Ahmadabad, Bhopal, Kolkata and Chennai	2001–2004	513 hypopharyngeal cancer cases	718 hospital based controls, frequency matched on age, sex and residence	67 food items
Sapkota (in press)	Ahmadabad, Bhopal, Kolkata and Chennai	2001–2004	502 laryngeal cancer cases	717 hospital based controls, frequency matched on age, sex and residence	67 food items
			Oral Precancer		
Carley et al. (1994)	Chickballapur, Karnataka	Not specified	158 oral precancer cases, all female tobacco and betel chewers	155 lesion–free controls, frequency matched for age, tobacco/betel habits, socioeconomic status	Survey of 108 food items
Gupta et al. (1999)	Kerala	1993–1994	226 oral precancer who are tobacco users	226 individuals from a survey, matched on age, sex, residence, use of tobacco	81 food items
Gupta et al. (1998)	Gujarat	Not specified	323 oral precancer cases, all male tobacco users	318 lesion–free controls, frequency matched on age and tobacco use	92 food items

TABLE 30.2
Fruit Intake and the Risk of Head and Neck Cancers in Epidemiologic Studies

Reference	Cases	Controls	Exposure Category	Risk Estimate (Odds Ratio)
		Head and Neck Cancer		
Rajkumar et al. (2003)	591 oral cavity cancer cases	582	Tertile 1	1.00
			Tertile 2	**0.24 (0.16–0.36)**
			Tertile 3	**0.55 (0.38–0.81)**
			p for trend	**0.001**
Heck et al. (2008)	513 hypopharyngeal cancer cases	718	*Never tobacco users*	
			Quartile 1	1.00
			Quartile 2	0.79 (0.38–1.62)
			Quartile 3	0.71 (0.30–1.66)
			p for trend	0.3

continued

TABLE 30.2 (continued)
Fruit Intake and the Risk of Head and Neck Cancers in Epidemiologic Studies

Reference	Cases	Controls	Exposure Category	Risk Estimate (Odds Ratio)
			Tobacco users	
			Quartile 1	1.00
			Quartile 2	1.53 (0.91–2.56)
			Quartile 3	**0.52 (0.30–0.91)**
			Quartile 4	**0.37 (0.20–0.69)**
			p for trend	**0.001**
Sapkota (in press)	502 laryngeal cancer cases	717	*Never tobacco users*	
			Quartile 1	1.00
			Quartile 2	0.67 (0.32–1.39)
			Quartile 3	0.53 (0.22–1.27)
			Quartile 4	0.46 (0.16–1.33)
			p for trend	0.11
			Tobacco users	
			Quartile 1	1.00
			Quartile 2	0.94 (0.57–1.55)
			Quartile 3	0.69 (0.40–1.19)
			Quartile 4	0.31 (0.16–0.61)
			p for trend	<0.01
		Oral Precancer		
Carley et al. (1994)	158 oral precancer cases, all female tobacco and betel chewers	155	Less than median vs. over median	1.50 (0.93–2.42)
Gupta et al. (1999)	226 oral precancer who are tobacco users	226	Quartile 1	1.00
			Quartile 2	0.59 (0.34–1.03)
			Quartile 3	0.64 (0.36–1.12)
			Quartile 4	1.01 (0.54–1.87)
Gupta et al. (1998)	323 oral precancer cases, all male tobacco users	318	*Submucous fibrosis* not specified	0.85 (0.70–1.04)

Most of the studies presented their results on fruit and vegetable intake while a subset had presented on meat and dairy intake. The analysis of the study data were generally presented in quantiles of dietary intake.

The results for fruit intake as a protective factor against oral precancers and head and neck cancers are presented in Table 30.2. The protective effect was observed for oral cavity cancer in general in the study by Rajkumar et al. (2003). However, in the studies by Heck et al. (2008) and Sapkota et al. (in press) which stratified the odds ratios estimate by tobacco use status, the inverse association was only observed among tobacco users. For oral precancers, the associations were not evident with fruit intake in the studies by Carley et al. (1994), and Gupta et al. (1999), whereas a protective effect was suggested for the study by Gupta et al. (1998).

TABLE 30.3
Vegetable Intake and the Risk of Head and Neck Cancers in Epidemiologic Studies

Reference	Cases	Controls	Exposure Category	Risk Estimate (Odds Ratio)[a]
		Head and Neck Cancer		
Rajkumar et al. (2003)	591 oral cavity cancer cases	582	Tertile 1	1.00
			Tertile 2	**0.87 (0.61–1.26)**
			Tertile 3	**0.44 (0.28–0.69)**
			p for trend	**0.002**
Heck et al. (2008)	513 hypopharyngeal cancer cases	718	*Never tobacco users*	
			Quartile 1	1.00
			Quartile 2	1.63 (0.76–3.48)
			Quartile 3	1.70 (0.72–4.00)
			Quartile 4	0.96 (0.30–3.06)
			p for trend	0.07
			Tobacco users	
			Quartile 1	1.00
			Quartile 2	1.16 (0.69–1.95)
			Quartile 3	1.22 (0.69–2.14)
			Quartile 4	**0.40 (0.18–0.87)**
			p for trend	0.2
Sapkota (in press)	502 laryngeal cancer cases	717	*Never tobacco users*	
			Quartile 1	1.00
			Quartile 2	1.10 (0.56–2.15)
			Quartile 3	0.65 (0.26–1.62)
			Quartile 4	0.19 (0.06–0.64)
			p for trend	0.03
			Tobacco users	
			Quartile 1	1.00
			Quartile 2	1.36 (0.86–2.16)
			Quartile 3	1.20 (0.66–2.20)
			Quartile 4	0.52 (0.26–1.02)
			p for trend	0.20
		Oral Precancer		
Carley et al. (1994)	158 oral precancer cases, all female tobacco and betel chewers	155	Less than median vs. over median	1.76 (1.09–2.85)
Gupta et al. (1999)	226 oral precancer who are tobacco users	226	Quartile 1	1.00
			Quartile 2	0.92 (0.53–1.60)
			Quartile 3	0.66 (0.37–1.18)
			Quartile 4	0.83 (0.42–1.67)

TABLE 30.4
Meat Intake and the Risk of Head and Neck Cancers in Epidemiologic Studies

Reference	Cases	Controls	Exposure Category	Risk Estimate (Odds Ratio)[a]
		Head and Neck Cancer		
Rajkumar et al. (2003)	591 oral cavity cancer cases	582	Tertile 1	1.00
			Tertile 2	0.67 (0.45–0.98)
			Tertile 3	1.54 (1.00–2.37)
			p for trend	0.35
Heck et al. (2008)	513 hypopharyngeal cancer cases	718	*Never tobacco users*	
			Quartile 1	1.00
			Quartile 2	0.98 (0.39–2.43)
			Quartile 3	0.85 (0.31–2.29)
			Quartile 4	0.86 (0.30–2.47)
			p for trend	0.7
			Tobacco users	
			Quartile 1	1.00
			Quartile 2	0.68 (0.38–1.23)
			Quartile 3	0.55 (0.29–1.05)
			Quartile 4	0.32 (0.15–0.66)
			p for trend	0.002
Sapkota (in press)	502 laryngeal cancer cases	717	*Never tobacco users*	
			Quartile 1	1.00
			Quartile 2	0.56 (0.24–1.32)
			Quartile 3	0.41 (0.16–1.03)
			Quartile 4	0.62 (0.24–1.59)
			p for trend	0.19
			Tobacco users	
			Quartile 1	1.00
			Quartile 2	0.59 (0.34–1.03)
			Quartile 3	0.80 (0.43–1.49)
			Quartile 4	0.62 (0.32–1.20
			p for trend	0.35
		Oral Precancer		
Carley et al. (1994)	158 female oral precancer cases	155	Less than median vs. over median	3.38 (2.07–5.54)

For vegetable intake (Table 30.3), a protective effect was observed against oral cavity cancer (Rajkumar et al., 2003), among tobacco users for hypopharygneal cancers (Heck et al., 2008), and among never tobacco users for laryngeal cancer (Sapkota et al., in press). The evidence for an association between vegetable intake and oral precancers was not strong.

Higher meat intake was observed to be a risk factor for oral cavity cancers (Rajkumar et al., 2003) and for oral precancers (Carly et al., 1994). An association between meat intake and laryngeal cancer risk was not observed. On the other hand, an inverse association between meat intake and hypopharyngeal cancers was observed among tobacco users.

For dairy intake, no associations were observed with head and neck cancers (Table 30.5). The association between meat intake and oral precancers was not presented in the publications.

TABLE 30.5
Dairy Intake and the Risk of Head and Neck Cancers in Epidemiologic Studies

Reference	Cases	Controls	Exposure Category	Risk Estimate (Odds Ratio)[a]
		Head and Neck Cancer		
Rajkumar et al. (2003)	591 oral cavity cancer cases	582	Tertile 1	1.00
			Tertile 2	0.99 (0.67–1.45)
			Tertile 3	1.14 (0.70–1.87)
			p for trend	0.60
Heck et al. (2008)	513 hypopharyngeal cancer cases	718	*Never tobacco users*	
			Quartile 1	1.00
			Quartile 2	1.97 (0.84–4.61)
			Quartile 3	1.98 (0.86–4.58)
			Quartile 4	1.54 (0.56–4.19)
			p for trend	0.3
			Tobacco users	
			Quartile 1	1.00
			Quartile 2	1.50 (0.88–2.57)
			Quartile 3	2.04 (1.23–3.38)
			Quartile 4	1.24 (0.64–2.41)
			p for trend	0.9
Sapkota (in press)	502 laryngeal cancer cases	717	*Never tobacco users*	
			Quartile 1	1.00
			Quartile 2	1.51 (0.68–3.33)
			Quartile 3	1.93 (0.87–4.30)
			Quartile 4	1.16 (0.43–3.14)
			p for trend	0.45
			Tobacco users	
			Quartile 1	1.00
			Quartile 2	1.15 (0.73–1.83)
			Quartile 3	1.74 (1.03–2.94)
			Quartile 4	1.50 (0.79–2.86)
			p for trend	0.06

DISCUSSION

In summary, the results for the protective effect of fruit intake in India was very consistent, whereas the results for vegetable intake was less consistent though fairly convincing. For meat intake in India, the associations observed were very inconsistent. For dairy intake, there appeared to be no evidence of an association for head and neck cancers in India. Thus, in contrast to the published results from European and North American studies, associations for meat and dairy intake with head and neck cancer or precancers were not convincing.

The epidemiologic literature on dietary intake and head and neck cancers and oral precancers in India was fairly limited. It may be possible that our review is subject to publication bias; the null associations were probably not published. In particular, even if the food frequency questionnaire may have been detailed, unfortunately, not all of the results are published.

There are various limitations in comparing the results of dietary data across studies and across geographic regions. Since most dietary analyses use quantiles based on the distribution of dietary intake in the control group, the categories are not comparable. In addition, case-control studies are subject to recall bias. The head and neck cancer and oral precancer patients may have recalled their dietary intake differently from controls, which would lead to a bias in the estimates.

Since some of the associations with dietary factors appeared to differ between tobacco users and never tobacco users, it would be of interest for future studies to focus on examining groups stratified by tobacco use as well as other strong head and neck cancer risk factors including alcohol drinking. In conclusion, high fruit and vegetable intake is protective against oral precancers and head and neck cancers in India. Cancer prevention messages to promote fruit and vegetable intake may be one possible way to curb the high incidence of head and neck cancers in India.

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Section IV

Nonbotanical Dietary Components

31 Vitamin D and Cancer

Arnaud Teichert and Daniel D. Bikle

CONTENTS

INTRODUCTION

In addition to its important role in the maintenance of the skeleton, there is mounting evidence that vitamin D has effects on other body systems, and that adequate supplies of vitamin D are likely to be required for optimal health. Vitamin D is obtained both from dietary sources and from cutaneous synthesis with exposure to sunlight. Some epidemiological studies have indicated that vitamin D deficiency and decreased exposure to solar UVB radiation increase the risk of some cancers.

Two forms of vitamin D exist: vitamin D3 or cholecalciferol and vitamin D2 or ergocalciferol. The former is produced in the skin under the influence of UVB radiation (UVR); the latter is produced by UVR in a variety of plant materials and yeast. Differences exist in their binding to the major transport protein in blood, vitamin D binding protein, and in their metabolism because of the differences in the chemistry of their side chains, with the result that single doses of D2 lead to lower levels of circulating 25OHD than single doses of D3 (Armas et al., 2004; Romagnoli et al., 2008), although daily administration of D2 and D3 maintains comparable levels of 25OHD (Holick et al., 2008). At the tissue level, these differences are minor in that the biologic activity of $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$ appear to be comparable at least with respect to binding to VDR.

$1,25(OH)_2D_3$ stimulates the expression of cell cycle inhibitors p21 and p27 (Ingraham et al., 2008) and the expression of the cell adhesion molecule E-cadherin (Palmer et al., 2001) and inhibits the transcriptional activity of β-catenin (Palmer et al., 2001; Shah et al., 2003, 2006). In keratinocytes, $1,25(OH)_2D_3$ has been shown to promote the repair of DNA damage induced by UVR (Dixon et al., 2005), reduce apoptosis and increase survival after UVR (De Haes et al., 2003), and increase p53 (Gupta et al., 2007). Epidemiological evidence supporting the importance of adequate vitamin D nutrition (including sunlight exposure) for the prevention of a number of cancers (Garland et al., 1985, 1990; Hanchette and Schwartz, 1992; Bostick et al., 1993; Kearney et al., 1996) is extensive. Although numerous types of cancers show reduction (Boscoe and Schymura, 2006), most attention has been paid to cancers of the breast, colon, and prostate.

VITAMIN D3 PRODUCTION

The hormonal or bioactive form of vitamin D is 1alpha,25 dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]. It is generated from sequential hydroxylations of vitamin D3 (D3), a secosteroid precursor that is obtained from the diet or produced in the skin from 7-dehydrocholesterol upon exposure to UV light (e.g., sunlight).

During exposure to sunlight, epidermal keratinocytes are the site of UVB-induced photochemical conversion of 7-dehydrocholesterol (provitamin D3) to previtamin D3. Holick et al. (1979, 1980, 1981) demonstrated that the formation of pre-D3 is relatively rapid, reaching a maximum within hours. Previtamin D3 is thermodynamically unstable and converts to the more thermodynamically stable vitamin D3 (cholecalciferol) (Holick, 2004). Both intensity of UVR and level of pigmentation in the skin regulate the rate of pre-D3 formation but not the maximal level achieved. With continued UVR exposure, pre-D3 is converted to the biologically inactive lumisterol. Tachysterol is also formed but, like pre-D3, does not accumulate with extended UVR. The formation of lumisterol and tachysterol is reversible and can be converted back to pre-D3 as pre-D3 levels fall. Thus, prolonged exposure to sunlight will not produce toxic amounts of D3 because of the photoconversion of pre-D3 to lumisterol and tachysterol as well as the photoconversion of D3 itself to suprasterols I and II and 5,6 transvitamin D3 (Holick et al., 1980). Melanin in the epidermis, by absorbing UVR, reduces D3 production. The intensity of UVR from sunlight varies according to season and latitude, so the farther one lives from the Equator, the less time of the year one can rely on solar exposure to produce D3. Clothing (Matsuoka et al., 1992) and sunscreen (Matsuoka et al., 1987) effectively prevent D3 production in the covered areas.

Vitamin D_3 is hydroxylated by the vitamin D3-25 hydroxylase (25OHase) to form 25-hydroxyvitamin D_3 and then by the 25 hydroxyvitamin D3-1alpha hydroxylase (1OHase) to form the biologically active metabolite 1,25$(OH)_2D_3$ (calcitriol) (Bikle et al., 1986b; Lehmann et al., 2001).

Although the proximal renal tubule is the major source of 1,25$(OH)_2D_3$ production for the body, the enzyme is also found in a number of extrarenal sites such as immune cells, epithelia of many tissues, bone, and parathyroid glands (Hewison et al., 2007), in which it functions to provide 1,25$(OH)_2D_3$ for local consumption as an intracrine or paracrine factor. Regulation of CYP27B1 in the proximal renal tubule is controlled by PTH and fibroblast growth factor (FGF)-23, which stimulate and inhibit, respectively, its expression. Additionally, 1,25$(OH)_2D_3$ negatively regulates its own levels by suppressing CYP27B1 expression (Kitagawa et al., 2003) and by inducing CYP24, a mitochondrial P450, that catabolizes both 1,25$(OH)_2D_3$ and 25OHD_3 (Zierold et al., 1995).

Control of 1,25$(OH)_2D_3$ production (and levels) by nonrenal tissues differs. When macrophages are activated via specific toll like receptors (TLRs), CYP27B1 is induced (Liu et al., 2006). In these cells 1,25$(OH)_2D_3$ production appears to be governed primarily by the availability of substrate (Liu et al., 2006). PTH and FGF23 do not regulate CYP27B1 in these cells due presumably to lack of their cognate receptors. Furthermore, macrophages may express a nonfunctional alternatively spliced form of CYP24 located in the cytoplasm that potentially interferes with substrate access to the mitochondrial CYP24 (Ren et al., 2005), thus reducing 25OHD_3 and 1,25$(OH)_2D_3$ catabolism in these cells. The keratinocyte also contains CYP27B1, which like the macrophage enzyme can be induced by activation of specific TLRs (Schauber et al., 2007). Both TNF-α and interferon (IFN)-γ stimulate 1,25$(OH)_2D_3$ production by keratinocytes (Bikle et al., 1989, 1991), suggesting that the keratinocyte like the macrophage uses 1,25$(OH)_2D_3$ for important host defense mechanisms. Unlike the macrophage, the keratinocyte has a fully functional CYP24, and its induction by 1,25(OH)2D3 is the major means by which 1,25$(OH)_2D_3$ limits its own levels in the epidermis (Xie et al., 2002).

Unlike serum concentrations of 25OHD_3, which decrease with distance from the Equator and are lower among persons with dark pigmentation, serum concentrations of 1,25$(OH)_2D_3$ are tightly regulated and do not vary with geographic latitude or race (Chesney et al., 1981). The demonstration that many tissues in addition to the kidney possess the enzyme CYP27B1 and, like the kidney, synthesize 1,25$(OH)_2D_3$ from circulating concentrations of 25OHD_3 eliminated this concern (Schwartz,

2007). Research has subsequently shown the autocrine synthesis of $1,25(OH)_2D_3$ by many other organs.

MOLECULAR MECHANISM OF ACTION

Actions of $1,25(OH)_2D_3$ and its synthetic analogs are mediated through the vitamin D receptor (VDR), which belongs to the superfamily of nuclear hormone receptors. The VDR contains several functional domains, including a ligand-binding domain (LBD), that mediates ligand-dependent gene regulation (Carlberg and Polly, 1998). A critical step in $1,25(OH)_2D_3$ action is the induction of a LBD conformational change to form activation function 2 (AF-2) (Moras and Gronemeyer, 1998), which serves as a binding surface for coactivators (Feng et al., 1998). Unliganded nuclear receptor dimers associate with corepressors (Chen and Evans, 1995; Kurokawa et al., 1995) and associated histone deacetylases (Heinzel et al., 1997; Nagy et al., 1997). These proteins function as adaptors to convey a repressive signal to the transcriptional apparatus by maintaining a closed chromatin structure (Belandia and Parker, 2003). Ligand binding promotes the dimerization of the VDR with the retinoid X receptor alpha (RXRα) (Koli and Keski-Oja, 1993), the release of corepressors and the binding of coactivators, resulting in either activation or repression of transcription of target genes by interacting with specific DNA sequences [vitamin D response elements (VDREs)] (Rachez and Freedman, 2000; Robyr et al., 2000; Christakos et al., 2003; Sutton and MacDonald, 2003; DeLuca, 2004). Some coactivators, such as the SRC family (Hong et al., 1996; Voegel et al., 1996; Zhu et al., 1996), recruit other coregulators with histone acetylase activity and remodel chromatin structure, enabling transcription to occur. Other coactivators, such as the DRIP factors (Yuan et al., 1998; Rachez et al., 1999), interact with the basal transcriptional machinery. In skin we have found that DRIP is more abundant in the proliferating keratinocyte, whereas SRC3 is more abundant in differentiated keratinocytes (Oda et al., 2003). Furthermore, different genes regulated by VDR in these cells require different coactivators (Hawker et al., 2007; Schauber et al., 2008), indicating that at least in the keratinocyte, these coactivator complexes serve different functions and different genes.

Another coregulator, Hairless (Hr), is of great interest because null mutations in either VDR or Hr induces alopecia, in both the mouse and human (Li et al., 1997; Yoshizawa et al., 1997; Cichon et al., 1998) and is known to repress VDR-mediated transcription (Hsieh et al., 2003; Xie et al., 2006). Hr is found primarily in brain, epidermis, hair follicles, and other epithelia, although trace levels of expression have been found elsewhere (Cachon-Gonzalez et al., 1999). Other transcription factors also modulate the activity of VDR. β-Catenin binds to VDR and regulates its ability to induce a number of genes (and vice versa) (Palmer et al., 2008). Similarly, YY1 and CCAAT enhancer binding proteins-β and -δ modulate VDR-mediated transcription (Guo et al., 1997; Raval-Pandya et al., 2001; Dhawan et al., 2005). These coregulators differ in their tissue distribution, providing for substantial tissue specificity in the actions of $1,25(OH)_2D_3$ and VDR.

However, it is not clear that all actions of VDR require $1,25(OH)_2D_3$. Hair follicle cycling, which is impaired in mice lacking the VDR, is normal in mice lacking the ability to produce $1,25(OH)_2D_3$ (CYP27B1 knockouts). A recent study even suggests that some of the best known $1,25(OH)_2D_3$ dependent actions of VDR such as CYP24 induction can occur in primary keratinocytes independent of $1,25(OH)_2D_3$ through a $1,25(OH)_2D_3$-independent VDR-RXR heterodimerization that is sufficient to drive transactivation of the 24-hydroxylase promoter (Ellison et al., 2007).

PHYSIOLOGICAL REGULATIONS BY $1,25(OH)_2D_3$

Regulation of Proliferation

$1,25(OH)_2D_3$ blocks proliferation at the G0/G1 to S transition, presumably following an upregulation of the cell cycle inhibitors p21 and p27 (Sebag et al., 1992; Kira et al., 2003; Pinette et al., 2003) and a reduction in the mRNA levels for c-myc (Matsumoto et al., 1990).

At subnanomolar concentrations, $1,25(OH)_2D_3$ has been found to promote proliferation in some studies (Itin et al., 1994; Bollag et al., 1995; Gniadecki, 1996), although antiproliferative actions are most frequently observed, especially when concentrations above 10^{-9} M are employed. The mechanisms underlying the proliferative actions are not known.

Cell cycle regulators CDKN1A and GADD45A contain a functional VDRE and are direct transcriptional targets of $1,25(OH)_2D_3$–VDR. $1,25(OH)_2D_3$–VDR transcriptional activation of CDKN1A induces cell cycle exit (differentiation) and cell cycle arrest in human U937 myelomonocytic cells (Liu et al., 1996). Treatment of human breast cancer MCF7 cells with $1,25(OH)_2D_3$ also increases the expression of CDKN1A and CDKN1B, (which encodes p27) and represses CCND1 (encoding cyclin D1), CCND3 (encoding cyclin D3), CCNA1 (which encodes cyclin A1) and CCNE1 (which encodes cyclin E1), and hence leads to the inhibition of CDK activity and pRb hypophosphorylation (Verlinden et al., 1998; Jensen et al., 2001).

Similarly, the treatment of SCC cells with $1,25(OH)_2D_3$ induces G0/G1 cell cycle arrest owing to the transcriptional activation of CDKN1B and consequent pRb hypophosphorylation (Hershberger et al., 1999). However, in this context CDKN1A expression was repressed, indicating that the cell cycle arrest is an indirect effect of $1,25(OH)_2D_3$ treatment or that cell-type specificity might determine the ability of activated $1,25(OH)_2D_3$–VDR to induce CDKN1A expression (Hershberger et al., 1999). Other genes have been shown to be transcriptionally affected by $1,25(OH)_2D_3$ in colon cancer, ovarian carcinoma, and leukaemia cells, such as activation of GADD45 (Jiang et al., 2003), which is involved in DNA damage responses, repression of TYMS (which encodes thymidylate synthetase) (Palmer et al., 2003) and TK1 (which encodes thymidine kinase) (Palmer et al., 2003), which are involved in DNA replication, and activation of the INK4 family (Wang et al., 1996) of cyclin D-dependent kinase inhibitors, which mediate G1 cell cycle arrest; whereas cyclin E–CDK2 and the SKP2 (S-phase kinase-associated protein 2) ubiquitin ligase, which targets CKIs to the proteasome, are downregulated (Li et al., 2004) by $1,25(OH)_2D_3$. $1,25(OH)_2D_3$ treatment also results in the repression of the protooncogene MYC (Caligo et al., 1996; Jensen et al., 2001), which significantly contributes to the antiproliferative effects of $1,25(OH)_2D_3$.

$1,25(OH)_2D_3$ can have many indirect effects on cell cycle regulation owing to cross-talk with other pathways; for example, $1,25(OH)_2D_3$ treatment can result in the upregulation of IGFBP3 (which encodes insulin growth factor binding protein 3) and transforming growth factor-β (TGFβ)–SMAD3 signaling cascades (Verlinden et al., 1998) and by downregulating the epidermal growth factor receptor (EGFR) signaling pathway (Huynh et al., 1998; Tong et al., 1999; Yanagisawa et al., 1999).

Activation of the VDR by $1,25(OH)_2D_3$ can also inhibit tumor cell proliferation by inducing differentiation in various myeloid leukaemia cell lines and freshly isolated leukaemia cells (Liu et al., 1996; Wang and Studzinski, 2001), which is dependent on the formation of activated VDR and phosphatidylinositol 3-kinase (PI3K) complexes (Hmama et al., 1999). However, in haematopoeitic progenitor cells, $1,25(OH)_2D_3$ inhibits differentiation through VDR-independent suppression of interleukin 12 (IL12) protein secretion and downregulation of other costimulatory molecules (CD40, CD80 and CD86) (Penna and Adorini, 2000). In cell lines of head and neck, colon and prostate tumors, administration of $1,25(OH)_2D_3$ or vitamin D analogues induces the expression of genes that are associated with the differentiated cell of origin (Akutsu et al., 2001; Palmer et al., 2003; Guzey et al., 2004). In various colon cancer cells, treatment with $1,25(OH)_2D_3$ induces differentiation either by increasing PKC- and JNK-dependent JUN activation (Chen et al., 1999) or by differentially regulating the expression of inhibitor of DNA binding 1 and 2 (ID1 and ID2), which encode proteins that are transcriptional regulators of epithelial cell proliferation (ID2) and differentiation (ID1); the repression of ID2 mediated the antiproliferative effects of $1,25(OH)_2D_3$ (Fernandez-Garcia et al., 2005). $1,25(OH)_2D_3$ promotes differentiation through the induction of E-cadherin in adenomatosis polyposis coli (APC)-mutated human colorectal cancer SW480 cells (Palmer et al., 2001). E-cadherin activation consequently restrained cell growth by facilitating the translocation of β-catenin from the nucleus to the plasma membrane. Again, there appears to be no specific mechanism regarding the ability of $1,25(OH)_2D_3$ to induce differentiation in tumor cells.

$1,25(OH)_2D_3$ and Apoptosis

In addition to the antiproliferative effects of $1,25(OH)_2D_3$, there is increasing evidence that $1,25(OH)_2D_3$ exerts antitumor effects by regulating key mediators of apoptosis, such as repressing the expression of the antiapoptotic, pro-survival proteins BCL2 and BCL-XL, or inducing the expression of proapoptotic proteins (such as BAX, BAK and BAD). It has been reported that $1,25(OH)_2D_3$ downregulates BCL2 expression in MCF-7 breast tumor and HL-60 leukaemia cells and upregulates BAX and BAK expression in prostate cancer, colorectal adenoma and carcinoma cells (Ylikomi et al., 2002). In addition to regulating the expression of the BCL2 family, $1,25(OH)_2D_3$ might also directly activate caspase effector molecules, although it is unclear whether $1,25(OH)_2D_3$-induced apoptosis is caspase-dependent (Ylikomi et al., 2002). In support of this idea, the treatment of mouse SCC tumor cells with $1,25(OH)_2D_3$ increased VDR expression and concomitantly inhibited the phosphorylation of ERK (McGuire et al., 2001). Upstream of ERK, the growth-promoting and pro-survival signaling molecule MEK is cleaved and inactivated in a caspase-dependent manner in cells that undergo apoptosis after treatment with $1,25(OH)_2D_3$. Another mechanism of $1,25(OH)_2D_3$-mediated apoptosis in epithelial ovarian cancer cells has been proposed, wherein they showed that $1,25(OH)_2D_3$ destabilizes telomerase reverse transcriptase (TERT) mRNA, therefore inducing apoptosis through telomere attrition resulting from the downregulation of telomerase activity (Jiang et al., 2004). The diverse effects observed for $1,25(OH)_2D_3$-mediated apoptosis suggest that although antiproliferative effects directed against the tumor are clear *in vitro* and *in vivo*, dissecting the exact mechanism(s) central to these activities remains a challenge.

Regulation of Angiogenesis

$1,25(OH)_2D_3$ inhibits the proliferation of endothelial cells *in vitro* and reduces angiogenesis *in vivo* (Merke et al., 1989; Iseki et al., 1999; Chung et al., 2006). Vascular endothelial growth factor (VEGF)-induced endothelial cell tube formation and tumor growth are inhibited *in vivo* by $1,25(OH)_2D_3$ administration to mice with VEGF-overexpressing MCF-7 xenografts (Mantell et al., 2000). $1,25(OH)_2D_3$ can increase VEGF mRNA levels in vascular smooth muscle cells (Cardus et al., 2006) and upregulate mRNA levels of the potent antiangiogenic factor thrombospondin 1 (THBS1) in SW480-ADH human colon tumor cells (Fernandez-Garcia et al., 2005). In SCC cells, $1,25(OH)_2D_3$ induces the angiogenic factor interleukin 8 (IL8) (Lin et al., 2002), but in prostate cancer cells $1,25(OH)_2D_3$ interrupts IL8 signaling leading to the inhibition of endothelial cell migration and tube formation (Bao et al., 2006). A significant inhibition of metastasis is observed in prostate and lung murine models treated with $1,25(OH)_2D_3$, and these effects may be based, at least in part, on the antiangiogenic effects described (Getzenberg et al., 1997; Nakagawa et al., 2005). Interestingly, in tumor-derived endothelial cells (TDECs), $1,25(OH)_2D_3$ induces apoptosis and cell cycle arrest; however, these effects are not seen in endothelial cells isolated from normal tissues or from Matrigel plugs (Matrigel-derived endothelial cells) (Chung et al., 2006). TDECs may be more sensitive to $1,25(OH)_2D_3$ owing to the epigenetic silencing of CYP24A1 (Chung et al., 2007). Therefore, direct effects of $1,25(OH)_2D_3$ on endothelial cells may have a primary role in the $1,25(OH)_2D_3$-mediated antitumor activity that is observed in animal models of cancer.

Regulation of Immune Function

The potential role for vitamin D and its active metabolite $1,25(OH)_2D_3$ in modulating the immune response was first appreciated 25 years ago with three important discoveries: (i) the presence of VDRs in activated human inflammatory cells (Provvedini et al., 1983), (ii) the ability of $1,25(OH)_2D_3$ to inhibit T cell proliferation (Rigby et al., 1984), and (iii) the ability of disease activated macrophages to produce $1,25(OH)_2D_3$ (i.e., express CYP27B1) (Adams et al., 1983). Vitamin D and CYP27B1 play important roles in both innate and adaptive immunity, which impact a number of

clinical conditions and thus could play a role in cancer. For example, vitamin D deficiency is a well-known accompaniment of various infectious diseases such as tuberculosis (Ustianowski et al., 2005), and $1,25(OH)_2D_3$ has long been recognized to potentiate the killing of mycobacteria by monocytes (Rook et al., 1986). The mechanism underlying these observations has recently been determined by the observation that the monocyte, when activated by mycobacterial lipopeptides, expresses CYP27B1, producing $1,25(OH)_2D_3$ from circulating $25OHD_3$ and in turn inducing cathelicidin, an antimicrobial peptide that enhances killing of the mycobacterium. Inadequate $25OHD_3$ levels fail to support this process (Liu et al., 2006). As a second example, it has been observed that vitamin D deficiency and/or living at higher latitudes (with less sunlight) are associated with a number of autoimmune diseases including type 1 diabetes mellitus, multiple sclerosis, and Crohn's disease (Hypponen et al., 2001; Ponsonby et al., 2002). Other studies have linked vitamin D deficiency to increased risk of multiple sclerosis (Munger et al., 2006), asthma (Litonjua and Weiss, 2007), and other immunologic diseases. A discussion of the mechanisms by which $1,25(OH)_2D_3$ regulates adaptive and innate immunity follows.

Adaptive Immunity

The adaptive immune response involves the ability of T and B lymphocytes to produce cytokines and immunoglobulins, respectively, to specifically combat the source of the antigen presented to them by cells such as macrophages and dendritic cells. Vitamin D exerts an inhibitory action on the adaptive immune system. In particular, $1,25(OH)_2D_3$ suppresses proliferation and immunoglobulin production and retards the differentiation of B cell precursors into plasma cells (Chen et al., 2007). In addition, $1,25(OH)_2D_3$ inhibits T cell proliferation (Rigby et al., 1984), in particular, the T helper (Th)-1 cells capable of producing IFN-γ and IL-2 and activating macrophages (Lemire et al., 1995). These actions prevent further antigen presentation to and recruitment of T lymphocytes (role of IFN-γ), and T lymphocyte proliferation (role of IL-2). In contrast, IL-4, IL-5, and IL10 production can be increased (Boonstra et al., 2001), shifting the balance to a Th2 cell phenotype. $CD4^+/CD25^+$ regulatory T cells (Treg) are also increased by $1,25(OH)_2D_3$ (Penna and Adorini, 2000) as shown by increased FoxP3 expression and IL-10 production (Daniel et al., 2008). The IL-10 so produced is one means by which Treg block Th1 development. At least in part, these actions on T cell proliferation and differentiation stem from actions of $1,25(OH)_2D_3$ on dendritic cells to reduce their antigen presenting capability. The impact of $1,25(OH)_2D_3$ on Th17 development and function is more recently discovered, and many of the effects of 1,25(OH)2D on various autoimmune diseases previously ascribed to inhibition of Th1 development and function are now being ascribed at least in part to inhibition of Th17 development and function (Daniel et al., 2008). The ability of $1,25(OH)_2D_3$ to suppress the adaptive immune system appears to be beneficial for a number of conditions in which the immune system is directed at self, that is, autoimmunity. In a number of experimental models (DeLuca and Cantorna, 2001; Adorini, 2005) including inflammatory arthritis, autoimmune diabetes, experimental allergic encephalitis (a model for multiple sclerosis), and inflammatory bowel disease, $1,25(OH)_2D_3$ administration has prevented and/or treated the disease process. As indicated previously, studies in humans also show promise. However, suppression of the adaptive immune system may come at a price if such suppression leads to decreased response to infectious agents or decreased immune surveillance.

Innate Immunity

Innate immune responses involve the activation of toll like receptors (TLRs) in polymorphonuclear cells, monocytes, and macrophages as well as in a number of epithelial cells including those of the epidermis, gingiva, intestine, vagina, bladder, and lungs. TLRs are transmembrane pathogen recognition receptors that interact with specific membrane patterns shed by infectious agents that trigger the innate immune response in the host (Medzhitov, 2007). Activation of TLRs leads to the

induction of antimicrobial peptides and reactive oxygen species, which kill the organism. Among those antimicrobial peptides is cathelicidin. The expression of this antimicrobial peptide is induced by $1,25(OH)_2D_3$ in both myeloid and epithelial cells (Wang et al., 2004; Gombart et al., 2005). As noted previously, both macrophages (Adams et al., 1983) and epithelial cells (Bikle et al., 1986a) are capable of responding to and producing $1,25(OH)_2D_3$ (i.e., they both have VDR and CYP27B1). Stimulation of TLR2 by an antimicrobial peptide in macrophages (Liu et al., 2006) or stimulation of TLR2 in keratinocytes by wounding the epidermis (Schauber et al., 2007) results in increased expression of CYP27B1, which in the presence of adequate substrate ($25OHD_3$) stimulates the expression of cathelicidin. Lack of substrate ($25OHD_3$), VDR, or CYP27B1 blunts the ability of these cells to respond to a challenge with respect to cathelicidin production (Wang et al., 2004; Liu et al., 2006; Schauber et al., 2007). As mentioned, the innate immune system is widely distributed and operates not only in cells within the lymphopoietic system but also within epithelia of those tissues facing the outside environment in which it contributes to the protective barrier of those tissues. Therefore, it seems that it is no accident of nature that both VDR and CYP27B1 can be found in those tissues.

VITAMIN D AND CANCER

$1,25(OH)_2D_3$ has been examined preclinically for its therapeutic efficacy in chemopreventive and anticancer activity. A chemoprevention study used *Nkx3-1;Pten* mutant mice to recapitulate prostate carcinogenesis, and showed that $1,25(OH)_2D_3$ administration delayed the onset of prostate intraepithelial neoplasias (PIN) and had better antitumor activity when administered to mice with early stage (PIN) rather than advanced-stage prostate disease (Banach-Petrosky et al., 2006). Furthermore, studies using model systems of prostate adenocarcinoma (Getzenberg et al., 1997), cancers of the ovary (Zhang et al., 2005), breast (Colston et al., 1992) and lung (Nakagawa et al., 2005) showed that the administration of $1,25(OH)_2D_3$ or vitamin D analogues had significant anticancer effects. The effects of $1,25(OH)_2D_3$ and its derivatives have been shown to function through the VDR to regulate proliferation, apoptosis and angiogenesis (Shabahang et al., 1993; Simboli-Campbell et al., 1996; Liu et al., 1996; Mantell et al., 2000; Wang and Studzinski, 2001; Ylikomi et al., 2002).

A prospective four-year trial with 1100 IU vitamin D and 1400–1500 mg calcium showed a 77% reduction in cancers after excluding the initial year of study (Lappe et al., 2007), including a reduction in both breast and colon cancers. In this study, vitamin D supplementation raised the $25OHD_3$ levels from a mean of 28.8 to 38.4 ng/mL with no changes in the placebo or calcium only arms of the study. However, this was a relatively small study in which cancer prevention was not the primary outcome variable. Trials of $1,25(OH)_2D_3$ and its analogs for the treatment of cancer have been disappointing. In a small study involving seven subjects with prostate cancer treated with doses of $1,25(OH)_2D_3$ up to 2.5 μg for 6–15 months, six of the seven subjects showed a decrease in the rise of prostate-specific antigen, a marker of tumor progression (Gross et al., 1998), and one patient showed a decline. However, hypercalciuria was common and limiting. A preliminary report of a larger study involving 250 patients with prostate cancer using 45 μg $1,25(OH)_2D_3$ weekly in combination with docetaxel demonstrated a nonsignificant decline in prostate-specific antigen, although survival was significantly improved (hazard ratio 0.67) (Beer et al., 2007). The incidence of either hypercalcemia or hypercalciuria was not reported. Most likely until an analog of $1,25(OH)_2D_3$ is developed that is both efficacious and truly nonhypercalcemic, treatment of cancer with vitamin D metabolites will remain problematic.

Increased CYP27B1 expression is observed in breast (Townsend et al., 2005) and prostate (Schwartz et al., 1998) cancers and during early colon tumor progression in well-to-moderately differentiated states, but decreased in poorly differentiated colon carcinomas (Bareis et al., 2001; Cross et al., 2001; Bises et al., 2004). Increased expression of CYP27B1 in cancer tissues could provide local conversion of $25OHD_3$ to $1,25(OH)_2D_3$, and may support the notion that $25OHD_3$ and $1,25(OH)_2D_3$ might have a role in the chemoprevention of these cancers. However, CYP24A1 mRNA

expression is upregulated in tumors, and may counteract $1,25(OH)_2D_3$ antiproliferative activity, presumably by decreasing $1,25(OH)_2D_3$ levels (Friedrich et al., 2003; Cross et al., 2005). Cross et al. (2005) have demonstrated that the upregulation of CYP24A1 and downregulation of CYP27B1 can occur in high-grade colon carcinomas. The chromosomal region containing the CYP24A1 gene is amplified in human breast tumors (Albertson et al., 2000), and CYP24A1 mRNA expression is upregulated in samples from human lung, colon, and ovarian tumors, suggesting that $1,25(OH)_2D_3$ levels would be reduced in these cases (Weiss et al., 2003; Cross et al., 2005; Anderson et al., 2006). This suggests that inhibition of CYP24A1 expression and activity is essential for prevention to be effective. Small-molecule inhibitors with varying specificity for 24-OHase (Zhao et al., 1996; Ly et al., 1999; Peehl et al., 2002; Parise et al., 2006) render tumor cells more sensitive to the action of $1,25(OH)_2D_3$ and its analogues.

VDR Polymorphism

Associations have been made between some VDR gene polymorphisms and cancer risk in prostate (Ingles et al., 1997, 2001; Kim et al., 2001; Xu et al., 2003; Andersson et al., 2006; Cicek et al., 2006), colorectal (Slatter et al., 2001, 2004; Wong et al., 2003), breast (Curran et al., 1999; Dunning et al., 1999; Ingles et al., 2000; McCullough et al., 2007) and bladder (Mittal et al., 2007). But those results remained contradictory as some studies have not shown an association between the VDR polymorphisms and prostate cancer risk (Chokkalingam et al., 2001; Li et al., 2007). Moreover, these polymorphism associations have in some cases to be paired with other factors to show a link with cancer risk. For example, VDR polymorphism has been associated with colorectal cancer in the presence of low calcium and low fat intake (Wong et al., 2003), with reduced breast cancer risk among individuals with low calcium intake (McCullough et al., 2007) and with colorectal adenomas in individuals with low dietary calcium and vitamin D intake (Ingles et al., 2001) and (Kim et al., 2001). VDR polymorphism has been associated with reduced risk of rectal cancer when paired with low calcium and low energy but the opposite association was observed for proximal colon tumors (Slattery et al., 2004).

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32 Dietary Selenium and Liver Cancer

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CONTENTS

INTRODUCTION

Dietary selenium (Se) is an essential mineral for both humans and animals. It functions as a component of several proteins, termed selenoproteins. These include glutathione peroxidases (GPx, several different isoforms), thioredoxin reductases (three isoforms), iodothyronine deiodinases (three isoforms), selenophosphate synthetase, selenoprotein P, and selenoprotein W (Burk and Levander, 2006). Because glutathione peroxidase and thioredoxin reductase function as antioxidants and because an inverse relationship between selenium intake and cancer risk was identified in several studies, increasing selenium intake has been proposed as a way to prevent the development of some forms of cancer in humans.

In the United States, liver cancer is the fifth-leading cause of death from cancer in men and the eighth leading cause in women (American Cancer Society, 2008). Worldwide, hepatocellular carcinoma (HCC) is the third leading cause of death from cancer (Ganne-Carrie and Trinchet, 2004). The incidence of and mortality from HCC is variable worldwide, with the Far East and sub-Saharan Africa having the highest incidences. The primary risk factors for HCC are infection with hepatitis B and hepatitis C viruses, and long-term exposure to aflatoxin (Feitelson et al., 2002). In the United States, chronic alcoholism leading to chronic liver disease is an important risk factor (Bosch et al., 2004). The prognosis for HCC is poor, with the 5 year survival rate at diagnosis being only 11% (American Cancer Society, 2008). A number of molecular changes have been identified. These include mutations in the p53, Rb, β-catenin, and insulin-like growth factor receptor 1 genes (Feitelson et al., 2002; McKillop et al., 2006). Other genes are overexpressed, including c-myc, c-jun, and cyclin D_1 (Feitelson et al., 2002; McKillop et al., 2006).

In this review, we will examine the role that selenium may play in the possible prevention of liver cancer. We first will discuss epidemiological studies as well as clinical trials in humans, and then will discuss experimental hepatocarcinogenesis studies in which selenium was studied.

Se AND HUMAN LIVER CANCER

Epidemiological data as well as supplementation trials support the hypothesis that Se is likely to be effective in humans. Epidemiological studies on the relationship between Se and cancer have found that Se status is inversely related to some cancer risks. Shamberger reported this association in

human subjects and also found that mortality attributed to lymphomas and cancers of the gastrointestinal tract (GIT), peritoneum, lung and breast were lower in subjects living in areas where Se concentration is high in forage crops compared with those living in areas with low-Se containing forage crops (Shamberger, 1970; Shamberger, et al. 1973). Clark and Stafford (1981), using the same forage data, indicated that colorectal cancer mortality is indeed associated with high Se. Using the estimated Se intake per capita, Schrauzer et al. (1977) noted an inverse association with total cancer mortality rate and age corrected mortality rate for leukemia and cancers of the colon, rectum, breast, ovary, and lung.

Using serum Se level, several studies reported that low serum or plasma Se level is associated with increased risk for some cancers such as GIT cancer, prostate cancer, thyroid cancer, malignant oral cavity lesions, esophageal and gastric cancers, cervical cancer and colorectal adenomas, and nonmelanoma skin cancer (Willett et al., 1983; Willett, 1986 ; Glattre et al., 1989; Toma et al., 1991; Taylor et al., 1994 ; Russo et al., 1997; Mark et al., 2000; Brooks et al., 2001). On the other hand, some studies have reported no significant association between serum Se concentration and cancer risks (Nomura et al., 1987; Coates et al., 1988; Helzlsouer et al., 1989; Kabuto et al., 1994). A recent case-control study found that individuals with higher toenail selenium had a decreased risk of developing hepatocellular carcinoma (Sakoda et al., 2005).

Se supplementation trials have been conducted to determine if Se is effective in reducing liver cancers in humans. Most of the supplementation trials were based in China and the rest in the United States, Italy, and India. The first China trial investigated the preventive effect of Se on primary liver cancer and found that Se supplementation using table salt fortified with sodium selenite (30–50 μg Se/day) resulted in an almost 50% decrease in the primary liver cancer incidence (Yu et al., 1991, 1997). Another study showed that selenite-fortified salt supplementation reduced the incidence rate of viral infectious hepatitis, a predisposing factor of primary liver cancer (Yu et al., 1989, 1999). Yu et al. (1997) reported a significant decrease in primary liver cancer among those receiving Se yeast (mainly selenomethionine) compared with controls. However, Qu et al. (2007) found that supplementation with a combination of β-carotene, α-tocopherol, and selenium (as selenium yeast) for 5.25 years in Linxian, China did not affect mortality from liver cancer.

A double-blind, randomized trial of Se-enriched yeast involving 1312 patients with nonmelanoma skin cancer led to the unexpected discovery that Se protects against colon, lung, and prostate cancers; data on liver cancer were not presented (Clark et al., 1996, 1998). After extending the trial to 10 years, the resulting trend was still the same (Duffield-Lillico et al., 2002). They found that Se significantly decreased the incidence of total cancer and prostate cancer, but the incidences of lung or colorectal cancers were not reduced significantly. However, Duffield-Lillico et al. (2002) found that subjects with low plasma Se levels had a lower incidence of cancer whereas those with high plasma Se levels did not correlate with cancer incidence. The results from this trial led to the initiation of other clinical intervention trials including Se and Vitamin E Cancer Prevention Trial (SELECT) in the United States and Prevention of Cancers by Intervention with Se (PRECISE) in Europe (Hoque et al., 2001; Klein et al., 2003). The SELECT trial has recently been published, but failed to detect inhibitory effects of Se on lung, colon, or prostate cancer; data on liver cancer were not presented (Lippman et al., 2009). The European clinical trials are currently ongoing (Facompre and El-Bayoumy, 2009).

Se AND HEPATOCARCINOGENESIS

Liver cancer can be induced experimentally by a number of agents, including aromatic amines, azo dyes, nitrosamines, and aflatoxin (Pitot and Dragan, 1994). In initiation–promotion protocols, the administration of a single subcarcinogenic dose of a carcinogen (such as diethylnitrosamine [DEN]) along with a proliferative stimulus (such as partial hepatectomy) followed by the long-term feeding of chemicals such as phenobarbital, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, or polyhalogenated biphenyls leads to a high incidence of hepatocellular adenomas and carcinomas (Pitot and Dragan, 1994; Glauert et al., 2001). Transgenic mouse models of liver carcinogenesis have also been developed

(Calvisi and Thorgeirsson, 2005). In addition, foci of putative preneoplastic hepatocytes appear before the development of gross tumors. These foci, known as altered hepatic foci or enzyme-altered foci, contain cells which exhibit qualitatively altered enzyme activities or alterations in one or more cell functions, such as iron or glycogen accumulation (Glauert, 1991; Pitot and Dragan, 1994). The enzymes most frequently studied include γ-glutamyl transpeptidase (GGT) and placental glutathione-*S*-transferase (PGST), which are normally not present in adult liver but which are often present in foci; and ATPase and glucose-6-phosphatase, which are normally present but which are frequently missing from foci (Hendrich et al., 1987; Pitot et al., 1985). Altered hepatic foci can also be identified on hematoxylin and eosin stained tissue (Bannasch et al., 1989; Harada et al., 1989). The appearance of foci has been correlated with the later development of malignant neoplasms (Emmelot and Scherer, 1980; Kunz et al., 1983).

Prior supplementation of Se before administering DEN significantly reduced the number of tumors in rats (Thirunavukkarasu et al., 2004). In a similar study using phenobarbital as the tumor promoter, Se supplementation was observed to have better protection against hepatoma when given to rats before the DEN initiation phase compared with rats receiving supplementation during the promotion stage (Thirunavukkarasu et al., 2001). Dorado et al. (1985) supplemented female rats with 4 and 6 ppm Se during the preinitiation stage, during the promotion stage only, or throughout the entire experiment (40 weeks). No Se effect was observed in relation to hepatic nodules or carcinoma incidence, and regardless of what stage the Se was given, Se supplementation did not produce protection against hepatocellular cancer (Dorado et al., 1985). An earlier similar study using a lower Se dose (2 ppm Se as selenite) produced the same finding (Aquino et al., 1985). LeBoeuf et al. (1985) also noted that Se (6 ppm) decreased focal growth (mean volume) with no corresponding effect on the number of GGT-positive foci in the liver when fed either after DEN or during AAF administration; when high (6 ppm) Se was fed between DEN initiation and phenobarbital promotion, however, the induction of altered hepatic foci was increased.

Using a modified Solt-Farber protocol, Bjorkhem-Bergman et al. (2005) found that Se doses of 1 and 5 ppm (as sodium selenite) administered to Fisher 344 rats had no effect on the number of hepatic nodules and the volume fraction of tumor tissue during the initiation and progression stages, respectively. However, rats receiving 1 ppm Se during the selection phase had a decreased density of liver nodules (25%) compared with the nonselenite group (38%) and a greater decrease was seen in the 5 ppm Se group (14%).

Aflatoxin B_1 (AFB_1) is a potent hepatocarcinogenic mycotoxin in experimental animals. Se (5 ppm) supplemented in drinking water to rats was found to inhibit altered hepatic foci induced by one dose of AFB_1 followed by partial hepatectomy and phenobarbital (Milks et al., 1985). Baldwin and Parker (1987) employed 12 doses of AFB_1 followed by phenobarbital and observed that on a standard diet Se was effective in reducing foci during the promotion stage but not during the initiation phase. It was found that inhibition of AFB_1 induced-hepatocarcinogenesis occurs mainly at the initiation phase although Se also has inhibitory effects on the progression stage of nodules to hepatocellular carcinoma (Lei et al., 1990). Male Wistar rats fed with 0, 3, and 6 ppm Se (as sodium selenite) given in drinking water for 30 weeks were administered repeated AFB_1 dosing during a period of 18–27 weeks. The AFB_1 + Se groups had decreased number of nodules per square cm and smaller average area of the gamma-glutamyl transpeptidase (GGT) positive foci than the Se deficient rats, but the 3 ppm group showed greater inhibitory effects than the 6 ppm group, which showed signs of toxicity. In addition, Se appeared to prevent progression of the nodules to full blown hepatocellular carcinoma even after cessation of AFB_1.

Other studies have used other chemicals as hepatic carcinogens or tumor promoters. Using *N*-nitroso-bis(2-oxopropyl)amine (BOP) to induce liver tumors in hamsters, Lee et al. (2008) found that Se (as sodium selenite) injected i.p. (1 mg/kg twice per week for 12 weeks) inhibited the volume and area of tumor foci. Stemm et al. (2008) examined the effect of feeding both deficient and supplemental Se levels during promotion by 3,3′,4,4′-tetrachlorobiphenyl (PCB-77), a coplanar PCB and Ah receptor agonist; and 2,2′,4,4′,5,5′-hexachlorobipenyl (PCB-153), a di-ortho substituted PCB

and constitutive androstane receptor (CAR) agonist. Feeding the highest level of Se was found to increase the number of placental glutathione-*S*-transferase (PGST)-positive foci but to decrease the focal volume. Using the peroxisome proliferator ciprofibrate, Glauert et al. (1990) found that Se inhibited the incidence of tumors and the number and volume of altered hepatic foci in rats.

Two studies have used transgenic models. In mice overexpressing both transforming growth factor (TGF)-α and c-myc, both selenium deficiency and high dietary selenium (as sodium selenite) inhibited the development of liver tumors (Novoselov et al., 2005). In Mdr2 knockout mice, the feeding of supplemental selenomethionine for 3 months followed by a latency period of 13 months resulted in a decrease in the incidence of large tumor nodules (Katzenellenbogen et al., 2007).

Xu et al. (2007) used a transplantable tumor model using HepG2 human hepatoma cells. Mice were given drinking water containing sodium selenite or green tea extracts in which the tea had been grown with different levels of Se. Both selenite and high Se-containing teas inhibited the growth of the transplantable tumors.

Several studies have examined if changes in oxidative stress correlated with effects on carcinogenicity by selenium. In the Glauert et al. (1990) study, in which ciprofibrate-induced carcinogenesis was reduced by dietary Se, high Se levels increased serum, and liver GPx activity, but did not decrease the oxidative damage indices such as thiobarbituric acid reactive substances (TBARs) and conjugated dienes, indicating that increased GPx activity may have no protection against oxidative damage. Hepatoma cells injected into Sprague Dawley rat livers resulted in decreased GPx1 activity, but no significant effect on oxidative stress markers, TBARs and 8-hydroxydeoxyguanosine (8-OHdG), were seen (Sung et al., 1999). Se supplementation reduced lipid peroxide levels in tissues (Thirunavukkarasu and Sakthisekaran, 2001). Se that was supplemented either before initiation or during initiation and selection/promotion phases of hepatocarcinogenesis was found to be effective in altering hepatic lipid peroxidation and antioxidant enzyme activities either in the hepatoma or in the normal liver tissues. Moreover, increased level of lipid peroxidation products and reduced levels of antioxidants, superoxide dismutase, and catalase, were observed in nontumor bearing organs; however, these conditions were reversed to normal upon Se supplementation. When 2-acetylaminofluorene (2-AAF) was used as selection agent in a modified Solt-Farber protocol, selenite did not produce an effect on GPx1 activity (Bjorkhem-Bergman et al., 2005). The liver GPx1 activity of rats given SeMet was also shown to be either not affected or only slightly increased as a result of long term 2-AAF feeding (Mukherjee et al., 1996). They also observed high GSH levels in the nodules as well as the surrounding parenchyma.

In contrast, it was shown that a high Se diet (2.0 ppm as sodium selenite) given to DEN initiated rats had no effect on DEN-induced 8-OHdG, and no correlation was observed between GPx activity and 8-OHdG levels (Wycherly et al., 2004). In fact, they found that a high Se diet increased liver 8-OHdG levels; therefore, instead of protecting against oxidative DNA damage, inorganic Se supplementation may be enhancing oxidative DNA damage *in vivo*. Using the Big Blue rat transgenic model, Zeng et al. (2009) found that dietary selenium did not affect the mutation rate in either the liver or colon in rats injected with 1,2-dimethylhydrazine, a colon carcinogen.

Thioredoxin reductase (TrxR) is another selenoprotein with antioxidant activity, which could mediate the effect of Se on carcinogenesis. Berggren et al. (1999) observed that hepatic TrxR activity of rats fed with high sodium selenite (1.0 ppm) diet was increased twofold; however, this increase was not sustained and was not accompanied by a corresponding increase in TrxR protein synthesis. They suggested that this may be caused by decreased Se incorporation leading to decrease in TrxR protein synthesis. Increased TrxR proteins in tumors of TGFα/c-myc transgenic mice was noted compared with normal liver tissues (Gladyshev et al., 1998). Stemm et al. (2008), however, did not find any changes in TrxR activity in response to PCBs or dietary selenium.

SUMMARY

Overall, studies examining the effect of dietary Se on human liver cancer or experimental hepatocarcinogenesis have shown contradictory results. Several studies have suggested that Se

supplementation could inhibit the development of liver cancer in humans, but other studies do not support this. In experimental studies, a wide variety of models have been used. Again, it has been difficult to observe consistent effects. Increasing dietary Se leads to an increase in hepatic GPx activity; however, this did not lead to expected decreases in oxidative stress, such as lipid peroxidation or oxidative DNA damage. It is possible that mechanisms other than GPx and TrxR activities and the antioxidant properties of these enzymes are mediating the cancer protective effects of selenium.

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33 Cancer, Probiotics, and Clinical Practice

Gian Vincenzo Zuccotti, Fabio Meneghin, and Dario Dilillo

CONTENTS

INTRODUCTION

Complementary and alternative medical therapies are defined as a group of diverse medical and healthcare systems, practices, and products that are not considered part of conventional medicine. Although alternative therapies are widely used by the general population, most of these practices are not well understood by conventional healthcare providers. Among cancer patients, the use of alternative medical therapies is widespread; a review (Ernst and Cassileth, 1998) reported an average prevalence in this population of 31%. Among children, surveys over the past decade have identified a 24–90% prevalence of complementary therapy custom (Kelly, 2007). Given this high level of usage, there is a need to educate families and healthcare providers about both the benefits and the potential risks of alternative therapies.

CANCER EPIDEMIOLOGY

Estimates of the incidence, mortality, and prevalence of cancers in 2002 (Parkin and Bray, 2002) revealed 10.9 million new cases of cancer, 6.7 million cancer-related deaths, and 24.6 million persons living with cancer (within 5 years of diagnosis). Lung cancer is the primary cancer in the world, whether considered in terms of number of cases (1.35 million, representing the 12.4% of all new cancer) or deaths (1.18 million). Breast cancer is the second most common cancer overall with an estimated 1.15 million new cases, but is the most frequent cancer among women (23% of all cancer diagnoses in women).

Colon and rectal cancers constitute the third most prevalent form of cancer in men and women, accounting for about 1 million new cases in 2002 (9.4% of all cancer diagnoses worldwide). The overall prognosis of colorectal cancer is relatively good—its associated mortality rate is about half that of its incidence (about 529,000 deaths in 2002), while its prevalence is second only to that of breast cancer worldwide, with an estimated 2.8 million persons alive with colorectal cancer within five years of diagnosis. Colorectal cancer has a five-year survival rate of 63%, decreasing to 10% in patients diagnosed with metastatic disease (Goldberg, 2005).

Stomach cancer was the second most common cancer worldwide until recently; now, with an estimated 934,000 new cases per year in 2002, it is in fourth place. However, it is the second most common cause of death from cancer (700,000 deaths annually). Regarding bladder cancer, an estimated 357,000 new cases occurred in 2002, making this the ninth most common cause of cancer for both sexes combined, with 145,000 deaths annually.

PROBIOTICS

Probiotics are defined as "live microorganisms which, when ingested in sufficient quantities, exert health benefits" (Salminen et al., 1998, p. 162). Several studies in the past decades have implicated probiotic bacteria in a number of beneficial effects within the host, such as suppression of allergies, control of blood cholesterol levels, and modulation of the immune system; moreover, probiotics have demonstrated efficacy in a number of pathological conditions including gastroenteritis, ulcerative colitis, pouchitis, Crohn's disease, and infections such as urinary tract infections and vaginitis (Zuccotti et al., 2008). In recent times, probiotics have also been suggested as a potential new preventative and treatment option for cancer. The vast majority of studies in current literature about the anticancer effects of probiotics deal with colorectal cancer, although some relate to breast and bladder cancers. The common microorganisms investigated for their probiotic properties are listed in Table 33.1.

ANTICARCINOGENIC MECHANISMS OF PROBIOTICS IN COLORECTAL CANCER

Colorectal cancer (CRC) represents a major public health problem, accounting for more than 1 million cases and about half a million deaths worldwide (Chau and Cunningham, 2006). Although

TABLE 33.1
Common Microorganisms Investigated for their Probiotic Properties

Lactobacillus Species	Bifidobacterium Species	Streptococcus Species	Saccharomyces Species	Other Species
L. acidophilus	*B. bifidum*	*S. thermophilus*	*S. boulardii*	*Bacillus cereus*
L. casei (rhamnosus)	*B. breve*	*S. salivarius subsp. thermophilus*		*Escherichia coli*
L. fermentum	*B. lactis*			*Enterococcus*
L. gasseri	*B. longum*			*Propionibacterium freudenreichii*
L. johnsonii	*B. infantis*			
L. lactis	*B. adolescentis*			
L. paracasei				
L. plantarum				
L. reuteri				
L. salivarius				
L. bulgaricus				

chemotherapy and radiotherapy have been applied as surgical adjuvant treatments for CRC, their success rates for local recurrence, disease-free survival, and overall survival vary. In addition, these treatments may present some side effects, such as an increased risk for infections, hair loss, fatigue, nausea, vomiting, diarrhoea, and bloody stools.

The reputed anticarcinogenic effects of probiotics arise from *in vivo* studies in animals and, to a limited extent, humans. This evidence is supported by *in vitro* studies with carcinoma cell lines and antimutagenicity assays. However, the mechanisms involved in any effect have not been completely elucidated and several studies are needed to completely understand the possible role of probiotics in cancer treatment and prevention.

There are presently very few epidemiological studies specifically aimed at assessing the potential effects of probiotics on the incidence of CRC/adenoma development. One such study (Ishikawa et al., 2005) examined whether dietary fiber and *L. casei* prevented the occurrence of CRC in tumor-free patients who had had at least two CRC removed. Results suggested that the occurrence rate of tumors with a grade of moderate or higher atypia was significantly lower in patients treated with *L. casei*.

The few human studies present in the literature are very difficult to interpret because they are extremely heterogeneous in terms of design, population examined, definitions of items considered, terminology, and endpoints evaluated. The conclusions reached are often completely divergent and inconclusive regarding the possible preventive role of probiotics in CRC development (Capurso et al., 2006).

Colorectal cancers arise through a well-defined series of histological changes (adenoma-carcinoma sequence), which is paralleled by mutations, activations, and deletions of oncogenes and tumor suppressor genes. It is not yet clearly understood at what point in this process probiotics may be exerting their effects, but it is likely that different strains may exert effects at different stages of carcinogenesis.

Commensal bacteria have been linked to CRC since the site of these intestinal cancers coincides with colonic regions in which bacterial concentration is greater (Huycke and Gaskins, 2004). The alterations of intestinal microflora facilitate the development of intestinal inflammation, possibly due to an increased host-immune response to the commensal bacteria (Guarner and Malagelada, 2003).

The importance of microflora in CRC pathogenesis has been evaluated in several animal model studies including inteleukin-10 (IL-10) knockout mice, *Muc2* knockout mice (Berg et al., 1996; Yang et al., 2005) and *TCRβ/p53* double knockout mice (Kado et al., 2001). When the latter are exposed to germ-free conditions they appear normal, but when intestinal bacterial colonization is promoted they spontaneously develop intestinal inflammation, which can progress to tumor formation. On the basis of previous experiments, manipulation of the intestinal bacterial milieu by administration of probiotics represents a rational therapeutic target.

The mechanisms by which probiotics may inhibit colon cancer are not yet fully characterized, but there is evidence for a reduced inflammatory response to host flora, alterations in the metabolic activities of intestinal bacteria, a reduction in the number of bacteria involved in procarcinogenic and mutagenic pathways, and the production of antitumorigenic and mutagenic substances.

Alteration of the Intestinal Microflora Composition/Competition with Pathogens

Several studies, including those of O'Mahony et al. (2001) and Gaudier et al. (2005) have shown that consumption of probiotic organisms could significantly reduce the population of fecal putrefactive bacteria (e.g., coliforms) and increase the population of commensal bacteria (e.g., lactobacillus and bifidobacteria). This alteration of intestinal microflora has been associated with a reduced incidence of colonic adenocarcinoma in IL-10 knockout mice treated with *L. salivarius* ssp. salivarius UCC118 (O'Mahony et al., 2001).

Reduction of Intestinal Inflammation

The relationship between chronic inflammation and several malignant diseases has been reported for a long time (Prescott and Fitzpatrick, 2000). There is evidence that the relationship is mediated by cytokines (Feghali and Wright, 1997) or reactive oxygen species generated by inflammatory phagocytes that can cause injury to cells and contribute to cancer development (Weitzman and Gordon, 1990). Intestinal inflammation has been linked to the development of CRC, and data has shown that inflammatory bowel disease (IBD) increases the likelihood of CRC development later in life. Several studies have reported that probiotics have an anti-inflammatory effect in IBD patients, and this reduction in inflammation has the potential to lead to a reduced incidence of CRC. Tooley et al. (2006) used Streptococcus thermophilus TH-4 as a vehicle to deliver a source of folate, a compound with important DNA repair properties, to rats with chemotherapy-induced mucositis in order to reduce the pro-inflammatory response. Very recently, Urbanska et al. (2009) studied the potential immunomodulatory and antitumorigenic properties of microencapsulated *L. acidophilus* in a yogurt formulation in Min mice carrying a germline mutation in the APC gene. Daily oral administration of *L. acidophilus* resulted in significant suppression of colon tumor incidence and multiplicity, as well as reduced tumor size. The authors concluded that *L. acidophilus* exerts beneficial action by maintaining constant body weight, minimizing intestinal inflammation, and delaying overall polyp progression in experimental mice.

Reduction of Mutagenic/Carcinogenic/Genotoxic Compounds

Many foreign compounds are detoxified by glucuronide formation in the liver before entering the intestine via the bile. A number of bacterial enzymes, including β-glucuronidase and nitroreductase, play an important role in cancer development. These enzymes hydrolyze many glucuronides, and thus may liberate carcinogenic aglycones in the intestinal lumen (de Moreno de LeBlanc et al., 2005). Animal studies (Kulkarni and Reddy, 1994; Rowland et al., 1998) have revealed that consumption of lactic acid bacteria is able to reduce the activity of these enzymes, indicating a possible mechanism by which probiotics can prevent CRC. Probiotics have also reduced the specific activities of fecal enzymes in human volunteer studies (Ayebo et al., 1980; Goldin et al., 1980; Ling et al., 1994; Spanhaak et al., 1998). Goldin and Gorbach (1984) studied the effect of *L. acidophilus* strain NCFM and N-2 on the activity of three bacterial enzymes (β-glucuronidase, azoreductase, and nitroreductase) in 21 healthy volunteers. Both strains had a similar effect and caused a significant decline in the specific activity of the three enzymes in all subjects after 10 days of feeding, but a reversal of the effect was observed within 10–30 days after supplementation was discontinued. These data suggested that continuous consumption of these probiotics was necessary to maintain their effect. Moreover, human studies have demonstrated that the capacity for probiotics to decrease the activity of these bacterial enzymes is strain-specific. To this end, *L. plantarum 299V* (Goossens et al., 2003), *L. rhamnosus DR20* (Tannock et al., 2000) and *L. acidophilus A1* (Marteau et al., 1990) were unable to decrease β-glucuronidase activity in healthy subjects. However, *L. casei Shirota* (Spanhaak et al., 1998) and *L. acidophilus* (Goldin et al., 1980) significantly decreased enzymatic activity, indicating that these last two strains could potentially reduce carcinogen production and the likelihood of CRC. Recently, Hatakka et al. (2008) performed a human randomized, double-blind, placebo-controlled, two-period crossover study in which they found that oral administration of *L. rhamnosus* LC705 together with *Propionibacterium freudenreichii* ssp. shermanii JS was followed by an increase in the fecal counts of lactobacilli and propionibacteria and a decrease in the activity of β-glucosidase with increasing counts of propionibacteria.

Reports published to date do not always find a reduction in the same enzymes, although findings with β-glucuronidase and nitroreductase are most consistently positive. Thus, in summary, animal and human studies do indicate that oral administration of certain probiotics can result in a decrease

of fecal enzymes that may be involved in carcinogenesis. However, it is still not completely understood how or whether this effect affects cancer rates in humans.

Alteration of Physiochemical Conditions in the Colon

Fermentation is a common characteristic of microflora. The anaerobic breakdown of substrates, such as undigested polysaccharides, resistant starch, and fiber, enhances the formation of lactic acid bacteria and also produces short-chain fatty acids (SCFA) as fermentation products. Increased production of SCFA leads to a decrease in the pH of colon content. A low pH in feces has been associated with a reduced incidence of colon cancer in various populations (Malhotra, 1977; Segal et al., 1995).

Modler et al. (1990) have suggested that large bowel cancer could be influenced directly by reducing intestinal pH, thereby preventing the growth of putrefactive bacteria. In rats given inulin-containing diets with or without *B. longum*, increases in cecal weight and β-glucosidase and a decrease in cecal pH were observed (Rowland et al., 1998), although some other studies did not detect a significant change in intestinal pH (Bartram et al., 1994; Abdelali et al., 1995).

Epidemiological studies indicate an association between the risk of colon cancer development and consumption of high-fat diets. It has been suggested that this phenomenon may be mediated primarily by an increased level of secondary bile acids in the colon, due to the action of bacterial 7α-dehydroxylase on primary bile acids (Weisburger and Wynder, 1987). These acids are believed to exert a cytotoxic effect on epithelial cells, thereby increasing cell proliferation and leading to a higher probability of colon cancer development (Bruce, 1987). Modulation of the intestinal microflora through probiotic consumption may affect the activity of 7α-dehydroxylase (Kithara et al., 2001). De Boever et al. (2000) suggest that probiotics may physically bind bile salts; using hydrolase-active *L. reuteri*, they attempted to control cell toxicity in the presence of bile salts. Theoretically, *L. reuteri* should have increased the bioavailabilty of secondary bile acids; however, decreased cell toxicity was observed with *L. reuteri* treatment, suggesting that, despite activating secondary bile acids, *L. reuteri* may protect against their toxic effects by binding the bile salts. Moreover, it has been demonstrated that a 6-week administration of *L. acidophilus* to 14 colon cancer patients resulted in lower concentrations of soluble bile acids in feces (Lidbec et al., 1991). Although the decrease in the concentration in this fraction of feces was not significant (perhaps due to the small number of patients), it was of interest that a definite trend towards decreased levels of soluble bile acids was observed in these patients. In another three-month study (Biasco et al., 1991), *L. acidophilus* was administered together with *B. bifidum* to patients with colonic adenomas. During this period, the fecal pH was reduced significantly and patients who had a higher proliferative activity in the upper colonic crypts than had been calculated for subjects at low risk for colon cancer showed a significant decrease after therapy with probiotics. This decrease in proliferative activity was in part due to decreased levels of bile acids in the aqueous phase of feces.

Another mechanism by which probiotics may play a role in host physiology is the production of metabolites that could affect the mixed function of cytochrome P450, and ultimately the conversion of compounds to carcinogens (Campbell and Hayes, 1976). There are 57 of these enzymes encoded in the human genome, most of which catalyze the metabolism of steroids, bile acids, eicosanoids, drugs, and xenobiotic chemicals. Some of the P450 cytochromes are also active carcinogens. The metabolic activation of foodborne heterocyclic amines to colon carcinogens in humans is hypothesized to occur via *N*-oxidation followed by *O*-acetylation to form metabolites that bind to DNA to create carcinogen-DNA adducts. One of these steps is catalyzed by hepatic cytochrome P4501A2.

Binding and Degradation of Potential Carcinogens

Meat products have been linked to an increased risk of CRC as a consequence of the production of toxins including *N*-nitroso compounds and heterocyclic aromatic amines (Zsivkovits et al., 2003; Ferguson et al., 2004).

Mutagenic compounds, which are commonly found in the Western meat-rich diet, can be bound to the intestinal and lactic acid bacteria *in vitro*, and this binding correlates well with the reduction in mutagenicity observed after exposure to certain bacterial strains. For example, Orrhage et al. (1994) have reported on the binding capacity of eight human intestinal or probiotic strains for mutagenic heterocyclic amines formed during the cooking of protein-rich food.

Morotomi and Mutai (1986) investigated the ability of 22 strains of intestinal bacteria to bind the mutagenic prolyzates and compared their ability with that of some dietary fibers. Some indoles (the aromatic heterocyclic organic compounds, such as Trp-P-1 and Trp-P-2) were effectively bound to all Gram-positive and some Gram-negative bacterial cells, corn bran, apple pulp, and soybean fiber. When the mechanism of binding of Trp-P-2 to *L. casei* YIT 9018 and corn bran was investigated, it was shown to be pH dependent, instantaneously occurring, and inhibited by the addition of metal salts. The mutagenicity of Trp-P-2 for *Salmonella thphimurium* TA98 in the presence of S9 mix was inhibited by the addition of *L. casei* YIT 9018 to the reaction mixture, indicating that bound Trp-P-2 did not cause mutation under the assay conditions. A more recent study demonstrated a reduced uptake of Trp-P-2 and its metabolites in various tissues of mice supplemented with dietary lactic acid bacteria (Orrhage et al., 2002).

Zhang and Ohta (1993) showed that freeze-dried cells of lactic acid bacteria, intestinal bacteria, and yeast significantly reduced the absorption of Trp-P-1 from the small intestine in rats, and that this was accompanied by a decreased level of this mutagen in the blood. In addition, the consumption of lactobacilli by human volunteers has been shown to reduce the mutagenicity of urine and feces associated with the ingestion of carcinogens in cooked meat (Hayatsu and Hayatsu, 1993). It is possible that the lactic acid bacteria supplements are influencing the uptake and excretion of mutagens by binding them in the intestine. Lactobacilli have also been shown to degrade nitrosamines (Rowland and Grasso, 1975), which have been revealed as carcinogens in animal models and have also been detected in human feces.

In a study by Zsivkovits et al. (2003), F344 rats were treated with heterocyclic aromatic amine (HCA) mixtures reflecting the HCAs found in beef and chicken. Oral administration of *L. bulgaricus* 291, *Streptococcus thermophilus* F4, *S. thermophilus* V3, or *B. longum* BB536 led to complete prevention of DNA damage in beef-mix treated rats, although little effect was observed in the chicken mix. In another study (Pool-Zobel et al., 1996), *L. acidophilus, L. gasseri, L. confuses, S. thermophilus, B. breve*, and *B. longum* have been demonstrated to decrease the extent of 1,2-dimethylhydrazine-induced DNA damage in rats. Burns and Rowland (2004) demonstrated that *L. plantarum* and *B.* Bb12 possessed significant antigenotoxic effects *in vitro* as they reduced fecal water genotoxicity toward HT-29 cells, suggesting that these probiotic strains may have been beneficial in preventing the early stages of colon cancer. Similar results were observed following treatment with probiotic yogurt containing *L. acidophilus* 145 and *B. longum* 913 (Oberreuther-Moschner et al., 2004).

Together, these data suggested that probiotic consumption may indeed prevent CRC by reducing the bioavailability of carcinogenic compounds and reducing DNA damage.

Production of Antitumorigenic or Antimutagenic Compounds

It has been suggested that probiotics or a soluble compound produced by the bacteria, may interact directly with tumor cells in culture and inhibit their growth (Reddy et al., 1983). Baricault et al. (1995) demonstrated that probiotics significantly reduced the growth and viability of the human colon cancer cell line HT-29 in culture, with an increase in dipeptidyl peptidase IV and brush-border enzymes, suggesting that these cells might have entered a differentiation process.

The production of SCFAs, such as butyrate, acetate and propionate, is another key mechanism by which probiotics may impart beneficial effects. The resident colonic flora ferments luminal substrates, such as nondigestible carbohydrates, proteins, lipids, mucins, and recyclable components of dead bacteria, to produce a variety of metabolites, particularly SCFAs. Probiotic bacteria compete

with other bacteria in the colonic lumen for fermentation of these substrates. Luminal SCFAs, particularly butyrate, are potential anticarcinogenic agents within the gut. Nadathur et al. (1995) measured the antimutagenicity of an acetone extract of a *L. bulgaricus* 191R fermented yogurt and observed a significant dose-dependent antimutagenic activity against several mutagens, suggesting that a metabolite of the probiotic may be responsible for its anticarcinogenic properties.

Butyrate administration in animal models of CRC has produced varying results, but it is now believed that the dose and mode of butyrate delivery, in addition to the stage of cancer development, are important factors in determining butyrate effects on the colon (Sengupta et al., 2006). Ohkawara et al. (2005) investigated the bacterial strain *Butyrivibrio fibrisolvens* MDT-1, which is able to produce high amounts of butyrate, in the context of CRC treatment. The administration of this strain of colon cancer in an induced mouse model led to a significant decrease in aberrant crypt foci (ACF), and the number of mice with an increased proportion of ACF was also reduced, indicating that progression of tumor development was inhibited. This new probiotic also reduced β-glucuronidase activity and enhanced the immune response, as indicated by an increase in the number of natural killer (NK) cells. Similar effects have been observed in the propionate- and acetate-producing probiotic, *Propionibacterium acidipropionici*, which has been demonstrated to induce apoptosis in HT29 and Caco-2 cancer cell lines (Jan et al., 2002).

The mechanisms behind the anticarcinogenic effects of SCFAs have been most clearly demonstrated for butyrate. This SCFA is the preferred energy source of colonocytes and has been implicated in the control of the biological machinery regulating apoptosis and cellular differentiation (Mariadason et al., 2000). At a molecular level, butyrate affects gene expression via histone phosphorylation and acetylation (Archer and Hodin, 1999). Certain probiotics may modify the ratio of SCFAs in the colon, and this remains one of their more likely mechanisms of anticarcinogenic action within the colon. These studies indicate that SCFA delivery via probiotic ingestion may be a potential new treatment option for CRC.

Enhancement of the Host's Immune Response

In addition to a potential role in the prevention of CRC, probiotics have been shown to possess antitumorigenic activity, suggesting a potential role in the treatment of established tumors. One mechanism by which this may occur is the modulation of the mucosal and systemic immune responses. Sekine et al. (1985) demonstrated that *B. infantis* stimulates the host-mediated response, leading to tumor suppression or regression. Now, many studies suggest that probiotics play an important role in the host's immune system by increasing specific and nonspecific mechanisms to have an antitumor effect. The potential mechanisms of probiotic-induced immune suppression of carcinogenesis are complex. An inflammatory immune response produces cytokine-activated monocytes and macrophages, which release cytotoxic molecules capable of lysing tumor cells *in vitro* (Philip and Epstein, 1986). The inflammatory cytokines IL-1 and tumor necrosis factor-α (TNF-α) exert cytotoxic and cytostatic effects on neoplastic cells in *in vitro* models (Raitano and Korc, 1993). NK cells are effective against tumor cells, and low cell activity has been associated with cancer risk (Takeuci et al., 2001). Studies report increased NK cell activity and inflammatory-type responses with the administration of some probiotic strains (Matsuzaki and Chin, 2000). Takagi et al. (2001) demonstrated that the administration of *L. casei* strain Shirota (LcS) was able to inhibit tumor-induced development in mice. Elevated levels of NK cell activity were observed in treated mice, as was the delayed onset of tumor development in comparison to the control group. Further, mice that were deficient in NK cells did not show delayed tumor development in response to probiotic treatment. Another study demonstrated that *L. casei* strain Shirota could have antitumor and antimetastatic effects on transplantable tumor cells to suppress chemically induced carcinogenesis in rodents and to induce the production of several cytokines (interferon-γ, TNF-α, and IL-1β), resulting in an inhibition of tumor growth and increased survival of tumor-bearing mice (Matsuzaki and Chin, 2000). Similar results have been reported recently after the administration of *L. acidophilus* SNUL,

L. casei YIT 9029 and *B. longum* HY 8001 (Lee et al., 2004). These strains significantly reduced tumor proliferation *in vitro*, increased the survival rate of mice injected with tumor cells, and promoted antitumor activity via increased cellular immunity. Sun et al. (2005) demonstrated *in vivo* that peptidoglycan from a lactobacillus species was able to dose dependently reduce the growth of CT26 colon cancer cells in BALB/c mice via an increased level of apoptosis. Interestingly, peptidoglycan had no effect on tumor cell apoptosis *in vitro*, implying that the *in vivo* activity may have been mediated by an immune response. Moreover, a recent study by Ghoneum et al. (2005) demonstrated that Caco-2 colonic adenocarcinoma cells underwent apoptosis *in vitro*, upon phagocytosis of the yeast strain *Saccharomyces cerevisiae.*

Effects of Probiotics on the Promotion Phase of Carcinogenesis

Aberrant crypt foci (ACF) are considered precancerous lesions and are observed in both humans and carcinogen-treated animals. Several studies have highlighted the effects of the oral administration of probiotics on colonic ACF formation, predominantly in animal models. Rowland et al. (1998) showed that dietary *B. longum* 25 inhibited azoxymethane (AOM) induced ACF formation and also demonstrated that this effect was increased with simultaneous administration of probiotics with the prebiotic inulin. The authors proposed that probiotics are acting during the promotion phase of carcinogenesis, as administration of the diets began one week after carcinogen exposure. In a similar experiment, Goldin et al. (1996) investigated the effect of diets enriched with *L. rhamnosus* strain GG (LGG) on the induction of tumors in rats by the carcinogen dimethylhydrazine. They observed a lower incidence of colonic tumors in rats fed LGG before, during, and after exposure to dimethylhydrazine than in rats receiving the probiotic after nine weekly injections of the carcinogen. Besides the effect on ACF formation, several studies have looked at the role of probiotics in other cellular and physiological events associated with tumor promotion in the colon, such as a decrease in epithelial barrier integrity. Probiotics have been shown to improve this barrier function *in vivo*, especially *L. brevis* (Garcia Lafuente et al., 2001) and *L. plantarum* 299 (Kennedy et al., 2000).

ROLE OF PROBIOTIC SUPPLEMENTATION ON EXTRA-INTESTINAL CANCERS

Although most published studies have shown the role of probiotics in the prevention and treatment of colorectal cancer, a few trials have tried to evaluate the possible application of probiotic strains to other types of cancer.

For example, Ghoneum et al. (2005) demonstrated that tongue squamous carcinoma cells, like the Caco-2 colonic adenocarcinoma cells, underwent apoptosis *in vitro* upon phagocytosis of the yeast strain *Saccharomyces cerevisiae*. This was also observed in a breast cancer cell line (Ghoneum et al., 2004) and in metastatic breast cancer cells (Ghoneum et al., 2008), suggesting that probiotic therapeutic interventions may not necessarily be restricted to cancers affecting gastrointestinal system.

An Italian study (Biffi et al., 1997) examined the direct effect of milk fermented by five bacterial strains (*B. infantis, B. bifidum, B. animalis, L. acidophilus*, and *L. paracasei*) on the growth of the MCF7 breast cancer cell line. The results showed that all strains induced growth inhibition, although *B. infantis* and *L. acidophilus* were the most effective, and the authors deduced that these strains may have potential as producers of compounds with antiproliferative activity useful in the prevention and therapy of solid tumors (e.g., breast cancer). de Moreno de LeBlanc et al. (2007) demonstrated that it is possible to obtain an immune stimulation in distant mucosal sites in a breast cancer model with the oral administration of fermented products. Seven days of cyclic feeding with milk fermented by *L. helveticus* R389 or L89 delayed tumor development and consequently decreased the number of cells secreting the cytokine IL-6, which has been implicated in the synthesis of oestrogen, a hormone that the tumor requires for growth. Milk fermented by *L. helveticus* R389

induced a decrease of IL-6 as well as an increase of regulatory cytokine IL-10 and cell apoptosis in the tumor.

In relation to the ability of probiotics to induce an immune response, there was evidence that orally administered probiotic strains inhibit extraintestinal cancer. In two double-blind studies of human cancer patients fed *L. casei* preparations, Aso and colleagues (1992, 1995) reported suppression of the recurrence of bladder tumors, indicating anticancer effects beyond the local environment of the colon. In another study, Ohashi et al. (2002) assessed the preventive effect of *L. casei* against bladder cancer, and concluded that the habitual intake of this probiotic strain reduced the risk of this tumor. In an animal study, Lim et al. (2002) were able to inhibit the subcutaneous development of an implanted bladder tumor cell line in mice that were fed *L. rhamnosus* strain GG, compared with the development observed in control mice. Again, they associated this tumor inhibition with observed increases in cellular immune activity. In a prospective, randomized, controlled trial, Naito et al. (2008) demonstrated that oral administration of a *L. casei* preparation enhanced the prevention of recurrence by intravesical instillation of chemotherapeutic epirubicin after transurethral resection for superficial bladder cancer.

Okawa et al. (1993) performed a randomized, controlled, comparative study on the efficacy and safety of radiation therapy combined with *L. casei* YIT9018 in 228 patients with Stage IIIB cervical cancer. Results showed that the probiotic enhanced radiation-induced tumor regression and the combined radiation-probiotic therapy prolonged overall survival and relapse-free interval. Radiation-induced leukopenia was significantly less severe in the LC9018-combined group, suggesting that this probiotic strain was an effective agent for adjuvant immunotherapy when combined with radiation therapy.

The aim of a study by El-Nezami et al. (2006) was to determine whether the administration of probiotic bacteria, a mixture of the strains *L. rhamnosus* LC705 and *Propionibacterium freudenreichii* subsp. *shermanii*, could block the intestinal absorption of aflatoxin B_1. Aflatoxins, a group of mycotoxins produced by the common fungi *Aspergillus flavus* and *A. parasiticus*, are established human hepatocarcinogens and are well-known hepatocellular carcinoma (HCC) risk factors when present in foodstuffs. Results clearly showed that a probiotic supplement reduces the biologically effective dose of aflatoxin exposure and may thereby offer an effective dietary approach to decreasing the risk of liver cancer.

CONCLUSIONS

Despite the many claimed protective effects of probiotics against CRC, not all investigations support this outcome. The contradictory results may be related to the complexity of carcinogenesis, experimental design, complications in obtaining the appropriate sample size, variation in the type and dose of probiotic strains, and variations in the tumor stages of subjects. Although the effects of probiotics on the interactions between the commensal microflora and the host appear to be beneficial, at least in animal models, a greater understanding of the mechanisms involved and the identification of the probiotic strains that confer the most benefit are required before we can fully justify the use of probiotics for cancer prevention or treatment in humans.

Regardless, the use of probiotics to prevent CRC has gained much attention due to positive outcomes from *in vivo* and molecular studies. The strongest evidence for the anticancer effects of probiotics comes from animal studies; evidence from epidemiological and experimental human studies is still limited. An important future goal should be the development of carefully designed human clinical trials to corroborate the wealth of experimental studies.

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34 Indian Herbal Medicine for Cancer Therapy and Prevention
Bench to Bedside

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CONTENTS

INTRODUCTION

Cancer is a hyperproliferative disease, and a cascade of events take place in order to cause a full blown disease. The major events include transformation, dysregulation of apoptosis, proliferation, invasion, angiogenesis, and metastasis. Meticulous research since the past few decades has yielded much information about the biology of cancer. Drugs used in the treatment of most cancers are those

that can interfere with cell signalling, like growth factor signalling, prostaglandin production, inflammation, drug resistant gene products, cell cycle proteins, angiogenesis, invasion, antiapoptosis, cellular proliferation and many others (Aggarwal et al., 2006; Arora, 2010; Arora et al., 2010a,b,c). Herbals have been used in nearly every culture on earth for medicinal purposes. This method of medicine was practiced by various ancient civilizations thriving in Asia, Africa, Europe, and the Americas. As modern chemistry developed, chemicals and various constituents were isolated from medicinal herbs. These phytoconstituents have served either as drugs that are being used widely today or as starting materials for their synthesis. Many modern day drugs being used widely have been developed as a result of knowledge obtained from studying the mechanism of actions of various chemicals present in the herbal plants. Thus, we can easily infer that medicinal herbs have played a pivotal role in the expansion of modern medicine and continue to be widely used in their native form as well (Matthews et al., 1999; Sharma and Arora, 2006; Arora et al., 2008). Modern medicines derived from herbs are gaining attention throughout the world today. For example, the transformation of foxglove, a folk medicine, going through digitalis, eventually to a modern drug, digoxin, illustrates potential of modern pharmacology that has played a supportive role in making drugs safer and more effective (Goldman, 2001; Tapsell et al., 2006).

TRADITIONAL COMPLEMENTARY AND ALTERNATIVE MEDICINE

Patients suffering from cancers of prostate and various other organs are increasingly exploring the benefits of alternative medicine, primarily because they experience side effects due to usage of modern anticancer drugs or wish to try new therapies in the hope of getting better (Wargovich et al., 2001; Steenkamp, 2003; MacLaughlin et al., 2006). Complementary and alternative medical therapies (CAM) are basically therapies that are different from conventional modern medicine. CAM therapies are utilized for treating the side effects of cancer or cancer therapy, and seldom as an alternate to conventional medicinal therapy (Kelly, 2004). Traditional Indian and Chinese medicine have received enormous attention in the recent years (Moyad et al., 1999; Arora, 2010). Notably, there are some striking contrasts between the Western medicine system and traditional medicine. While the former system presently makes use of purified, single compounds, which can be either natural or synthetic, the latter medicine system has always used multiple combinations of processed natural products to treat many different human ailments. Several medicines, including those in traditional Indian, Chinese, and Tibetan herbal medicine have been successfully used for preventing and treating cancers in recent years (Lee, 2000; Wargovich et al., 2001; Steenkamp, 2003; Arora, 2010).

Indian herbal medicines have had a history of safe usage for the past few thousands of years. In recent years, the work on Indian herbal medicine has gained momentum due to both treatment and preventive options. Ayurveda, Siddha, and Unani systems involve an assortment of prophylactic and therapeutic approaches consisting of diet, herbs, metals, minerals, precious stones, and their combinations as well as some nondrug therapies. Ayurveda can easily be said to be the oldest system of medicine in the world and especially is the most commonly practiced form of medicine in India (Dev, 1999; Gogtay et al., 2002; Garodia et al., 2007). The difference between modern Western medicine and these systems arises from the fact that the knowledge base of the latter is based on years of experience, observations, and intuition and has been passed on from generation to generation both through word of mouth and treatises. On the contrary, Western medicine system has not been developed in this manner (Gogtay et al., 2002). While Western medicine is capable of handling acute medical crises, traditional systems of medicine like Ayurveda display the ability to control and prevent several chronic disorders in a suitable manner. Hence, in view of its inclusive approach, Ayurveda's emphasis on prevention, ability to manage chronic disorders, and its widespread use among the masses, it is widely accepted that it improves the quality of life of the people in general (Sharma et al., 2007) and is specifically useful for treating cancers.

WHY HERBAL MEDICINES ARE IN DEMAND?

The increasing popularity of herbs as medicines over the more common allopathic system is primarily a result of the risk of mortality and long-term morbidity linked to surgeries and side effects of allopathic medicines. Phytomedicines have been shown to benefit patients by providing relief from a plethora of ailments. It also gives the users the power to choose the medications that they want to use (Wargovich et al., 2001; Steenkamp, 2003). Hence, herbs have been used since a long time either directly or as dietary supplements. This is also due to the realization that herbal products act in a pathway similar to pharmaceuticals yet pose no side effects. Natural anti-inflammatory compounds are found in plenty in the herbal plants and have been already reported in green tea, the spices turmeric and rosemary, feverfew, and many others. It is a well-known fact that phenolic compounds are present in all plants. Some of these include flavonoids, carotenoids, vitamin C, vitamin E, selenium, dithiolthiones, isothiocyanates, indoles, phenols, protease inhibitors, allium compounds, plant sterols, limonene, and so on. These have been studied in some of the plants like cereals, legumes, nuts, olive oil, vegetables, fruits, tea, and so on. Many of these phenolic compounds possess antioxidant properties, and some have revealed favorable effects on tumorogenesis and inhibit cancer promotion and progression. Some components like hydroxytyrosol, a phenolic present in olives and olive oil, is a potent antioxidant and anticancer agent; resveratrol, isolated from nuts and red wine, shows antioxidant, antithrombotic, and anti-inflammatory properties, and also inhibits carcinogenesis. While lycopene, a potent antioxidant carotenoid found in tomatoes, is considered to provide protection against prostate and other cancers, and to inhibit tumor cell growth in animals. Organosulfur compounds in garlic and onions, isothiocyanates in cruciferous vegetables, and monoterpenes in citrus fruits, cherries, and herbs have been reported to exert anticarcinogenic effects in experimental models (Arora, 2010). The various chemopreventive mechanisms of actions of these components vary. Some of these like glucosinolates and indoles, thiocyanates and isothiocyanates, phenols, and coumarins can both inhibit phase I enzymes and induce an array of phase II (solubilizing and inactivating) enzymes; ascorbate and phenols act as an obstruction in the development of carcinogens such as nitrosamines; flavonoids and carotenoids act as antioxidants, which disable the carcinogenic potential of specific compounds. Lipid-soluble constituents such as carotenoids and sterols have potential to modify membrane structure or integrity. Also, carotenoids can inhibit DNA synthesis and enhance differentiation (Steinmetz and Potter, 1991; Potter and Steinmetz, 1996; Waladkhani and Clemens, 1998; Kris-Etherton et al., 2002; Pan and Ho, 2008).

Some of the various Indian herbs, which have shown immense potential in cancer treatment and prevention, are described in Table 34.1. These can be of great help for the mankind in their fight against cancer.

Catharanthus roseus

Catharanthus roseus belongs to the family Apocynaceae. Some of its common names in India are Nayantara, Nityakalyani, Periwinkle, Rattanjot, and Sadabahar. Out of the seven known species of the genus, only one is restricted specifically to India. The plant has been extensively used in traditional medicine since a long time. Ayurvedic medicines have been prepared from various parts of the plant like the stem, leaves, flowers, and roots. Extracts of the plant have been reported to be used for treatment of many ailments like ocular inflammation, diabetes, hemorrhage, in treatment of insect stings, and cancers. It contains more than 120 terpenoid indole alkaloids (TIAs), several of which have exhibited strong pharmacological properties. Some of these alkaloids are used widely in modern medicine as immunosuppressive and antitumor agents. The *Catharanthus* alkaloids have proved to be of utmost importance in clinical medicine, and mainly, vincristine (VCR), vinblastine (VLB), and vinorelbine (VRL) form essential components of many standard chemotherapy regimens like ABVD, BCVPP, CHOP, MOPP, STANFORD V, VC, and so on. (Figures 34.1 through 34.3) Vincristine is used mainly for Hodgkin's lymphoma, while vinblastine is effective in case of

TABLE 34.1 Plant-Derived Anticancer Compounds and Their Mode of Action against Different Types of Cancers

Plant	Compound	Action against Cancer Type	Mode of Action	Combinatorial Regimens
C. roseus	Vincristine	Hodgkin's lymphoma, cervical cancer	Arrest proliferation of cancer cells by binding tubulin filaments in mitotic spindle; induce apoptosis	BOMP, CHOP, MOPP, STANFORD V, etc.
C. roseus	Vinblastine	Childhood leukemia, Hodgkin's lymphoma, Kaposi's sarcoma, bladder cancer, malignant melanoma	Arrest proliferation of cancer cells by binding tubulin filaments in mitotic spindle; induce apoptosis	ABV, ABVD, BCVPP, CMV, CVD, STANFORD V
C. roseus	Vinorelbine	Non small cell lung cancer	Arrest proliferation of cancer cells by binding tubulin filaments in mitotic spindle; induce apoptosis	NP, VC, etc.
Azadirachta indica	Nimbolide	–	Antiproliferative; induces apoptosis	–
A. indica	Gedunin	–	Inhibits 90 kDa heat shock protein folding machinery	–
Bauhinia	Bauhiniastatin1 (2a)	Human P388 cancer cell line	–	–
P. hexandrum	Podophylloxin derived etoposide (VP-16)	Lung cancer, testicular cancer, neuroblastoma, hepatoma	Inhibition of microtubule assembly; inhibits DNA topoisomerase II	CEA, CEC, EI, etc.
P. hexandrum	Podophylloxin derived teniposide	Lung cancer, testicular cancer, neuroblastoma, hepatoma	Inhibition of microtubule assembly; inhibits DNA topoisomerase II	–
Allium sativum	Diallyl trisulphide	–	Modulation of carcinogen metabolism; inhibition of DNA adduct formation; upregulation of antioxidant defences and DNA repair systems; suppression of cell proliferation and inducing apoptosis	–
Combretum	Combretastatin A4	Human cancer cell lines like PTC, PA1, SKOV3, Miapaca 2	Inhibits microtubule assembly; disrupt tumor blood flow	CA4P in association with liposomal doxorubicin
Camptotheca	Camptothecin derived topotecan	–	Interferes with enzyme activity promoting Top1-mediated DNA breaks and inhibits DNA and RNA synthesis	–
Camptotheca	Camptothecin derived irinotecan (CPT-11)	Ovarian and lung cancer	Interferes with enzyme activity promoting Top1-mediated DNA breaks and inhibits DNA and RNA synthesis	–

Camptotheca	Camptothecin derived CKD-602	Anticancerous to glioma cells	Interferes with enzyme activity promoting Top1-mediated DNA breaks and inhibits DNA and RNA synthesis	–
Taxus baccata	Taxol (paclitaxel)	–	Cause mitotic arrest in cancer cells; apoptosis due to inhibition of depolymerization of microtubules	–
Taxus baccata	Taxotere (docetaxel)	NSCLC	Binds to tubulin and induces polymerization; promotes stable microtubule formation	Carboplatin, cisplatin, docetaxel, gemcitabine and vinorelbine; docetaxel and cisplatin
Curcuma longa	Curcumin (diferuloylmethane)	–	Arrests cell cycle; inhibits inflammatory response and the oxidative stress; induces apoptosis in cancer cells, p21-mediated cell cycle arrest; possesses marked antiangiogenic properties; inhibits phosphorylation of p70 S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1), downstream effector molecules of the mammalian target of rapamycin complex 1 (mTORC1)	–
Zingiber officinalis	[6]-gingerol and [6]-paradol	Human promyelocytic leukemia (HL-60) cells	Act against cyclo-oxygenase-2 (COX-2); inhibits tumor-promoter-stimulated inflammation, TNF-alpha production, and activation of epidermal ornithine decarboxylase in mice	–
Piper nigrum	Piperine	–	Inhibits hepatic and intestinal aryl hydrocarbon hydroxylase and UDP-glucuronyl transferase	–
Black tea	Theaflavins	Cancers of GI tract, liver, and prostate	Suppresses inducible nitric oxide synthase by blocking nuclear translocation of transcription factor nfκb due to decrease iκb kinase activity	–
Capsicum	Capsiate	–	Inhibitor of angiogenesis; inhibits VEGF-induced proliferation, DNA synthesis, chemotactic motility, and capillary-like tube formation of primary cultured human endothelial cells	–
Cannabis sativa	Cannabinoids	–	Limits cell proliferation and induces tumor-selective cell death; induces palliative effects as it prevents nausea, vomiting, pain and improves appetite	–

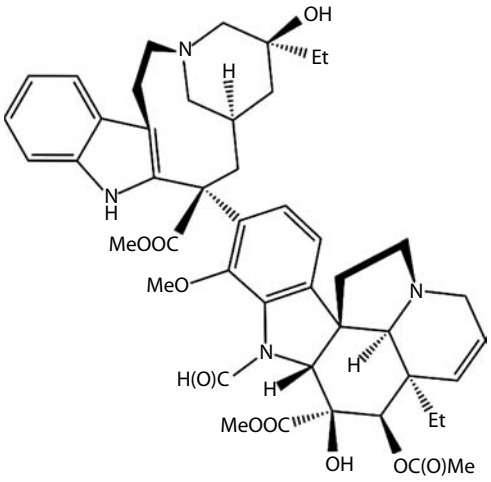

FIGURE 34.1 Chemical structure of vincristine.

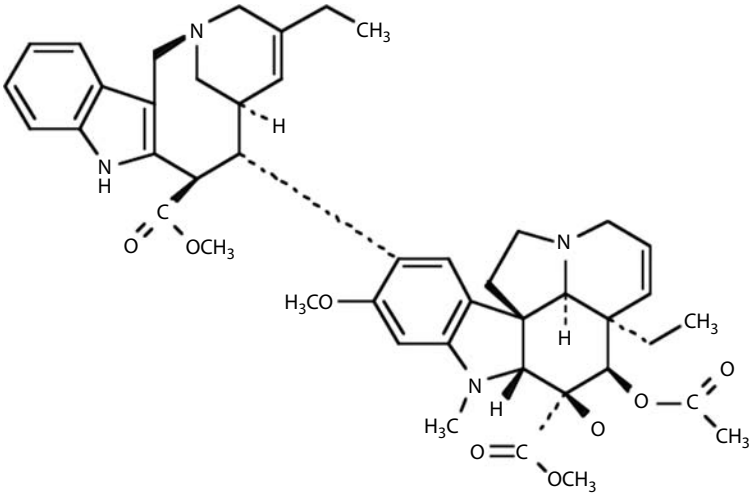

FIGURE 34.2 Chemical stucture of vinorelbine.

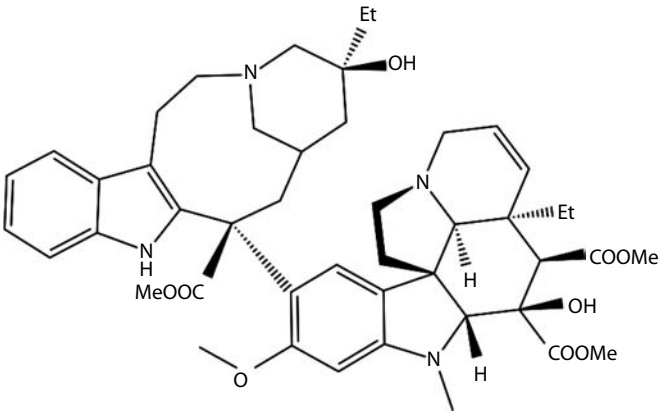

FIGURE 34.3 Chemical structure of vinblastine.

childhood leukaemia. These alkaloids arrest proliferation of cancer cells by binding to tubulin filaments in the mitotic spindle. They also have capability to induce apoptosis (programmed cell death) and hence inhibit spread of many types of cancers like breast, ovary, lung, colon, rectum, testis, neuroblastoma, Hodgkin's disease and leukaemia (Arora et al., 2010a,b,c).

Azadirachta indica

Neem (*Azadirachta indica*) belongs to the mahogany family Meliaceae (Figure 34.4). It is native to India, Myanmar, Bangladesh, Sri Lanka, and Pakistan and grows well in tropical and semi-tropical regions. Some other names of the plant are Nimba (Sanskrit and Marathi), Nimtree, "Divine Tree," "Heal All," "Nature's Drugstore," "Village Pharmacy," and Indian Lilac (English). It is a fast growing, evergreen tree and famous for its drought resistance. Formulations prepared from neem have reported medicinal properties and antihelmintic, antifungal, antidiabetic, antibacterial, antiviral, antifertility, and sedative effects. It is regarded as a major component in ayurvedic medicine and is prescribed mainly for skin diseases. Various extracts of this plant have been used against many insects, mainly Lepidoptera and more commonly against mosquitoes, and so on. Neem oil, derived from the seeds of *Azadirachta indica*, is rich in saponins, tannins, flavonoids, polysaccharides, peptides, terpenoids, limonoids, and volatile sulphur modified compounds (Ricci et al., 2008). Neem oil contains various compounds like nimbin, nimbinin, and nimbidin. Azadirachtin is a major phytoconstituent and a secondary metabolite isolated from the seeds and intensively studied. Some active constituents are present in the *n*-hexane soluble fraction prepared from fresh flowers of the plant. This possesses larvicidal activity mainly against *Anopheles stephensi* Liston, a vector of malarial parasite (Siddiqui et al., 2009). The plant is known to exert gastroprotective and antiulcer effects (Maity et al., 2009). Nimbolide, a limonoid, is useful in chemoprevention as well as therapeutic purposes as it exerts antiproliferative and apoptosis inducing effects (Harish Kumar et al., 2009). The leaves of the plant possess anticancerous properties. It has been observed that the chemopreventive effect of neem in the case of oral squamous cell carcinoma is mediated by modulation of glutathione and its metabolizing enzymes. Low dosage of neem leaf has been observed to be more potent in inhibiting occurrence of cancer incidences as compared to higher doses (Arora et al., 2008). Gedunin, another constituent of the neem tree, a tetranortriterpenoid, has been shown to exert anticancer activity. The mechanism of action is by inhibition of the 90 kDa heat shock protein

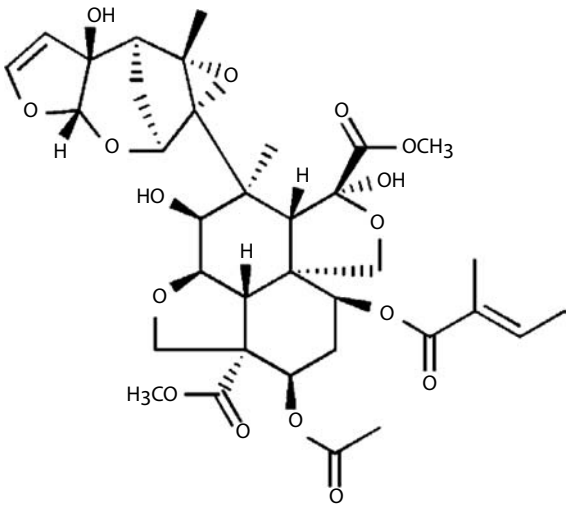

FIGURE 34.4 Chemical structure of azadirachtin.

(Hsp90) folding machinery, and as a result, it induces the degradation of Hsp90-dependent proteins (Brandt et al., 2008).

Bauhinia

Bauhinia belongs to the subfamily Caesalpinioideae of the flowering plant family Fabaceae. It has a pantropical distribution. The name of the genus was coined after the Bauhin brothers, Swiss-French botanists. Various species are planted in the tropics as orchid trees in the tropical regions like northern India. *Bauhinia variegata* is commonly known as Kachnar and has been used as a tonic to the liver in Ayurvedic medicine. The plant has been used extensively in folk medicine since a long time as it shows antidiabetic, anti-inflammatory, antimicrobial, analgesic, astringent, and diuretic effects (Shang et al., 2008). It has been reported that the major therapeutic properties exhibited by the plant are mainly due to the presence of steroids, terpenoids, and flavonoids (Filho, 2009). Extracts from the leaves, stems, pods, and roots of *Bauhinia purpurea* have been reported to yield four new dibenz[*b,f*]oxepins (2a, 3–5) coined as bauhiniastatins 1–4. Also, pacharin along with these bauhiniastatins is considered as cancer cell growth inhibitors. Various bauhiniastatins were observed to inhibit human cancer cell lines, and bauhiniastatin 1 (2a) was reported to inhibit P388 cancer cell line specifically (Pettit et al., 2006).

Podophyllum

Podophyllum hexandrum Royle belongs to the family Berberidaceae. Nowadays, it has been termed as a critically endangered medicinal plant. It is native to eastern Asia. All plant parts, except the fruit, are poisonous. It is even capable of causing unpleasant indigestion when ingested. Rhizomes of *P. hexandrum* provide several lignans which possess antitumor activity. Podphyllotoxin is an active cytotoxic natural product used as starting natural compound for the synthesis of anticancer drugs like etoposide and teniposide (Figure 34.5). The mechanism of action of podophyllotoxin is by inhibition of microtubule assembly. However, the anticancer action of etoposide (VP-16) and teniposide is due to their interaction with DNA and inhibition of DNA topoisomerase II. Some of the recent modifications of podophyllotoxins follow an unknown third mechanism of action (Damayanthi and Lown, 1998). These drugs which are derived from the plant constituents are used against lung cancer, testicular cancer, neuroblastoma, hepatoma and many others (Giri and Lakshmi,

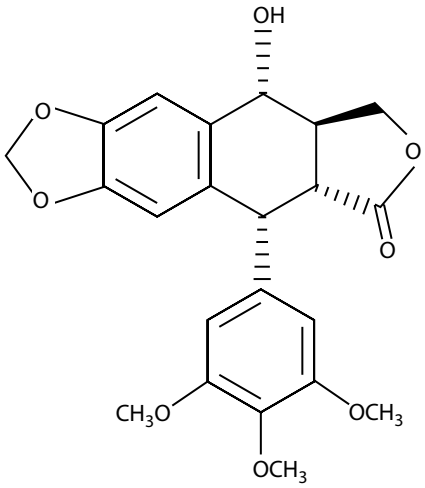

FIGURE 34.5 Chemical structure of podphyllotoxin.

2000). However, the major setback in using these for treatment is their cytotoxicity for normal cells and as a result lack of selectivity against cancer cells (Gordaliza et al., 2000).

Ocimum

Ocimum grows as a small herb throughout India and is commonly known as Tulsi in Hindi. Traditionally, different parts of the plant like leaves, stem, flower, root, seeds, and sometimes even whole plant have been used for the treatment of various ailments like bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite. Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), which is an active constituent present in it, has been observed to be capable of providing therapeutic properties to the plant (see Figure 34.6). It also possesses antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic, adaptogenic, and diaphoretic activities (Prakash and Gupta, 2005). Its antitumor activity against human nonsmall-cell lung carcinoma (NSCLC) A549 cells has not been investigated so far (Magesh et al., 2009). In case of MNNG-induced gastric carcinogenesis, the key proteins which are involved in development of the tumor by causing the proliferation, invasion, angiogenesis, and apoptosis of cells are molecular targets for chemoprevention using ethanolic leaf extract of the plant (Manikandan et al., 2007). Leaf extract prepared from *O. sanctum* has been reported to protect against chemical carcinogenesis using some of the mechanisms of action as antioxidant, modulating phase I and II enzymes, and antiproliferative activity (Rastogi et al., 2007).

Allium sativum

Allium sativum has been used for treating ailments as a home remedy since ancient times. It exerts many beneficial effects such as antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic and antitumor activity. The plant shows potential against cancer due to the presence of organosulfur compounds (Thomson et al., 2003). Alk(en)yl sulfide components provide the characteristic flavor present in garlic. Diallyl trisulfide is a major constituent of the garlic oil. It has been reported that the growth of human colon cancer cells HCT-15 and DLD-1 is significantly suppressed by diallyl trisulfide. The results showed that diallyl trisulfide shows anticancer effect for garlic eaters (Seki et al., 2008). There are multiple mechanisms present for organosulfur compounds (OSCs) to exert their anticancarcinogenic properties. Some of them include modulation of carcinogen metabolism, inhibition of DNA adduct formation, upregulation of antioxidant defences and DNA repair systems, and suppression of cell proliferation by blocking cell cycle

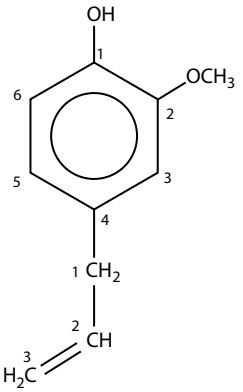

FIGURE 34.6 Chemical structure of eugenol.

progression and/or inducing apoptosis. As seen earlier, multiple signaling pathways are dysfunctional in cancer and new oncogenic mutations often accumulate with time; hence, dietary agents such as garlic with its rich array of bioactive OSCs offer promise as potential chemopreventive and chemotherapeutic agents (Nagini, 2008). *S*-allylcysteine, an organosulfur compound derived from garlic has been reported to retard the progress of both chemically induced as well as transplantable tumors in several animal models (Thomson et al., 2003).

Combretum

Combretastatin A4 (CA4), a novel vascular-disrupting agent (VDA), has emerged as a promising anticancer agent (Figure 34.7). It exerts its effect by inhibiting microtubule assembly resulting in disruption of tumor blood flow. These VDAs block blood flow to the tumor tissues but do not cause such harmful effects in normal ones (Tozer et al., 2008). It has also been proven that U0126, a compound that selectively inhibits mitogen-activated protein kinase (MEK), acts synergistically with CA4 in exerting cytotoxic effects in BEL-7402 cells, independent of MEK inhibition (Quan et al., 2009). 2,3-diaryl-5-hydroxycyclopent-2-en-1-one class contains CA-4 analogue 11 and 42 which have been evaluated for anticancer and antiangiogenic activity. Analogue 42 has reported cytoxic activity against a number of human cancer cell lines like PTC, MDA.MB.453, PA1, SKOV3, DU145 and Miapaca2. On the contrary, analogue 11 was effective only in case of Miapaca2. The mode of action in both the compounds mediates inhibition of growth factor-stimulated endothelial cell proliferation, migration, and capillary tube formation. Overall, in all respects, the analogue 42 was found to be superior to 11 (Sanna et al., 2010). It has been observed that when CA4P is administered in alternation with liposomal doxorubicin, an antineoplastic agent, it is more effective in inhibiting tumor growth. Hence use of CA4P is more efficient in combination therapies as compared to monotherapies (Mitrus et al., 2009).

Camptotheca

Nothapodytes foetida and *Camptotheca acuminata* secrete a number of significant anticancer alkaloids like camptothecin (Figure 34.8). To increase its yield commercially, an endophyte named ZP5SE, a *Neurospora crassa* strain, has been reported to be isolated from the seeds of *Nothapodytes foetida* and tested as a potential source of camptothecin (CPT). The fungus was grown in Sabouraud liquid culture media under shake flask conditions (Sirikantaramas et al., 2007; Rehman et al., 2008). Camptothecin is produced by various other parts of the plant also such as the hairy root cultures. The mode of action of CPT is centered on topoisomerase I (Top1). It interferes with enzyme activity promoting Top1-mediated DNA breaks and inhibition of DNA and RNA synthesis (Lotito et al., 2009). CPT and its derivatives are unique because they represent the only family of topoisomerase I inhibitors. Lack of cross-resistance has also been proved with most anticancer agents (Ardizzoni, 1995). Two semi-synthetic derivatives of CPT are topotecan and irinotecan (CPT-11), which are

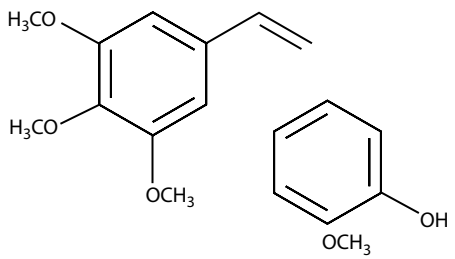

FIGURE 34.7 Chemical structure of combretastatin A4.

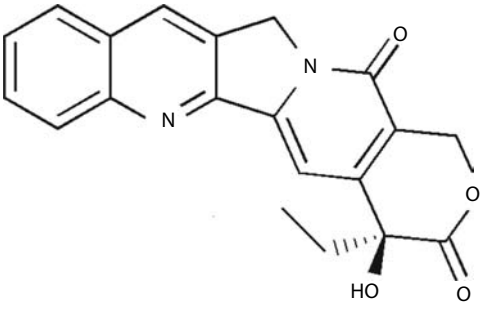

FIGURE 34.8 Chemical structure of camptothecin.

currently prescribed as anticancer drugs (Sirikantaramas et al., 2007). But phase I studies on these have indicated topotecan to cause neutropenia as a side effect while for CPT-11, either neutropenia, or diarrhoea was showing a dose-limiting toxicity (Ardizzoni, 1995). CKD-602, 7-[2-(N-isopropylamino)ethyl]-(20S)-camptothecin, belotecan), another synthetic water soluble camptothecin derivative and a topoisomerase inhibitor has expressed clinical anticancer potential against ovarian and lung cancer. CKD-602 has also shown significant anticancer effect on glioma cells *in vitro* and has proved to be promising for use against malignant gliomas (Kim et al., 2009). The mode of action of irinotecan requires hydrolysis by carboxyesterase enzyme to generate the active metabolite SN-38. But on further metabolizing, this becomes inactive SN-38 glucuronide (SN-38G) and so diminishes the levels of active SN-38. It has been experimentally observed that its anticancer activity can be enhanced by converting relatively high levels of endogenously generated SN-38G to SN-38 in tumors. Also it has been hypothesized that clinical response to CPT-11 can be improved by elevating beta-glucuronidase activity in tumors (Prijovich et al., 2009). It has been proved that human cells with decreased Top1 levels are significantly more resistant to killing by camptothecin than otherwise isogenic cells (Toyoda et al., 2009).

Withania somnifera L.

Ashwagandha is known as a wonder shrub of India and is widely used in Ayurvedic medicine and health tonics claiming a variety of health-promoting effects. *Withania somnifera* L. has been traditionally used as a sedative, to reduce stress, enhance health, and as a hypnotic (Oza et al., 2010; Xu et al., 2009). It enhances efficiency of radiation therapy while potentially reducing the undesirable side effects. It also reduced harmful effects of chemotherapeutic agents like cyclophosphamide and paclitaxel but did not alter the tumor-reducing action of the drug in any way. These effects have been proved *in vitro* on human cancer cell lines, and *in vivo* on animal subjects, but no human trials have been carried out till date. Hence, *W. somnifera* has a potential to act as a novel complementary therapy for integrative oncology care (Winters, 2006). It has been proved on molecular basis, that the leaf extract of ashwagandha selectively kills tumor cells; thus it qualifies as a natural source for safe anticancer medicine (Devi, 1996; Widodo et al., 2007). The plant produces a potent component, steroidal lactone withaferin A, which exerts significant antitumor and radiosensitizing effects (Devi, 1996; Xu et al., 2009). Also extracts of *W. somnifera* root, have shown a reproducible, dose-dependent inhibition of colony formation of CHO cells. The Chinese Hamster ovary (CHO) cell line has been widely used for measuring drug cytotoxicity and resistance (Sumantran et al., 2007). Many studies done on the plant have reported that it possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoietic, and rejuvenating properties. Also preliminary studies have indicated that a variety of constituents of the plant exhibit many therapeutic effects with little or no associated toxicity (Mishra et al., 2000).

Taxus baccata

Taxol (paclitaxel) and Taxotere (docetaxel) are included among the important anticancer drugs used in cancer chemotherapy. The anticancer activity of these is due to their ability to cause mitotic arrest in cancer cells, leading to apoptosis caused by inhibition of the depolymerization of microtubules. Although both drugs possess potent antitumor activity, treatment with these often results in few undesirable side effects, as well as multidrug resistance (MDR) (Miller and Ojima, 2001). Paclitaxel (Taxol) is a widely used anticancer isoprenoid synthesized by the secondary metabolism of yew (Taxus sp.) trees (Besumbes et al., 2004; Tabata, 2004). Docetaxel, is a new semi-synthetic anticancer agent derived from bacatin III of the needles of *Taxus baccata*. Its novel mechanism of action has been investigated and described; it binds to tubulin and hence induces its polymerization and promotes stable microtubule formation. Preclinical and phase II studies have reported docetaxel to be active against NSCLC (nonsmall-cell lung cancer) (Georgoulias, 2002). A taxane, diterpneoid 2-deacetoxytaxinine J (2-DAT-J) 1 has been derived from the bark of Himalayan yew or *Taxus baccata* L. spp. wallichiana and its anticancer potential has been investigated against breast cancer cell lines (MCF-7 and MDA-MB-231). It exhibited significant *in vitro* activity against breast cancer cell line. Few novel taxoids have also been derived from the naturally occurring 2-DAT-J (1) and these also have been investigated for their anticancer effects (Reddy et al., 2009).

Hippophae rhamnoides

Natural fruits or berries of *Hippophae rhamnoides* (seabuckthorn) are a rich source of vitamins A, C, and E, carotenes, flavonoids, and microelements like sulfur, selenium, zinc, and copper. These also have exhibited protection from many ailments like atopic dermatitis, hepatic injury, cardiac disease, ulcer, and atherosclerosis. Studies have indicated that Hippophae fruit is capable of decreasing carcinogen-induced forestomach and skin tumorigenesis, which might involve up-regulation of phase II and antioxidant enzymes as well as DNA-binding activity of IRF-1 (interferon regulatory factor-1), an antioncogenic transcription factor resulting in growth suppression and inducing apoptosis for exerting its anticancer effect (Padmavathi et al., 2005). Five types of flavonols were isolated from seabuckthorn and the proliferations of human promyelotic leukemia HL-60 cells were found to be inhibited along with increasing concentrations of these flavonols. The order of the extent of growth inhibition was observed to be: pentamethylquercetin > syringetin > isorhamnetin > quercetin > kaempferol > myricetin. Various other experiments carried out on HL-60 cells indicate that mechanisms of growth inhibition caused by pentamethylquercetin, syringetin and isorhamnetin are different from those of quercetin, kaempherol, and myricetin (Hibasami et al., 2005).

Curcuma longa

Curcumin (diferuloylmethane), a polyphenol natural product obtained from the rhizome of *Curcuma longa*, and the major active constituent of the dietary spice turmeric (López-Lázaro, 2008), is currently being checked for its potential as a novel anticancer agent (Kim et al., 2008; Villegas, 2008; Beevers et al., 2009; Iqbal et al., 2009) (Figure 34.9). However, the anticancer mechanism of curcumin remains unclear. Many researchers have reported its pharmacological properties like chemosensitizing, radiosensitizing, wound healing, antimicrobial, antiviral, antifungical, immunomodulatory, antioxidant and anti-inflammatory (Sun et al., 2008; Villegas, 2008). Another active component derived from *Curcuma longa* is tetrahydrocurcumin (THC), a major colourless metabolite of curcumin. It also possesses antidiabetic, anti-inflammatory, and antioxidant activity (Murugan and Pari, 2007). Curcumin exerts its anticancer properties using various mechanisms like arresting cell cycle, inhibiting inflammatory response and the oxidative stress, and by inducing apoptosis in cancer cells, sometimes through p21-mediated cell cycle arrest (López-Lázaro, 2008; Villegas, 2008; Watson et al., 2008). Also, it has been proved that it possesses marked antiangiogenic properties. It is also capable of potentiating the growth inhibitory effect of

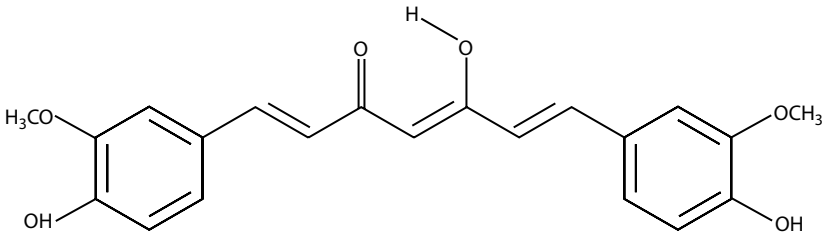

FIGURE 34.9 Chemical structure of curcumin.

cyclo-oxygenase (COX)-2 inhibitors and some other traditional chemotherapy agents (Villegas, 2008). Recently, it has been shown that curcumin inhibits phosphorylation of p70 S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1), downstream effector molecules of the mammalian target of rapamycin complex 1 (mTORC1) in numerous cancer cell lines. It has also been observed that curcumin is capable of dissociating raptor from mTOR, which eventually results in inhibition of mTORC1 activity. Therefore, it has been hypothesized that curcumin can be a representative of a new class of mTOR inhibitors (Beevers et al., 2009). Curcumin is also reported to inhibit breast cancer cell motility and invasion by directly interfering with the function of alpha(6)beta(4) integrin, and it has been suggested that it has potential to serve as an effective therapeutic agent in tumors that overexpress alpha(6)beta(4) (Kim et al., 2008). Curcumin has shown activities in accordance to some of the recently discovered tumor necrosis factor blockers like HUMIRA, vascular endothelial cell growth factor blocker (AVASTIN), human epidermal growth factor receptor blockers (GEFTINIB), and a HER2 blocker (HERCEPTIN). So taking into consideration the recent scientific ideology that multitargeted therapy is more beneficial as compared to monotargeted therapy for diseases, curcumin can be easily be regarded as an ideal "Spice for Life" (Aggarwal et al., 2007).

Zingiber officinalis

Ginger, the rhizome of *Zingiber officinalis*, is a widely used species of the ginger family. It is a common condiment used in India in various foods and beverages. It has been used medicinally since 2500 years. It has been traditionally used for many different human ailments like digestion, diarrhea, nausea, common colds, fever, rheumatic disorders, gastrointestinal complications, motion sickness, diabetes, and cancer (Shukla and Singh, 2007; Kundu et al., 2009). Some pungent constituents present in it have potent antioxidant and anti-inflammatory activities, and some also exhibit cancer preventive activity (Figures 34.10 and 34.11). The anticancer properties of ginger are explained by the presence of certain nonvolatile pungent vallinoids, [6]-gingerol and [6]-paradol, as well as some constituents like shogaols, zingerone, paradols, and gingerols, and so on (Lee and Surh,

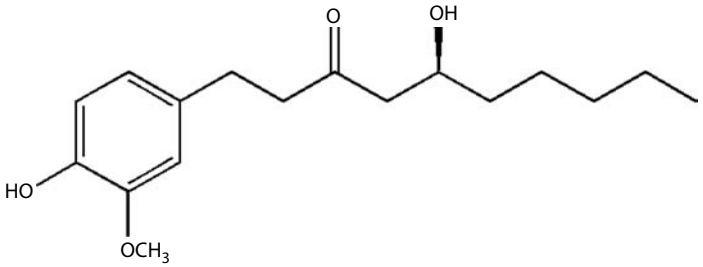

FIGURE 34.10 Chemical structure of gingerol.

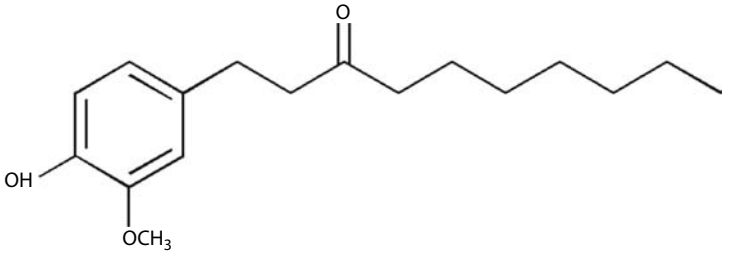

FIGURE 34.11 Chemical structure of paradol.

1998; Surh, 2002; Shukla and Singh, 2007; Kundu et al., 2009). The chemopreventive effects exerted by these are often linked to their antioxidative and anti-inflammatory activities. Many anti-inflammatory and chemopreventive chemicals like these, act against cyclo-oxygenase-2 (COX-2) (Surh, 2002). It has been reported that these substances also inhibit tumor-promoter-stimulated inflammation, TNF-alpha production, and activation of epidermal ornithine decarboxylase in mice. In another research, [6]-gingerol and [6]-paradol suppressed superoxide production stimulated by TPA (12-*O*-tetradecanoyl-phorbol-13-acetate) in differentiated HL-60 cells (Surh et al., 1999). [6]-Gingerol and [6]-paradol have been reported to exert inhibitory effects on the viability and DNA synthesis of human promyelocytic leukemia (HL-60) cells. The cytotoxic and antiproliferative effects of both were linked to apoptotic cell death (Lee and Surh, 1998; Wei et al., 2005).

Piper Species

Black pepper (*Piper nigrum*) is widely used as a spice throughout India. It has a characteristic biting quality due to the presence of a pure and pungent alkaloid, piperine (Figure 34.12). It acts as an antioxidant, antimutagenic and anticancer agent (Selvendiran et al., 2005, 2006; Srinivasan, 2007). Apart from being used as dietary agent, black pepper is used for a variety of purposes such as medicinal, preservative, and in perfumery. It has been observed that piperine is capable of suppressing benzo[*a*]pyrene (B[*a*]p)-induced lung cancer in Swiss albino mice. It has been reported that administering piperine to tumor-induced mice significantly lowers the phase I enzymes (NADPH-C reductase, cyt-p450 and cyt-b5) and rise in glutathione-metabolizing enzymes (GPx, GR and G6PDH). This indicated antitumor and anticancer effect of piperine (Selvendiran et al., 2005). It has been suggested that piperine can extend its chemopreventive effect by controlling and altering the protein bound carbohydrate levels, as these are indicators of tumorigenesis (Selvendiran et al., 2006). The most important phenomenon of piperine has been exerting its inhibitory effect on enzymatic drug biotransforming reactions in liver. It inhibits hepatic

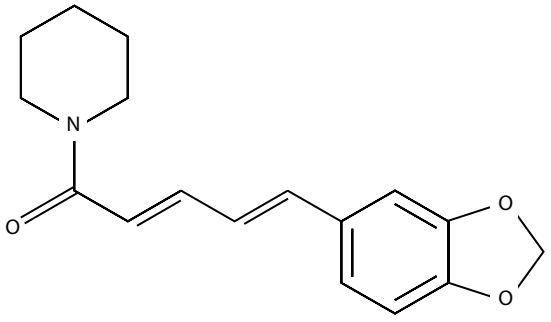

FIGURE 34.12 Chemical structure of piperine.

and intestinal aryl hydrocarbon hydroxylase and UDP-glucuronyl transferase. It has been observed to enhance the bioavailability of a number of therapeutic drugs by this property (Srinivasan, 2007).

Black Tea

Tea is a ancient beverage widely consumed throughout the world, and black tea exerts many biological effects on the organisms. It is a potent antioxidant due to its free radical-scavenging and metal-chelating properties (Sharma and Rao, 2009). There are a number of polyphenols present in black tea which include theaflavin (TF), theaflavin-3-gallate (TF-2a), theaflavin-3′-gallate (TF-2b), theaflavin-3,3′-digallate (TF-3), theaflavin gallate (TFG), and theaflavin digallate (TFdiG). It has also been suggested that the gallate structure of theaflavins is important for growth inhibition in tumor cells (Liang et al., 1999; Yang et al., 2000) (Figure 34.12). So by virtue of all these, it can prevent inflammation, clastogenesis, and several types of cancer. It reduces DNA damage and mutagenesis caused as a result of oxidative stress or due to the presence of pro-mutagens, through various mechanisms like antioxidation, blocking activation pathways of mutagens, suppression of transcription of enzymes involved, and so on. Although its role in cancers of GI tract, liver, and prostate is known and studied but its effect against urinary tract cancer is still uncertain (Sharma and Rao, 2009). Nitric oxide production was reduced by theaflavins present in black tea. It occurs mainly by suppressing inducible nitric oxide synthase by blocking nuclear translocation of the transcription factor NFκB as a result of decreased IκB kinase activity (Beltz et al., 2006).

Green Tea

Green tea has been found to be rich in polyphenolic compounds with catechins being the major constituent. The protective and preventive actions of green tea are mainly due to the presence of polyphenols like epigallocatechin-3-gallate (EGCG), epicatechin, epicatechin-3-gallate, epigallocatechin (EGC). These polyphenols comprise about one-third of the weight of the dried leaf of the plant. These catechins have been reported to possess diverse pharmacological properties including antioxidative, anti-inflammatory, anticarcinogenic, antimutagenic, antiarteriosclerotic and antibacterial effects (Lin et al., 1999; Koo and Cho, 2004; Shankar et al., 2007; Butt and Sultan, 2009). In the GI tract, green tea was observed to activate intracellular antioxidants, inhibit procarcinogen formation, and suppress angiogenesis, metastasis, and cancer cell proliferation. Tea consumption has been reported to be inversely proportional to the occurrence of various cancers like that of the stomach, oral, and colon (Lin et al., 1999; Hsu et al., 2002; Kazi et al., 2002; Koo and Cho, 2004; Beltz et al., 2006; Shankar et al., 2007). Green tea has shown the ability to reduce cellular damage arising due to oxidative stress. It is thought that it enhances humoral and cell-mediated immunity, and hence decreasing the risk of certain cancers. The major contributor in providing chemopreventive abilities to green tea is EGCG. Its mechanism of action includes inducing apoptosis and enhancing cell growth arrest by altering the expression of cell cycle regulatory proteins, activating killer caspases, altering Bcl-2 family member expression and inhibiting nuclear factor kappa-B (NF-κB) activation, PI3-K/Akt, Ras/Raf/MAPK, and AP-1 signaling pathways, as well as regulating expressions of VEGF, matrix metalloproteinases, urokinase-type plasminogen activator (uPA), insulin-like growth factor-I (IGF-I), epidermal growth factor receptor (EGFR), and cell cycle regulatory proteins. Metastasis in tumor cells was inhibited by exerting effects on urokinase and matrix metalloproteinases (Kazi et al., 2002; Lin, 2002; Beltz et al., 2006; Shankar et al., 2007). Apart from all this, it has also shown to take part in regulating and promoting IL-23-dependent DNA repair and stimulating cytotoxic T cells activities in a tumor microenvironment. It is also suggested that green tea polyphenols may involve a p57-mediated survival pathway in normal epithelial cells as a way of exerting chemopreventive effects, while carcinoma cells undergo an apoptotic pathway (Hsu et al., 2002). It is also known to block carcinogenesis by modulating the signal transduction pathways

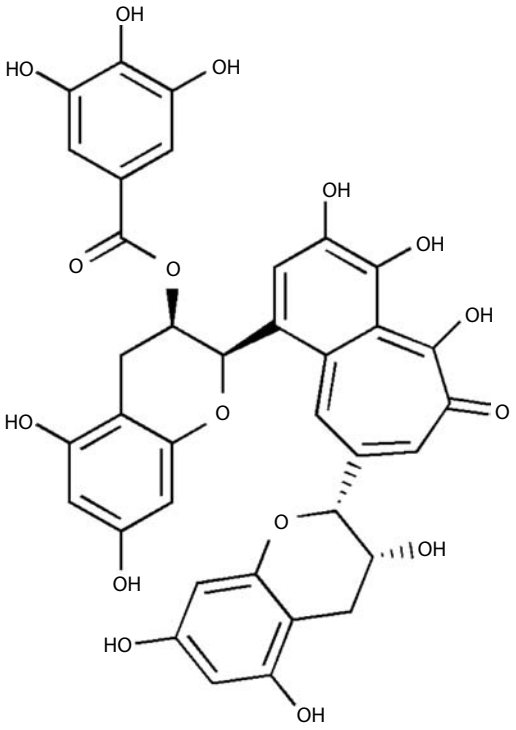

FIGURE 34.13 Chemical structure of theaflavin-3-gallate.

involved in cell proliferation, transformation, inflammation, and metastasis. Hence, it causes strong chemopreventive effects (Lin et al., 1999; Lin, 2002; Shankar et al., 2007; Butt and Sultan, 2009).

Capsicum

Capsaicin (trans-8-methyl-*N*-vanillyl-6-nonenamide), is an alkaloid produced mainly from the genus Capsicum (Figure 34.13). It causes the pungency factor and is a bioactive molecule of food and also of medicinal importance. It induces excitation of nociceptive terminals which are mainly involved in pain perception (Min et al., 2004). It is also useful as a counterirritant, antiarthritic, analgesic, and antioxidant. It has also been proven that capsaicin has chemopreventive as well as anticancer properties. Capsaicin is synthesized by the plant using capsaicin synthase (CS) enzyme and the process involves condensation of vanillylamine and 8-methyl nonenoic acid (Prasad et al., 2006). Another component of capsicum is capsiate, a nonpungent capsaicin analogue, and its dihydroderivative dihydrocapsiate (Pyun et al., 2008). A cancer-specific cell surface protein, tNOX, has been reported as a suitable target for low-dose cell killing using capsicum vanilloid. This protein is known to be associated specifically with cancer cells and is absent from normal cells. Its activity is linked to cancer growth. When the protein is blocked, cancer cells die as a result. Vanilloid capsaicin is a potent inhibitor of this protein. The effectiveness of inhibiting the protein increases 10-fold to 100-fold when catechin-vanilloid combinations are used as compared with using either of them alone (Morré and Morré, 2006). It has been reported that capsaicin possesses ability to act as a novel inhibitor of angiogenesis. It has been shown that *in vitro*, capsaicin, capsiate, and dihydrocapsiate inhibit vascular endothelial growth factor (VEGF)-induced proliferation, DNA synthesis, chemotactic motility, and capillary-like tube formation of primary cultured human endothelial cells (Min et al., 2004; Pyun et al., 2008). Signaling experiments have shown inhibition of VEGF-induced p38

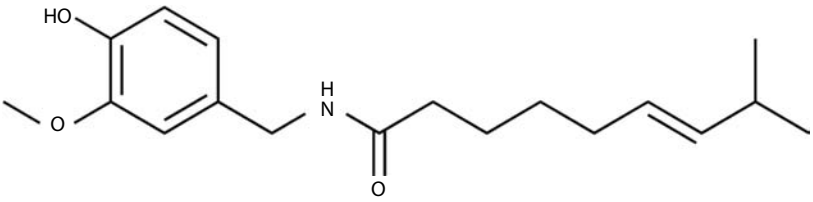

FIGURE 34.14 Chemical structure of capsaicin.

mitogen-activated protein kinase, p125(FAK), and AKT activation due to capsaicin and capsiate action (Min et al., 2004; Pyun et al., 2008).

Cannabis sativa

Cannabinoids, synthesized from *Cannabis sativa*, exert an effect on human body by mimicking endogenous substances—the endocannabinoids which activate specific cell surface receptors (Bifulco et al., 2006; Velasco et al., 2007). The cannabinoids prove their potential as antitumor drugs mainly due to their mechanism which involves limiting cell proliferation and inducing tumor-selective cell death. Synthetic cannabinoids, like nabilone, dronabinol, and HU211 (Williamson and Evans, 2000; Walsh et al., 2003), are thought to have pro-tumor effects *in vivo* because of their immunosuppressive properties, which include inhibiting tumor growth and migration, angiogenesis, metastasis, and inflammation. Till date, two types of cannabinoid receptors, CB1 and CB2, are reported. While CB1 receptors are known to be expressed mainly in the central and peripheral nervous system whereas CB2 receptors have been reported in certain nonneuronal tissues, mainly in the immune cells (Walsh et al., 2003). Today it is being suggested that agonists of cannabinoid receptors expressed by tumor cells may prove to be a novel strategy to follow in order to treat cancer (Pisanti et al., 2009). Delta(9)-tetrahydrocannabinol (THC) has potential to treat the symptoms and side effects of cancer. (Figures 34.15 through 34.17) Some others which are known

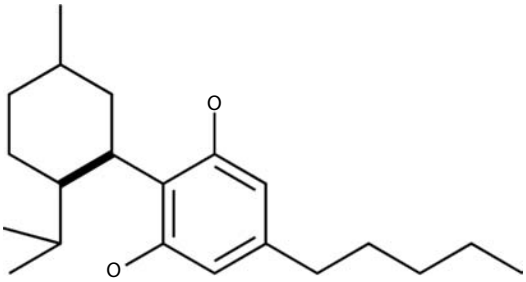

FIGURE 34.15 Chemical structure of CBD.

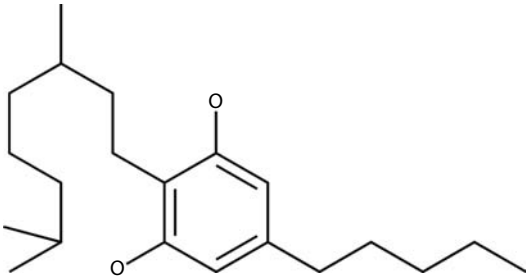

FIGURE 34.16 Chemical structure of CBG.

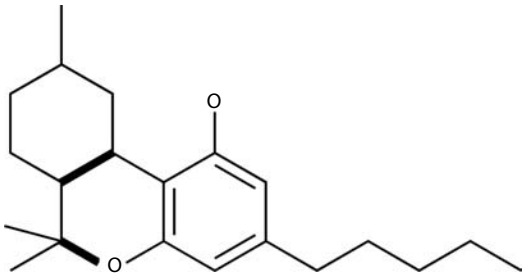

FIGURE 34.17 Chemical structure of THC.

include cannabidiol (CBD) and cannabigerol (CBG). Cannabinoids are known to induce palliative effects mainly by preventing nausea, vomiting, and pain and improving appetite (Dow and Meyers, 1981; Guzmán, 2003; Walsh et al., 2003; Hall, 2005; Patsos et al., 2005). It has been said that smoking of cannabis preparations may result in cancers of the aerodigestive and respiratory system, but it has not been proven yet. The major concern for using these cannabinoids for medical purposes is the development of safe and effective methods of use which will lead to therapeutic effects but avoid adverse psychoactive effects (Hall, 2005). Cannabinoids inhibit tumor cells growth in *in vitro* and *in vivo* experimental models mainly by modulating key cell-signalling pathways. Cannabinoids generally do not produce toxic side effects of conventional chemotherapies (Guzmán, 2003; Patsos et al., 2005; Bifulco et al., 2006; Velasco et al., 2007).

FROM BENCH TO BEDSIDE

A number of modern day anticancer drugs, for example, vincristine, vinblastine, combretastatin, taxol, etoposide, teniposide, camptothecin, and so on have been derived from Indian herbal medicine and find routine use in the clinic, either alone or as a part of the combination regimen (Figure 34.18). Cisplatin, etoposide, cytarabine, and paclitaxel can be used in combined chemotherapy regimens. This is due to the fact that they are able to penetrate the intact blood-brain barrier and also exhibit a unique mechanism of action and lack cross-resistance ability (Kollmannsberger et al., 1999). In a separate study, docetaxel was combined successfully with cisplatin (ORR 33–46%), carboplatin (ORR 30–48%), vinorelbine (ORR 20–51%), gemcitabine (ORR 37–47%), with a median survival ranging from 5–14 months. Docetaxel also demonstrated radiosensitizing properties, and promising results were observed when it was used along with irradiation. Randomized trials showed that second-line docetaxel confered a survival benefit over either BSC or ifosfamide/vinorelbine in pretreated patients with NSCLC (Georgoulias, 2002). Chemotherapy regimens using vinblastine derived from *C. roseus*, are being used for treatment of various cancers include ABV (doxorubicin, bleomycin, VLB) for treating Kaposi's sarcoma, ABVD (doxorubicin, bleomycin, VLB and/or dacarbazine), STANFORD V (mechlorethamine, doxorubicin, VLB, VCR, bleomycin, ETOPOSIDE, prednisone) and BCVPP (carmustine, cyclophosphamide, VLB, procarbazine, prednisone) for Hodgkin's lymphoma. CMV (cisplatin, methotrexate, VLB) is used for bladder cancer while CVD (cisplatin, VLB, dacarbazine) is used in case of malignant melanoma. Vincristine is also used in various combination therapies for cancer treatment. Some of them are CHOP (cyclophosphamide, doxorubicin, VCR, prednisone) for non-Hodgkin's lymphoma, MOPP (mechlorethamine, VCR, procarbazine, prednisone) for Hodgkin's lymphoma, BOMP (bleomycin, vincristine, mitomycin and cisplatin) for treating cervical cancer and many other combination therapies also make use of it. Vinorelbine also contributes in the combinatorial therapies. Some of them are NP (vinorelbine, cisplatin), VC (vinorelbine, cisplatin), and cisplatin, gemcitabine, vinorelbine regimens are used for the treatment of nonsmall-cell lung cancer (Comella et al., 2000; Arora et al., 2010a,b,c). For the

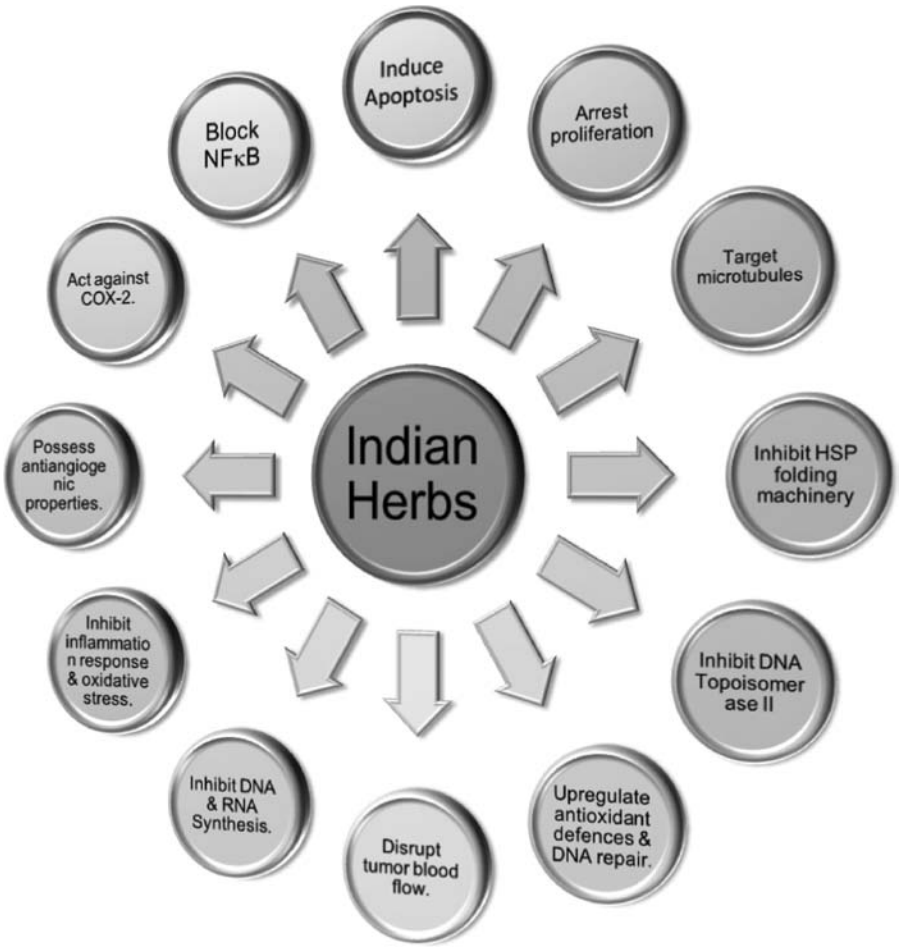

FIGURE 34.18 How Indian medicinal herbs render cancer chemopreventive and therapeutic effects.

treatment of small cell lung cancer either of the two combination regimens can be utilized: (CEC) cytoxan, etoposide and cisplatin or (CEA) cytoxan, etoposide and adriamycin (Kwiatkowski et al., 1987). cisplatin and etoposide can be used synergistically for the treatment of adrenal cancer and if used in association with ifosfamide (EI), it can provide protection against soft tissue sarcoma (Johnson and Greco, 1986; Miser et al., 1987). Cytoxan, adriamycin, fluorouracil, vincristine, and prednisone (CAFVP), vincristine, prednisone, cyclophosphamide, methotrexate, and fluorouracil (VPCMF) are used in combination for the treatment of breast cancer patients (Muss et al., 1978; Cooper et al., 1979). Docetaxel and cisplatin are used in combination for nonsmall-cell lung cancer therapy (Fossella et al., 2003).

INDIAN HERBAL MEDICINE: WEIGHING THE PROS AND CONS FOR THE JOURNEY AHEAD

An important fact is sidelined at times that in herbal extracts several active components contribute to the pharmacological action. It has been reported that extracts of medicinal herbs, once isolated in their pure state, can exert pharmacological effects significantly different from those of the whole herb (Chang, 2000). It is also known that the use of nonsteroidal anti-inflammatory

drugs (NSAID) is related to lowering of the risk of occurrence of several cancers. Therefore, it is a feasible option to look for natural NSAID that can be used as cancer preventive agents (Wargovich et al., 2001). The uncertain composition of several traditional medicines, including Chinese and Indian herbal preparations, available commercially over-the-counter is the main reason why sometimes doubt is raised about their safe usage. The evidence that certain herbs may have risky interactions when coadministered with modern prescription drugs also complicates the issue. Therefore, adequate studies are required to ensure efficacy and safety and to develop new, effective and safe world-class drugs (Goldman, 2001). More evidence-based studies would lead not only to establishment of safety of Indian herbal medicine, but also the discovery of several new chemopreventive and anticancer drugs. Indian herbal medicine is virtually a goldmine of new cancer preventive and therapeutic molecules and beckons researchers to investigate them in great detail.

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35 Dietary Intake and the Development of Lung Cancer

Lauri Wright, Catherine Christie, and Julia A. Watkins

CONTENTS

INTRODUCTION

Worldwide, lung cancer is the most common cancer and is the leading cause of cancer deaths. Prevention, early detection, and treatment are key approaches for reducing cancer-related morbidity and mortality. Early detection of lung cancer has not been successful and the survival rate remains low. Therefore, prevention is becoming increasingly important. Strategies for reducing lung cancer risk include avoiding primary and secondary tobacco smoke, as well as avoiding environmental agents such as asbestos and air pollution. Increasingly, diet is receiving attention as a preventative modality in lung cancer. This chapter reviews current literature on fruits and vegetables, antioxidants, folic acid, phytoestrogens, alcohol, fat, and β-carotene supplements in the prevention of lung cancer.

EPIDEMIOLOGY

Lung cancer is the most common cancer worldwide accounting for 1.2 million new cases annually, as well as the leading cause of cancer deaths with 17.8% of all cancer deaths (WHO, 2009). In the United States, lung cancer is the second most commonly diagnosed malignancy and the leading cause of cancer-related death for both men and women (American Cancer Society, 2008). In 2005, the most recent year for which statistics are currently available, lung cancer accounted for more deaths than breast cancer, prostate cancer, and colon cancer combined in the United States (CDC, 2009). In that year, 107,416 men and 89,271 women were diagnosed with lung cancer while 90,139 men and 69,078 women died from lung cancer (CDC, 2009). Among men in the United States, lung cancer is the second most common cancer among white, black, Asian/Pacific Islander, American Indian/Alaska Native, and Hispanic men. Among women in the United States, lung cancer is the second most common cancer among white, black, and American Indian/Alaska Native women and the third most common cancer among Asian/Pacific Islander and Hispanic women.

RISK FACTORS

Cigarette smoking is the predominant cause of lung cancer, accounting for approximately 90% of all cases (Alberg and Samet, 2003). Despite the fact that 10% of lung cancer patients in the United States have never smoked, relatively few studies have examined the risk factors for lung cancer in nonsmokers (Subramanian and Govindan, 2007). Studies among nonsmokers point to an increased risk associated with existing lung diseases such as tuberculosis and childhood pneumonia (Wu et al., 1995). Environmental tobacco smoke (ETS) exposure has been estimated to confer an increased risk of 20–30% in pooled analyses (Hackshaw et al., 1997). There are also indications that family history of lung cancer or of any cancer confers an increased risk, particularly of adenocarcinoma of the lung (Wu et al., 1996; Hackshaw et al., 1997). Studies in China and Taiwan have identified other forms of indoor air pollution such as burning coal and cooking fumes as possible risk factors among nonsmoking women (Gao et al., 1987).

TYPES OF LUNG CANCER

Lung cancers can arise in any part of the lung, but 90–95% of cancers of the lung are thought to arise from the epithelial or lining cells of the larger and smaller airways (bronchi and bronchioles). For this reason, lung cancers are sometimes called bronchogenic carcinomas or bronchogenic cancers (Stoppler, 2009). Cancers can also arise from the pleura (the thin layer of tissue that surrounds the lungs), called mesotheliomas, or rarely from supporting tissues within the lungs, such as blood vessels.

Lung cancer is broadly classified into two types: small cell lung cancers (SCLC) and non-small-cell lung cancers (NSCLC). This classification is based upon the microscopic appearance of the tumor cells themselves. These two types of cancers grow and spread in different ways and may have different treatment options so a distinction between these two types is important.

SCLC comprise about 20% of lung cancers and are the most aggressive and rapidly growing of all lung cancers (Stoppler, 2009). SCLC are strongly related to cigarette smoking with only 1% of these tumors occurring in nonsmokers (Stoppler, 2009). SCLC metastasize rapidly to many sites within the body and are most often discovered after they have spread extensively. Referring to a specific cell appearance often seen when examining samples of SCLC under the microscope, these cancers are sometimes called oat cell carcinomas.

NSCLC are the most common lung cancers, accounting for about 80% of all lung cancers. NSCLC can be divided into three main types that are named based upon the type of cells found in the tumor: adenocarcinomas, squamous cell carcinomas, and large cell carcinomas. Adenocarcinomas

are the most commonly seen type of NSCLC in the United States and comprise up to 50% of NSCLC (Stoppler, 2009). While adenocarcinomas are associated with smoking like other lung cancers, this type is observed as well in nonsmokers who develop lung cancer. Most adenocarcinomas arise in the outer, or peripheral, areas of the lungs. Squamous cell carcinomas account for about 30% of NSCLC (Stoppler, 2009). Also known as epidermoid carcinomas, squamous cell cancers arise most frequently in the central chest area in the bronchi. Large cell carcinomas, sometimes referred to as undifferentiated carcinomas, are the least common type of NSCLC. Mixtures of different types of NSCLC are also seen.

PREVENTION RATIONALE

Prevention, early detection, and treatment are three approaches for reducing cancer-related morbidity and mortality. Early detection of lung cancer has not been successful since symptoms often do not appear until the disease is advanced. In fact, only 15% of lung cancer are discovered while the disease is still localized (Ziegler et al., 1995). Treatment also is not as effective as for other cancers; the five-year relative survival rate for lung cancer is only 16% (American Cancer Society, 2008). These statistics emphasize that the most viable strategy for reducing lung cancer mortality is prevention.

The National Cancer Institute's strategies for preventing lung cancer are to avoid primary and secondary tobacco smoke, avoid environmental agents such as asbestos and air pollution, consume a diet rich in fruits and vegetables, and increase physical activity (National Cancer Institute, 2009). Although cessation is the most effective preventive strategy among smokers, it is exceedingly difficulty to quit. Even if successful, former smokers continue to have increased risk of lung cancer compared to nonsmokers throughout their lifetime (Alberg and Samet, 2003). Diet, therefore, emerges as an important additional method to prevent lung cancer.

OVERALL DIET AND LUNG CANCER

Bjelke (1975) was the first to raise the possibility that diet might have an effect on lung cancer risk after making the observation that, allowing for smoking, a low dietary intake of vitamin A was associated with an increased risk of lung cancer. Since then, numerous observational studies have found that lung cancer patients generally report a lower intake of fruits, vegetables, and related nutrients such as carotenoids than controls (WCRF/AICR, 1997; COMA, 1998). Observational studies have demonstrated an inverse relationship with vitamin C, folate, vitamin E, and carotenoids, as well as a positive association with fat and alcohol (Bandera et al., 1997). The following sections will review the relationship between dietary components and lung cancer.

Fruits and Vegetables in Prevention

Mechanism of Action

A myriad of substances in fruits and vegetables have been studied or postulated to have anticarcinogenic properties. These substances include carotenoids, selenium, vitamin C, vitamin E, folic acid, allium compounds, and flavonoids. Some of the biological mechanisms through which these substances may help prevent cancer include as antioxidants, changes in cell differentiation, DNA methylation and maintenance of DNA repair, decreased cell proliferation, and blocked formation of nitrosamines (Steinmetz and Potter, 1996). Because fruits and vegetables contain multiple substances, their anticarcinogenic effects are enhanced. For example, citrus fruit is known for its high content of vitamin C and coumarins. Vitamin C is an antioxidant which may protect cell membranes and DNA from oxidative damage. Vitamin C may further help prevent cancer by its ability to scavenge and reduce nitrites. This reduces substrates for the formation of nitrosamines which are known carcinogens. In addition, coumarins, which are also in citrus fruits, have been

shown to increase the activity of glutathione transferase, a detoxification enzyme (Steinmetz and Potter, 1996).

Review of the Evidence

A prospective cohort study is in many ways the ideal method for studying dietary risk factors for cancer. In this study design, a large group of healthy individuals provide information regarding a risk factor of interest and then researchers followed up with the individuals for several years to see which individuals develop the disease of interest. Early cohort studies have shown an inverse association for vegetable and/or fruit consumption and lung cancer in populations of Norwegian men (Kvale et al., 1983), postmenopausal women in Iowa (Steinmetz et al., 1994), and Seventh-Day Adventists in California (Fraser et al., 1991). More recent cohort studies have also looked at the relationship between fruit and vegetable consumption and lung cancer. Wright et al. (2008) examined the association between lung cancer risk and intake of fruit and vegetables in 472,081 participants in the National Institutes of Health—Association for Advancement of Retired Persons (AARP) Diet and Health Study. Diet was assessed at baseline with a 124-item dietary questionnaire. Higher intake of fruit and vegetables, overall and when analyzed separately, was strongly inversely associated with lung cancer risk. The European Prospective Investigation into Cancer and Nutrition (EPIC) study also evaluated the association of fruit and vegetable consumption and lung cancer incidence (Linseisen et al., 2007). Participants were 478,590 adults living in Europe. Lifestyle questionnaires and 24-hour diet recalls were conducted on participants who were followed for an average of 6.4 years. In the whole study population, fruit consumption was significantly inversely associated with lung cancer risk while no association was found for vegetable consumption. In addition, lung cancer risk significantly decreased with higher fruit and vegetable consumption in the smoker population. Feskanich et al. (2000) examined the association between lung cancer risk and fruit and vegetable consumption in 77,283 women in the Nurses' Health Study and 47,778 men in the Health Professionals' Follow-up Study. The participants' diet was assessed using a food-frequency questionnaire. Five hundred-nineteen women and 274 men developed lung cancer in the study period. Overall, total fruit and vegetable consumption was associated with a modestly lower risk of lung cancer in women not men. Among the nonsmoker subgroup, however, fruit and vegetable consumption was associated with a lower risk of cancer in both women and men. Cohort studies on the relationship between fruit and vegetable intake and lung cancer risk have generally demonstrated an inverse relationship.

In case-control studies, individuals who develop cancer are asked about their past diet and other potential risk factors; control subjects are similarly asked about their diet. The diets of the case and control subjects are then compared. Early case-control studies have consistently found an association between fruit and/or vegetables consumption and lung cancer risk in populations of women in China (MacLennan et al., 1977), men in New Jersey (Ziegler et al., 1986), and smokers in Japan (Gao et al., 1993). More recent case–control studies have continued to find an inverse relationship. Galeone et al. (2007) investigated the relationship between dietary intake of vegetables and fruit and lung cancer risk in men and women in China using a case-control study. Cases were 218 newly diagnosed lung cancer patients who were hospitalized. The controls were patients admitted to the same hospital for cancer diseases. For each case, two controls were individually matched. Participants completed a food frequency questionnaire. The results showed an inverse relationship between vegetable and fruit intake and lung cancer risk in both genders and regardless of smoking history. Rylander and Axelsson (2006) also conducted a case-control study on lung cancer risks in relation to vegetable and fruit consumption. Five hundred thirty-six lung cancer cases and 916 population controls in West Sweden were interviewed using a food frequency questionnaire. After adjusting for smoking, the risk for those who seldom consumed vegetables was twice that among those who consumed vegetables frequently, both among nonsmokers, smokers, and former smokers. A similar tendency, although less pronounced, was found for fruit consumption. The overall results from the case-control studies on fruit and vegetable intake and lung cancer risk have strongly shown an inverse relationship.

The study results for the association between fruit and vegetable consumption and lung cancer incidence are somewhat mixed. Some studies have demonstrated an association for both fruits and vegetables, some for vegetables only, and some for fruits only and many based on smoking status. The association seems to be strongest in case–control studies than in cohort studies. Taken together though, the case-control and cohort study evidence regarding lung cancer supports a protective effect of vegetable and fruit intake.

Vitamin C, Vitamin E, and Carotenoids

Review of Evidence

Vitamin C, vitamin E, and carotenoids have been shown to have antioxidant properties. Some studies have investigated the potential of antioxidant intake and lung cancer. The research on vitamin C in lung cancer has been mixed. A protective effect of vitamin C on lung cancer risk has been reported in a population-based case-control study in Hawaii (Le Marchand et al., 1989) and a cohort study (Knekt et al., 1991). In contrast, two case–control studies did not find an association with vitamin C (Byers et al., 1987; Jain et al., 1990). A cohort study by Bandera et al. (1997) in New York State found a relationship between vitamin C and lung cancer for men but not women. Bandera also found a protective effect associated with carotenoid intake in males but not in women. This is in agreement with other studies that have failed to find an association between carotenoid and lung cancer in women (Hinds et al., 1984; Byers et al., 1987). In general, epidemiologic studies have not consistently shown an association between vitamin E intake and lung cancer. It is hypothesized that this may be due to difficulties in measuring vitamin E intake. Bandera et al. (1997) found a slight reduction in lung cancer risk associated with vitamin E consumption in males and females, although confidence intervals included the null value. These results are in agreement with those of Byers (1987) who found a weak and nonsignificant relationship with vitamin E. Overall, more research is needed to determine the effects of antioxidant intake on the prevention of lung cancer.

Folic Acid

Review of Evidence

There is a growing interest in the role of folic acid in carcinogenesis given its involvement in the process of DNA syntheis, methylation, and repair. However, the relationship between lung cancer and dietary folic acid has not been widely investigated in epidemiologic studies. Two case–control studies have tested the relationship between folic acid and lung cancer and failed to find an association after adjusting for relevant cofounders (Le Marchand et al., 1989; Bandera et al., 1991). In the New York State Cohort Study, folic acid seemed to exert a protective effect on lung cancer risk in men but not in women and only for squamous cell carcinomas (Bandera et al., 1991). Once again, more research is needed in this area.

Phytoestrogens

Mechanism of Action

Phytoestrogens are a broad group of nonsteroidal compounds of different structure that bind to estrogen receptors (ER). There are three main classes of phytoestrogens: isoflavones, lignans, and coumestans. Isoflavones are the most common form and the most investigated of the phytoestrogens (Adlercreutz and Mazur, 1997). The two major forms of isoflavones are genistein and daidzein. Precursors of these major forms of isoflavones are found in soy, chickpeas, and red clover. Lignans are found in rye grains, linseeds, carrots, spinach, broccoli, and other vegetables. Coumestrol is found in beans, peas, clover, spinach, and sprouts.

Phytoestrogens can exert both estrogenic and antiestrogenic effects depending on factors such as their concentration, the concentration of endogenous sex hormones, and the relative levels of estrogen receptors (Gallo et al., 2008). Phytoestrogens can also interact with pathways of cellular activity that do not involve estrogen receptors. Phytoestrogens are thought to decrease the risk of cancer through a number of mechanisms including cell cycle regulation, inhibition of invasion and metastasis, antioxidant activity, induction of apoptosis, inhibition of endothelial cell proliferation, and inhibition of angiogenesis (Fournier et al., 1998).

Review of Evidence

Epidemiological and experimental studies have suggested an association between higher intake of phytoestrogens and reduced risk for cancer of the breast and prostate (Magee and Rowland, 2004). Recent and growing epidemiologic evidence supports a protective effect of phytoestrogens and the risk of lung cancer. Seow et al. (2002) investigated the dietary and reproductive factors associated with lung cancer risk among Chinese women. A hospital-based case-control study of 303 cases and 765 age-matched controls was conducted. Data on demographic background and dietary intake of fruit, vegetables and soy foods were obtained by in-person interview. Soy intake was found to be an independent predictor of lung cancer risk in nonsmokers. A study by Schabath et al. (2005) reported results from a case-control study supporting evidence that phytoestrogens are associated with a decreased risk of lung cancer. The study enrolled 1674 patients with lung cancer and 1735 healthy controls from 1995–2003 in the United States. Participants were questioned about their diets from the previous year. Results demonstrated a protective effect for the highest intake of isoflavones (≥1mg/day) which was significant for both women and men and resulted in a 32% overall reduction in risk (44% for men and 22% for women). A recent study explored the relationship of soy and the risk of non-small-cell lung cancers (NSCLCs) that express the epidermal growth factor receptor mutations (EGFR) (Matsuo et al., 2008). EGFR is a receptor tyrosine kinase that activates several signaling pathways resulting in cell proliferation, escape from apoptosis, and increased likelihood of invasion or metastasis, all of which are associated with the cancer phenotype (Hunter and Cooper, 1981). Elevated levels of EGFR are frequently seen in a variety of epithelial tumors (Nicholson et al., 2001) including NSCLCs (Bunn and Franklin, 2002). A case-control study using 353 patients with NSCLCs and 1765 age-sex matched cancer control subjects was conducted in Japan. Dietary exposure was based on a semiquantitative food frequency questionnaire. Soy consumption was demonstrated to have a protective association with EGFR mutated NSCLCs. In summary, the research presented represents a growing body of epidemiologic evidence supporting the phytoestrogens' protective effect against lung cancer risk.

Alcohol

Mechanicsm of Action

Alcoholic beverages have been demonstrated to be carcinogenic in humans and are related to cancers in several sites, including the mouth, pharynx, larynx, esophagus, liver, colorectum, and female breast (IARC, 2007). The metabolism of alcohol generates acetaldehyde which causes mutation in the genetic material of cells thus allowing for the development of cancer cells. Additionally, alcohol metabolism creates radicals such as lipid hydroperoxides which are also considered carcinogenic (Ames, 2008). The question then is, do the carcinogenic effects of alcohol relate to the development of lung cancer? Epidemiologic studies on the association between alcohol consumption and lung cancer have been inconsistent (Bandera, 2001). In general, though, high levels of alcohol consumption are associated with increased risk for lung cancer (Ames, 2008). The relationship between alcohol and lung cancer was evaluated among participants of the New York State Cohort (Bandera, 1997). The cohort consisted of 27,544 men and 20,456 women who completed a mailed questionnaire on dietary habits. Results found a significant positive dose–response relationship between alcohol consumption and development of lung cancer (Bandera, 1997). The

European Prospective Investigation into Cancer and Nutrition (EPIC) also found an increase in risk among heavy drinkers (>60 g ethanol/day), though it was not significant (Rohrmann et al., 2006). Additionally, a meta-analysis of prospective cohort studies on lung cancer risk in heavy drinkers (≥467 g/week) showed a significant increase in risk of lung cancer compared with nondrinkers (Korte et al., 2002). In contrast to these studies, Nishino et al. (2006) found no association between the amount of alcohol consumed and lung cancer risk. Studies examining the association between alcohol and lung cancer risk are confounded by the positive correlation between smoking and alcohol. When smoking is controlled for, the studies on the association between alcohol and lung cancer continue to be conflicting. A recent pooled analysis of seven cohort studies showed a significant increase in risk for lung cancer according to alcohol consumption category among nonsmokers but not smokers (Freudenheim, 2005). Korte (2002) also reported the results for nonsmokers in the Cancer Prevention Study I and II. Compared with nondrinkers, the risk of lung cancer in smoking male drinkers who consumed >117 g ethanol/week was significantly higher. On the contrary, Shimazu et al. (2008) found an association between alcohol consumption and lung cancer only in smokers. The population-based prospective cohort study of over 46,000 men in Japan found alcohol consumption was significantly associated with an increased risk of lung cancer in men who drank ≥300 g/week ($p = 0.07$) and smoked. Even among participants with an ethanol intake of >450 g/week, no association between alcohol drinking and lung cancer risk was seen among nonsmokers. In conclusion, the research tends to point to a positive association between alcohol consumption and lung cancer risk.

Fat

Review of Evidence

Although the exact biological mechanism by which fat intake may increase lung cancer risk is not known, it is hypothesized that the effects of fat in immune and endocrine processes, intercellular communication, and cell proliferation may explain the relationship. The New York Cohort Study (Bandera et al., 1997) found a significant dose–response relationship between lung cancer and total fat, monounsaturated fat, and saturated fat in men but not in women. Other studies have also confirmed a relationship between total fat intake and lung cancer in men (Byers et al., 1987; Jain et al., 1990). A study conducted among Finnish men also found a positive association of lung cancer with saturated fatty acids, particularly among smokers and for squamous cell carcinoma (Knekt et al., 1991). The New York Cohort Study found no association between dietary cholesterol or polyunsaturated fat intake and lung cancer. In agreement with the New York Cohort Study, several case-control studies have found no association between polyunsaturated fat intake and lung cancer (Shekelle et al., 1981; Jain et al., 1990). The studies on the relationship between dietary cholesterol and lung cancer have been mixed. While the New York Cohort Study (Bandera, 1997) and other studies (Heilbrun et al., 1984; Knekt et al., 1991) found no relationship, Shekelle et al. (1981) and other case-control studies suggested a relationship with cholesterol in men (Byers et al., 1987; Jain et al., 1990). Although a definitive conclusion cannot be drawn between dietary fats and lung cancer risk, a diet low in total fat and saturated fat is protective against other major health issues.

β-Carotene Supplements

Review of Evidence

The observation that fruits and vegetables may reduce lung cancer risk led to the implementation of two randomized clinical trials in which high doses of β-carotene were used. The Beta-Carotene and Retinol Efficacy Trial (CARET) intervention tested the efficacy of 30 mg of β-carotene plus 25,000 IU of retinyl palmitate daily in male and female heavy smokers and men exposed to asbestos in the

United States (Omenn et al., 1996). The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) trial tested 20 mg of β-carotene plus 50 IU of vitamin E daily in male heavy smokers in Finland (Albanes et al., 1995). Both trials found that β-carotene, alone or in combination with vitamin E or retinyl palmitate, increased the incidence of lung cancers by 36% in CARET and 16% in ATBC compared with placebo. Satia et al. (2008) examined the associations of supplemental β-carotene, retinol, vitamin A, lutein, and lycopene with lung cancer risk. The VITamins And Lifestyle (VITAL) cohort study was conducted between 2000–2002 in Washington State. Over 77,000 persons aged 50–76 years participated, completing a 24-page questionnaire on supplement use. The study found longer duration of use (more than four years) of individual β-carotene, retinol, and lutein supplements was associated with a statistically significantly elevated risk of lung cancer. Long-term use of individual β-carotene, retinol, and lutein supplements should not be recommended for lung cancer prevention, particularly among smokers.

CONCLUSION

Due to the lack of early detection and high mortality rate, prevention of lung cancer is imperative. The most important measure to prevent lung cancer is avoidance or cessation of smoking. Additional preventative measures include avoiding second-hand smoke and environmental pollutants, and potentially dietary changes. This chapter reviewed dietary factors that may provide protection against lung cancer. A diet high in fruits and vegetables, low in fat and alcohol provides differing degrees of prevention. Fruit and vegetable consumption showed the strongest association with prevention of lung cancer. Inconsistencies in the research on diet and cancer are not uncommon. These inconsistencies likely reflect the multifactorial and complex nature of cancer and the specificity that individual dietary constituents have in modifying cancer risk. Despite the varying strengths of the associations, the dietary recommendations for lung cancer prevention also promote overall health and wellness.

ABBREVIATIONS

ATBC	Alpha-Tocopherol, Beta-Carotene Cancer Prevention
CARET	Beta-Carotene and Retinol Efficacy Trial
CDC	Centers for Disease Control and Prevention
EGFR	Epidermal growth factor receptor mutations
EPIC	European Prospective Investigation into Cancer and Nutrition
ER	Estrogen receptors
ETS	Environmental tobacco smoke
NSCLS	non-small-cell lung cancers
SCLC	small cell lung cancers
VITAL	Vitamins and Lifestyle Study

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36 Herbs and Bioactive Compounds in Prevention and Treatment of Hepatocellular Carcinoma

Anuradha Sehrawat and Vijay Kumar

CONTENTS

INTRODUCTION

The maintenance of a healthy liver is vital to overall health and well-being. However, hepatotoxicity caused by certain environmental toxins, poor eating habits, alcohol consumption, and some therapeutic drugs may cause serious diseases like hepatitis, cirrhosis, alcoholic liver disease that eventually lead to hepatic cancers. Chronic viral hepatitis B and C, alcoholic liver disease, nonalcoholic fatty liver disease are other etiological factors associated with hepatocellular carcinoma (HCC).

HCC is the fifth most common malignancy worldwide and is the third leading cause of cancer mortality, occuring with great frequency especially in Asia and Africa and is becoming more common in the Western countries as a complication of chronic hepatitis C and alcoholic liver disease (Bosch et al., 2004; El-Serag, 2004). Most commonly, HCC develops in patients with chronic liver disease, the aetiology of which includes alcohol, viral hepatitis (B and C) and dietary carcinogens, in particular aflatoxin (Kew, 2002). Surgery, including transplantation, is currently considered the most effective treatment for HCC, but the long-term prognosis for these patients is poor and recurrent disease occurs in the majority of cases. Liver tumors are highly resistant to available chemotherapeutic agents leading to high prevalence and increased death rates. For these reasons, prevention and treatment of HCC is a challenging project in both basic and clinical medicine and their is strong need for searching new agents and developing new strategies against HCC.

Over the past decade, herbal medicines have been accepted universally as good therapeutic agents for cancer and thus have made an impact on both world health and international trade. The concept "herbal drug and food are of the same origin" encourages many food and herbal medicines

which are consumed in daily life as antitumor therapies. In fact, there are several medicinal plants which are being used traditionally for the prevention and treatment of cancer, and more than 60% of drugs employed against cancer are of natural origin. In recent years, researchers have reexamined the therapeutic potential of many traditionally used herbs and bioactive compounds to support liver function and treat liver diseases and also confirm their therapeutic effectiveness in clinical studies (Stickel and Schuppan, 2007; Manna et al., 2009). Further, serious efforts are being made to understand the mode and mechanisms of their action.

The aim of this chapter is to provide a general outline on the current knowledge regarding potential use of several well-characterized herbs and bioactive compounds in HCC. In addition, it also covers some efficient and effective therapies for liver cancer using hepatoprotective herbs with anticancerous properties with an aim to raise awareness and encourage implementation of alternative therapies in HCC.

LIVER DISEASES

The liver performs hundreds of critical functions in the body to maintain homeostasis and health, therefore it is foreseeable that liver diseases severely affect health and can be life-threatening. There are four major types of liver diseases: cirrhosis, fatty liver, hepatitis, and HCC, with the latter two being among the most serious global public health problems. Primary causes of chronic liver diseases are shown in Figure 36.1.

Cirrhosis

Cirrhosis, a scarring damage to the liver, is a potentially life-threatening condition that develops after years of liver inflammation. Chronic alcoholism is the most common cause of cirrhosis with chronic viral hepatitis (types B, C, and D and autoimmune hepatitis), blocked bile ducts, steatohepatitis, and many inherited diseases. Prolonged exposure to environmental toxins can also lead to cirrhosis and this is a well-known fact that cirrhosis is associated with HCC, especially in men. Overall, 75% of HCC develop in cirrhotic livers (Macdonald, 2001).

Fatty Liver

Fatty liver is a nonserious condition in which excess fat accumulates in the liver of people who drink little or no alcohol. By itself it probably does not damage the liver but may lead to liver cell inflammation to severe liver scarring and cirrhosis. Factors that may contribute to the development of fatty

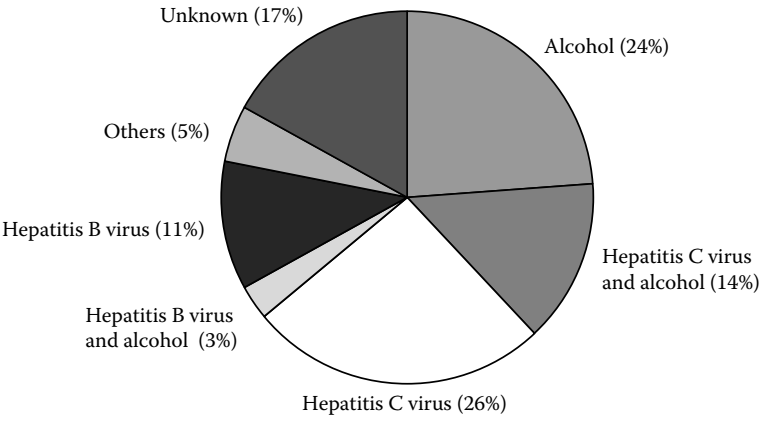

FIGURE 36.1 Primary causes of chronic liver disease (based on a report of Centers for Disease Control and Prevention).

liver include oxidative stress, excessive production and release of toxic inflammatory proteins by own inflammatory cells, liver cells or fat cells, and necrosis or apoptosis (Schütte et al., 2009).

Fibrosis

Fibrosis, a precondition for cirrhosis, is the excessive accumulation of extracellular matrix proteins including collagen in most types of chronic liver diseases. Advanced liver fibrosis results in cirrhosis, liver failure, and portal hypertension, and often requires liver transplantation. The main causes of liver fibrosis in industrialized countries include chronic HCV infection, alcohol abuse, and nonalcoholic steatohepatitis (Betaller and Brenner, 2005; Koike, 2009).

Hepatitis

In Latin, hepatitis means "inflammation of the liver," and the most common etiologies for hepatitis are infections by one of five hepatitis viruses, called hepatitis A, B, C, D, and E, cytomegalovirus, Epstein-Barr virus, yellow fever, parasites, drugs, and toxins. All these hepatitis viruses can cause acute disease with symptoms lasting several weeks; however, hepatitis B and C viruses can also establish persistent infections and thereby cause chronic hepatitis. According to WHO statistics, more than two billion people alive today have been infected with hepatitis B virus (HBV) at some time in their lives, and of these, about 350 million people are chronically infected and are carriers of the virus. An estimated 180 million people, 3% of the world's population, are infected with hepatitis C virus (HCV), 130 million of whom are chronic HCV carriers at risk of developing liver cirrhosis and liver cancer. It is estimated that three to four million people are newly infected each year, with 70% of them developing chronic hepatitis (Farinati et al., 2007).

Hepatocellular Carcinoma (HCC)

HCC has become an important issue in medicine as it is a major cause of death in both the developed and developing countries. Pathogenesis of HCC indicates that various factors such as aflatoxins, viral infections, alcoholism result in liver cell generation, proliferation, cell cycle activation, and uncontrolled cell growth associated with hyperplasia, dedifferentiation, and development of HCC. Thus, it can be said that HCC is a continuity of regeneration, proliferation, unregulated hyperplasia, dysplasia, and malignant transformation (Figure 36.2), and is closely linked with a histological progression to HCC (Feitelson et al., 2002).

Epidemiology

HCC is estimated to be the third leading cause of cancer death worldwide. It has a worldwide incidence of 2,50,000 to 1,000,000 patients annually with high prevalence in Asia and Africa where

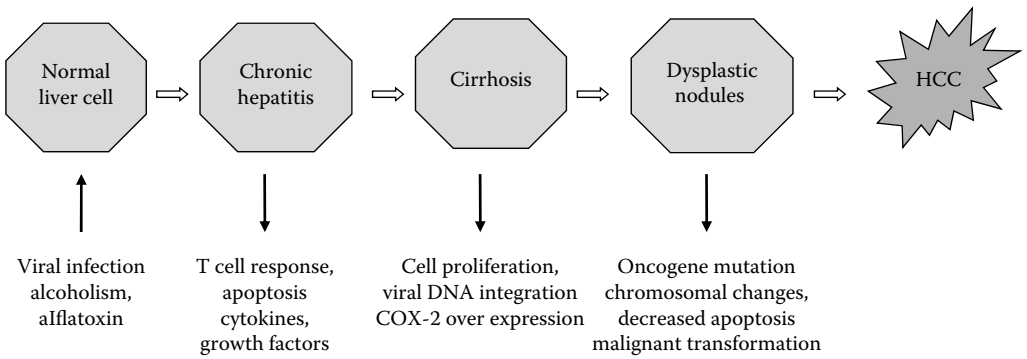

FIGURE 36.2 Histological evolution of HCC.

annual incidence of 500 cases occurs per 100,000 population. It can be estimated that around 3,72,000 new cases of HCC are diagnosed annually. In addition, HCC is more frequent in men than in women and the incidence generally increases with age. Data from the International Classification of Disease coding manual depicts that the annual mortality rate from HCC is virtually the same as its annual incidence, that is, the fatality ratio is close to 1.0 (Wong and Ng, 2008).

Risk Factors

Progression of HCC is a multistage, multifactorial, and complex process which is associated with a background of chronic and persistent infection. Hepatitis B virus or hepatitis C virus along with exposure to aflatoxin and heavy alcohol consumption are widely recognized etiological agents in HCC. About 80% of individuals with HCC have cirrhosis, which is the most severe form of liver fibrosis, the end point of chronic liver damage. Other factors contributes to HCC includes the occupational exposure to vinyl chloride or similar toxic chemicals, long-term use of oral contraceptives, and angiogenic therapy.

Molecular Changes Involved in HCC

Ozturk (1999) has reported that four genetic pathways are involved in the process of malignant transformation of liver cells: (1) p53 pathway involved in DNA damage and response, (2) pRB/$p16^{INK4A}$ pathway involved in cell cycle control, (3) TGF-β1 pathway involved in growth inhibition, and (4) apoptosis and β-catenin/Axin1 pathway involved in morphogenesis and signal transduction. More than 20 genes have been reported to be involved in HCC development.

Prevention of HCC

HCC prevention includes primary prevention aimed at interference with chronic liver diseases of diverse etiology and secondary prevention aimed at preventing the recurrence and development of new HCC lesions after successful surgery. Most of the HCC patients die rather early because of the high local invasiveness and metastatic efficiency of tumors. Hepatic resection or transplantation is the only possible curative treatment for HCC patients. There are no strong evidence to substantiate that any of the above treatment regimens benefit survival in HCC. Rather these treatments severely deteriorate the liver function. The prognosis of HCC is still poor, the tumor recurrence rate rather high (>50% at three years, even after tumor ablation or surgery), and it has become the most important factor that limit the long-term survival of patients. Therefore, promising new preventive strategies for HCC, in particular those present in medicinal plants and diet, are needed so that they can be included in the human diet at minimal cost. Chemopreventive agents are natural or synthetic chemicals, which reverse, suppress, or prevent carcinogenesis through inhibition of carcinogen-activating hepatic enzymes or by inducing phase II hepatic enzymes. These agents can prevent interaction of the carcinogen with cellular DNA, alter mitogenic signaling pathways, prevent the progression of normal cells through preneoplastic changes into a malignant cells, inhibit angiogenesis, induce cell cycle arrest, or trigger pro-apoptotic cell death. Focusing on such agents in patients at high risk of malignancy or following curative treatment may, therefore, offer potential therapeutic benefit (Mukhtar and Ahmed, 1999; Seeff et al., 2001). The majority of patients who are at risk of developing HCC have chronic liver disease and are therefore an ideal group for targeted chemoprevention. As the prognosis of HCC is extremely poor and is largely unresponsive to current chemotherapeutic agents, this should be an important area of research.

HERBAL TREATMENT OF HCC

Herbal treasure chest of complementary alternative medicine (CAM) offers a host of new phytochemicals that can be used both in a preventive mode and clinically to manage a spectrum of liver related imbalances including HCC (Table 36.1).

TABLE 36.1
Hepatoprotective Herbal Therapies

Disease	Herbal Therapy
Liver diseases	*Adhatoda vasica*
Viral hepatitis	*Acacia catechu, Bacopa monnieir, Citrullus lanthus, Solanum nigrum, Sphaeramthus indicus, Tephrosia purpurea*
Chronic hepatitis	*Chichorium intybus*
Hepatitis B	*Aegle marmelos, Azadirachta indica, Phyllanthus niruri, Vitex nigundo*
Hepatitis C	*Emblica officinalis, Fummaria parviflora*
Hepatoma	*Aloe vera, Anacardium occidentale, Glycyrrhiza glabra, Gynandropis pentaphylla, Plumbago zeylanica, Saussurea lappa, Withanea somnifera*

Herbal therapies have a very long history, and many food and herbs which are consumed in daily life are also used for the prevention and treatment of cancer despite little understanding of their molecular and cellular basis of action. Clinical practice has shown that traditional medicines elicit good effects by delaying tumor growth or in certain cancers, improve the quality of life and survival, or complement the effects of radiotherapy or chemotherapy. Plants like *Andrographis paniculata*, *Annona atemoya/muricata*, *Boerhavia diffusa*, *Eclipta alba*, *Piper longum*, *Phyllanthus amarus*, *Podophyllum hexandrum*, *Terminalia chebula,* and *Semecarpus anacardium Linn* have shown antitumor activity in humans also. Some of these plant products have been found to contain chemically defined components that can protect the liver from oxidative injury, promote virus elimination, block fibrogenesis, or inhibit tumor growth. Dietary powdered green tea shows both antiproliferative activity in hepatoma cells and hypolipidemic activity in hepatoma bearing rats (Khan et al., 2008). Numerous natural ingredients contribute to the immunoenhancing and antitumor properties, for example: leaves of *Macaranga triloba*, antioxidants in foods such as phenolic compounds and caretenoids, curcuminoids, resveratrol, reishi, triterpenoids, Korean red ginseng extract, polysaccharide rich substance, saikosaponins, baicalin and baicalein, organic gemanium, quercetin, β-carotene, citrus bioflavonoid complex, bilberry extract, rutin, zinc, and selenium as well as compounds released in fermented milks (Kelloff et al., 2000; Stickel and Schuppan, 2007; Pan et al., 2008; Manna et al., 2009). Recent meta-analyses have confirmed the utility of Chinese herbs in controlling viral-induced HCC and reducing side effects of chemotherapy (Shu et al., 2005). In tribal belts of India where viral hepatitis is common, dried and powdered rhizomes of *Acorus calamus* Linn (Buch) is consumed with water whereas in case of jaudice, leaves of beetle vine and *Andrographis paniculata* are given to the patients (Rai and Nath, 2003). Recently, a complete regression of HCC was reported after taking herbal medicine but the mechanism leading to regression is still far from being understood (Cheng and Tsai, 2004).

SOME IMPORTANT HERBS OF AYURVEDA AND THERAPY OF LIVER DISEASES

Picrorhiza kurroa (Kutuki)

History/Traditional Use

Picrorhiza kurroa (Scrophulariaceae) is a small perennial herb found in the Himalayan region growing at elevations of 3000–5000 m. It is a well-known herb in the traditional system of medicine in India and has been used in the treatment of disorders of the liver and upper respiratory tract,

dyspepsia, chronic diarrhea, and scorpion sting, and even in reducing fever. Medicinally used parts of the plant are the roots and rhizomes.

Active Constituents

The most important active principle of *P. kurroa* is a kutkin comprising of iridoid glycoside, picrosides I, II, III, and kutkoside. Other identified active constituents of the plant are apocynin, drosin, and nine cucurbitacin glycosides. Apocynin is a catechol that has been shown to inhibit oxidative burst in neutrophil addition to being a powerful anti-inflammatory agent whereas curcubitacins have been shown to be highly cytotoxic and thus, possess antitumor effects (Simons et al., 1990).

Mechanisms of Action

The mechanism by which Picrorhiza offers hepatoprotective action is poorly understood. However, several possibilities have come to light from new research (Figure 36.3).

The therapeutic activity of the herb may be based on three mechanisms: (1) Kutkins alter the structure of the outer membrane of the hepatocytes in such a way as to prevent penetration of liver toxins into the interior of cell. (2) Kutkins stimulate the action of nucleolar polymerase A, resulting in increased ribosomal protein synthesis, regenerative ability of the liver, and formation of new hepatocytes. (3) Apocynin, one of its constituents, has been found to exhibit powerful anti-inflammatory effects on a variety of inflammatory models (Verma et al., 2009).

Clinical Indications

Viral Hepatitis

Picrorhiza may be of therapeutic value in treating viral hepatitis. An *in vitro* study suggested anti-hepatitis B-like activity of Picrorhiza through reduction in the levels of surface antigens (Mehrotra et al., 1990). In a randomized, double-blind, placebo-controlled trial of 33 patients diagnosed with acute viral hepatitis (HBsAg negative), 15 patients in the treatment group were given 375 mg Picrorhiza root powder three times daily for two weeks. The remaining 18 subjects acted as controls and received placebo. Bilirubin, SGOT, and SGPT values were significantly lower in the treatment

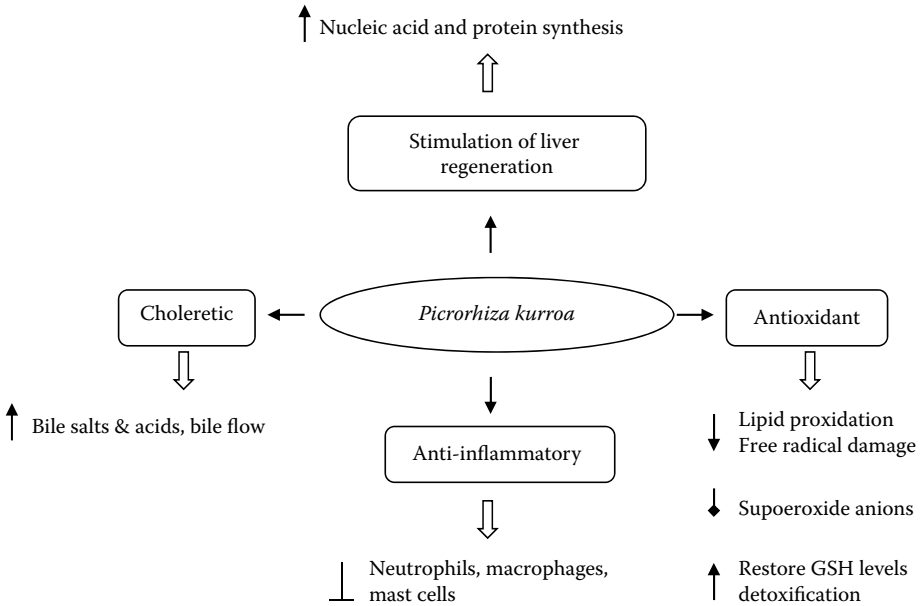

FIGURE 36.3 Possible mechanism of action of *Picrorhiza kurroa*.

group, and the time required for bilirubin values to drop to 2.5 mg was 27.4 days in the treatment group versus 75.9 days for the placebo group (Vaidya et al., 1996).

General Hepatoprotective

Numerous animal studies, primarily in rats, have demonstrated that Picrorhiza can prevent liver toxicity and subsequent biochemical changes caused by various toxic agents including carbon tetrachloride, galactosamine, ethanol, thioacetamide, aflatoxin B1, acetaminophen, oxytetracyline, amanita mushroom poisoning, and monocrotaline. The hepatoprotective effect was found to be similar, even superior, to the effect of silymarin. *In vitro* studies indicated the antioxidant activity of Picrorhiza in hypoxic glioma and Hep 3B cells with reduced cellular damages (Luper, 1998; Thyagarajan et al., 2002).

Dosage and Toxicity

Since Picrorhiza is poorly soluble in water and has a bitter taste, it is administered as a standardized (4% kutkin) encapsulated powder extract. Usual adult dosage is 400 to 1500 mg/day, with dosages up to 3.5 g/day sometimes being recommended for fevers. Picrorhiza use is widespread in India with no adverse effects have been reported. The LD_{50} of kutkin is greater than 2600 mg/kg in rats with no data available for humans (CSIR/RRL, 1989–1990).

Silybum marianum (Milk Thistle)

History/Traditional Use

Silybum marianum (Asteraceae) is indigenous to Europe and also found in some parts of the United States. The common name, milk thistle, is derived from the "milky white" veins on the leaves, which, when broken open, yield a milky sap. *S. marianum* is cited as one of the oldest known herbal medicines and is currently the most well researched plant in the treatment of liver disease with over 450 peer-reviewed and published papers. Its use for liver disorders dates back to Pliny the Elder, a Roman naturalist, who described milk thistle as being "excellent for carrying off bile" (Ross, 2008).

Active Constituents

S. marianum contains silymarin which is composed of the flavanolignans silybin, silydianin, and silychristine. Silybin is the biologically most active component. Milk thistle extracts are usually standardized to contain 70–80% silybin. Silymarin is found in the entire plant but is concentrated in fruits and seeds. The seeds also contain betaine (a proven hepatoprotector) and essential fatty acids, which may contribute to silymarin's anti-inflammatory effect.

Mechanisms of Action

The mechanism by which silymarin offers hepatoprotective action is fairly well understood. As illustrated in Figure 36.4, the therapeutic action of silymarin seems to involve multiple mechanisms such as antioxidant, antitumor, immunomodulatory, anti-inflammatory, antifibrotic, chemopreventive, and by tissue regeneration. All these mechanisms show molecular responses typical of each of the pathway (Varghese et al., 2005; Agarwal et al., 2006).

Clinical Indications

Amanita Mushroom Poisoning

Silymarin is used in the treatment of *Amanita phalloides* mushroom poisoning whose accidental ingestion results in about 60 cases of poisoning per year in the United States and Europe, with a mortality rate of about 30%. In animal studies, silymarin given within 10 minutes after Amanita toxin ingestion completely counteracts the toxic effects, while if given within 24 hours of toxin

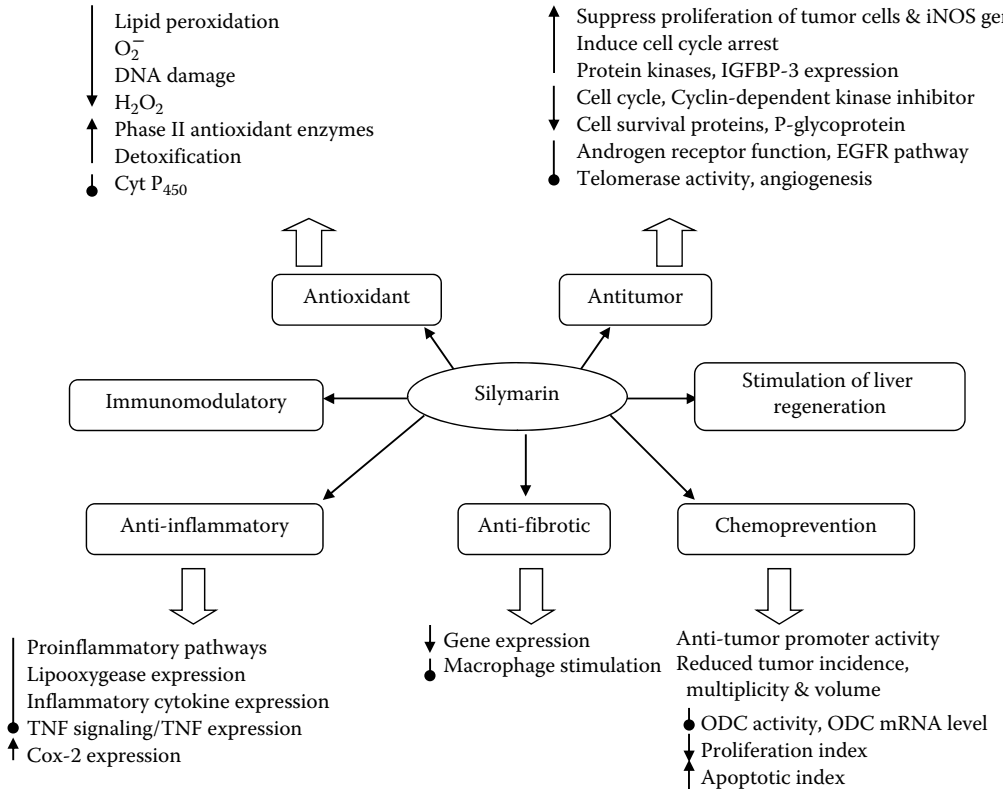

FIGURE 36.4 Possible mechanism of action of *Silybum marianum*.

ingestion, silymarin can prevent death and greatly reduce liver damage. The hepatoprotective effects of silymarin in humans after ingestion of Amanita toxins have been repeatedly demonstrated. In one series of 18 patients treated with silymarin, all patients survived except one, a particular case of high-dose suicide. In a 1995 study of 41 mushroom poisoning victims, none died in the group which included silymarin in the treatment regimen (Luper, 1998).

Hepatitis

Silymarin is quite effective in the treatment of both acute and chronic hepatitis. In acute viral hepatitis, administration of silymarin shortens treatment time and lowers serum bilirubin, AST, and ALT. In patients with chronic hepatitis, 420 mg silymarin per day for six months has been shown to improve the levels of serum liver enzymes (Trinchet et al., 1989; Buzzelli et al., 1993). Treatment of acute clinical hepatitis patients with silymarin has shown potential improvement in symptoms despite lack of a detectable effect on biomarkers of the underlying hepatocellular inflammatory process (El-Kamary et al., 2009).

Alcoholic Liver Disease and Cirrhosis

Studies conducted in Austria and Hungary have demonstrated that silymarin administration can normalize serum liver enzyme and total bilirubin levels in patients with alcoholic liver disease and improved liver histology. However, silymarin does not have direct effect on ethanol metabolism (Feher et al., 1989; Trinchet et al., 1989). In a study involving 170 patients with liver cirrhosis, 420 mg/day of silymarin for an average of 41 months resulted in a significant improvement in survival [58% in silymarin-treated patients and 39% in the placebo group ($p = 0.036$)]. No side effects of silymarin were noted in this study (Ferenci et al., 1989).

Dosage and Toxicity

S. marianum is not water soluble and usually given as a standardized extract (70–80% silymarin) in encapsulated form, 100–300 mg three times daily being the typical adult dose. Both animal and human studies have shown silymarin to be generally nontoxic. At high doses (>1500 mg per day), silymarin may produce a laxative effect due to increased bile secretion and flow. Mild allergic reactions have also been noticed but were not serious enough to discontinue treatment. Silymarin is cleared from the body predominantly via the bile and to a lesser extent via the kidney with a half-life of six to eight hours (Luper, 1998).

Podophyllum hexandrum (Indian May Apple)

History/Traditional Use

Podophyllum hexandrum (Podophyllaceae), a Himalayan herb, also known as Himalayan May apple or Indian May apple is poisonous, but when processed has medicinal properties. It has been extensively used in traditional Ayurvedic system of medicine for treatment of colds, constipation, septic wounds, burning sensation, erysipelas, mental disorders, rheumatism, plague, allergic, and inflammatory conditions of skin, cancer of brain, bladder, and lung.

Active Constituents

Podophyllotoxin the main active constituent of *P. hexandrum* is used to produce two cytostatic drugs, etoposide and teniposide. In addition a number of lignans isolated from *Podophyllum* species have shown a wide range of biological activities, such as antitumor, antimitotic, and antiviral activities. Some of them have also shown toxicity to fungi, insects, and vertebrates.

Mechanisms of Action

The mechanism of action of *Podophyllum* is illustrated in Figure 36.5 (Pandey, 2002).

Clinical Indications

HCC

P. hexandrum (active constituent: Podophyllotoxin) prescribed in liver disorders is a potent hepatic stimulant, a blood purifier, and an anticancer drug. Etoposide (VP-16), a semi-synthetic derivative of podophyllotoxin, has been used against HCC for more than 15 years (Park et al., 1994). In a phase

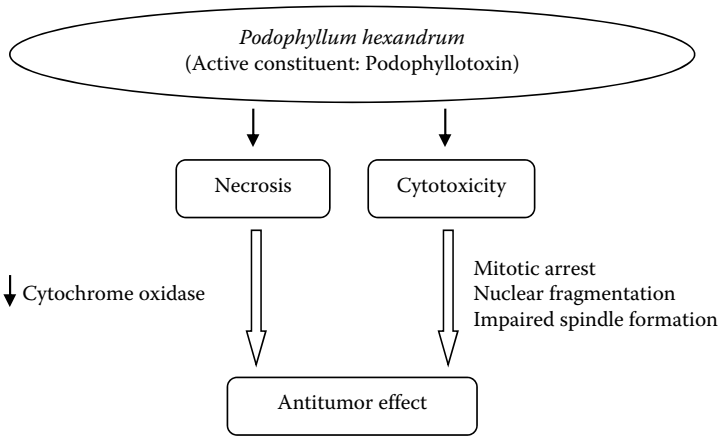

FIGURE 36.5 Mechanism of action of *Podophyllum hexandrum*.

II study on anticancer effectiveness of VP-16 in combination with epirubicin (one of the anticancer agents used in HCC chemotherapy), 3% of 36 HCC patients achieved complete response, 36% had partial response, and 31% exhibited stable disease; the disease progressed in another 31% of patients. Thus, this combination appears to be an active and tolerable therapeutic option for HCC patients (Pallavacini et al., 1997).

Dosage and Toxicity

Pharmacokinetic studies on etoposide showed that this drug is safer even for the above therapeutic doses and is without any hepatotoxic effects (Aita et al., 1999).

Phyllanthus amarus (Chanca Piedra)

History/Traditional Use

Phyllanthus amarus (Euphorbiaceae), is usually found in central and southern India, Philippines, Cuba, Nigeria, and Guam. It has a long tradition of use as a medicinal agent in cultures around the world. Traditional applications of *P. amarus* includes the treatment of many types of biliary and urinary conditions including kidney and gall bladder stones, hepatitis, common cold, flu, tuberculosis, and other viral infections; liver diseases and disorders including anemia, jaundice and liver cancer; and for bacterial infections such as cystitis, prostatitis, venereal diseases, and urinary tract infections.

Active Constituents

The main chemicals in *P. amarus* includes alkaloids, astragalin, brevifolin, carboxylic acids, corilagin, cymene, ellagic acid, ellagitannins, gallocatechins, geraniin, hypophyllanthin, lignans, lintetralins, lupeols, methyl salicylate, niranthin, nirtetralin, niruretin, nirurin, nirurine, niruriside, norsecurinines, phyllanthin, phyllanthine, phyllanthenol, phyllochrysine, phyltetralin, repandusinic acids, quercetin, quercetol, quercitrin, rutin, saponins, triacontanal, and tricontanol.

Mechanism of Action

The mechanism of action of *P. amarus* is illustrated in Figure 36.6 (Ott et al., 1997; RajeshKumar and Kuttan, 2000; Prabhakar, 2002).

Clinical Indications

Viral Hepatitis

In a clinical study conducted on 55 patients with chronic viral hepatitis, all the 30 patients under *P. amarus* treatment were cured within three months with remarkable recovery of liver functions and inhibition of HBV replication (Wang et al., 2001). In another study, aqueous extract of *P. amarus* cleared the HBV surface antigen in 59% of the patients (Mehrotra et al., 1991). Chronic HBV patients treated with *P. amarus* showed an equal effectiveness of 83% when compared with interferon treated group. The anti-HBV effects of the plant is attributed mainly to its four chemicals: niranthin, nirtetralin, hinokinin, and geraniin (Liu et al., 2001).

Hepatoprotection

The liver-protecting activity of *P. amarus* has been attributed to two novel plant chemicals *phyllanthin* and *hypophyllanthin*. Various *in vitro* and *in vivo* studies document that extracts of *P. amarus* effectively protect against liver damage from various chemical liver toxins (Prabhakar, 2002). In patients with hepatitis and jaundice, *P. amarus* showed liver protective and detoxifying actions and also increased the life span of the *N*-nitrosodiethylamine-induced HCC-bearing rats from 33 to 52 weeks (Rajeshkumar and Kuttan, 2000).

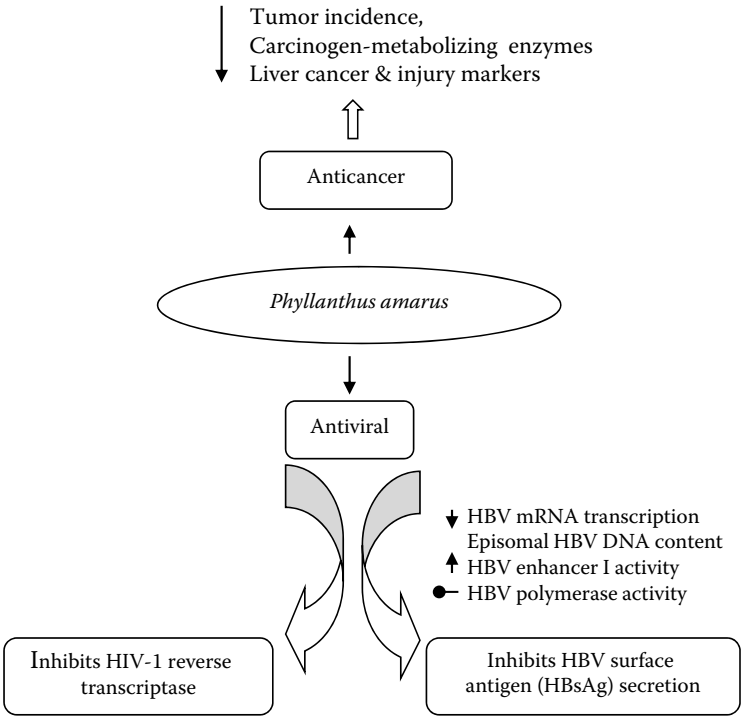

FIGURE 36.6 Mechanism of action of *Phyllanthus amarus*.

Toxicity

Most common dosage of *P. amarus* is 200 mg, three times a day, and to date, this dietary supplement is considered safe. However, the safety of this dietary supplement in children and during pregnancy or breast-feeding is not known.

Tinospora cordifolia (Guduchi)

History/Traditional Use

Tinospora cordifolia (Menispermaceae), a deciduous climbing shrub is distributed throughout tropical Indian subcontinent and China. It is widely used in veterinary folk and Ayurvedic system of medicine for its general tonic, antiperiodic, antispasmodic, anti-inflammatory, antiarthritic, antiallergic, and antidiabetic properties. Guduchi is used in ayurvedic "Rasayanas" to improve the immune system and hence the body's resistance against infections.

Active Constituents

A variety of constituents have been isolated from *T. cordifolia* plant which belongs to different classes such as alkaloids (berberine, palmatine, tembetarine, magnoflorine, choline, tinosporin, isocolumbin, tetrahydropalmatine) diterpenoid lactones (furanolactone, clerodane derivatives, tinosporon, jateorine, columbin), glycosides, steroids (β-sitosterol, δ-sitosterol, β-hydroxy ecdysone, ecdysterone, makisterone A, giloinsterol), sesquiterpenoid, phenolics, aliphatic compounds, and polysaccharides.

Mechanisms of Action

The mechanism by which *T. cordifolia* affords various actions are shown in Figure 36.7 (Panchabhai et al., 2008).

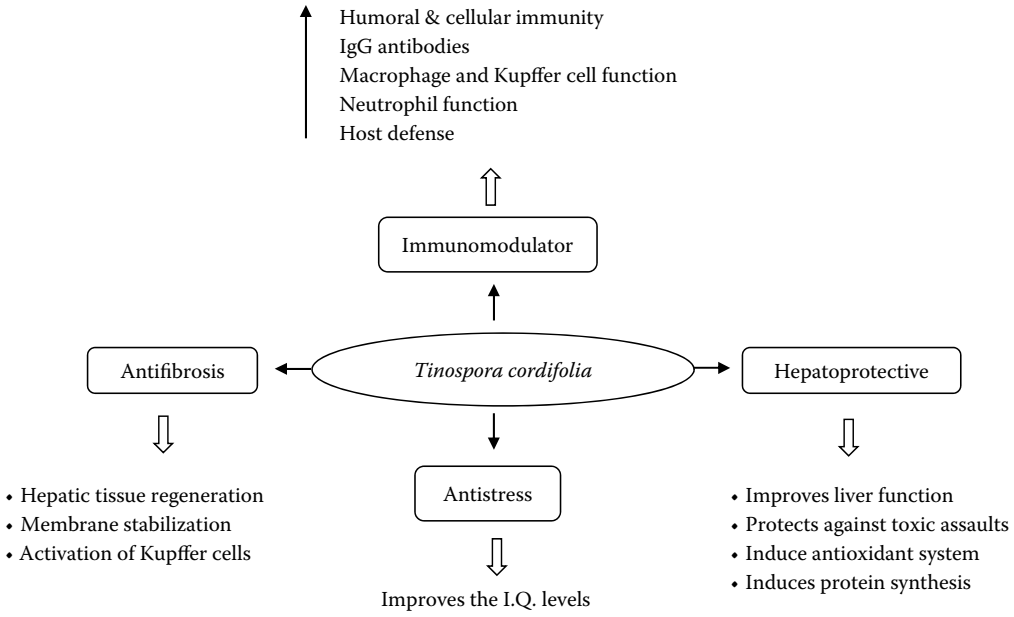

FIGURE 36.7 Mechanism of action of *Tinospora cordifolia*.

Clinical Indications

Obstructive Jaundice

Treatment of patients with malignant obstructive jaundice with *T. cordifolia* has shown decrease in morbidity and mortality due to liver failure without blood poisoning, and amazingly, 92.4% survived after surgery as compared to 40% survival rate in a conventionally treated group. Further, in another study, *T. cordifolia* administration reduce the risk involved and improve the outcome after surgery in obstructive jaundice patients, strongly favoured its use in the preoperative management of the jaundiced patients. (Rege et al., 1993; Bapat et al., 1995), perhaps by strengthening host defenses, normalizing phagocytic, and the killing capacities of neutrophils (Sohini and Bhatt, 1996).

Infective Hepatitis

In this study, a total of 20 patients were selected. All of the 20 patients treated with the *T. cordifolia* showed symptomatic relief with improvement in yellow discoloration of urine and body and reduction of LFT parameters. Treatment also reduced bilirubin and serum marker levels with 75% cure and 25% improvement (Prakash and Rai, 1996).

Liver Fibrosis

T. cordifolia prevents liver fibrosis, a major complication during HCC, by stimulating regeneration of hepatic tissue mediating through the activation of kupffer cells in CCl_4-induced liver damage in rats (Nagarkatti et al., 1994).

Immunosuppression

T. cordifolia is a known immunostimulant that works through enhancing cell-mediated as well as humoral immune response in mice. Treatment of mice with crude extract of dried stem can prevent cyclophosphamide-induced myelosuppression as well as immunosuppression (Sohini and Bhatt, 1996; Kapil and Sharma, 1997). In experimental animal tumors, it can reduce solid tumor volume

by 58.8%, which is comparable to a well-known anticancer drug cyclophosphamide (Matthew and Kuttan, 1999). These beneficial properties can be useful in the prevention of tumor progression and hence *T. cordifolia* could be a drug choice for HCC therapy where immunosuppression is a major complication.

Dosage and Toxicity

According to the Ayurvedic literature, *T. cordifolia* has no side effect and toxicity. Whereas according to some reports its regular uptake in high doses can cause constipation. Therefore, the safety and potential indications of *T. cordifolia* in human beings need to be established.

Glycyrrhiza glabra (Licorice)

History/Traditional Use

Glycyrrhiza glabra (Fabaceae), also, known as the grandfather of herbs has been used medicinally in parts of both Western and Eastern herbal traditions since over 500 BC. It has been cultivated in Europe since 16th century. Traditional uses of *G. glabra* includes the treatment of peptic ulcers, asthma, pharyngitis, malaria, abdominal pain, and infections. The traditional medicinal properties of Glycyrrhiza include demulcent, expectorant, antitussive, and mild laxative activity.

Active Constituents

The primary active constituent of Glycyrrhiza related to hepatic disorders is Glycyrrhizin (glycyrrhizic acid or glycyrrhetinic acid). It is 50 times sweeter than sucrose and found in concentrations ranging from 6–14%. Other constituents of Glycyrrhiza include flavonoids (liquiritin and isoliquiritin), isoflavonoids (isoflavonol, kumatakenin, licoricone, and glabrol), chalcones, coumarins (umbelliferone, herniarin), triterpenoids, and phytosterols.

Mechanisms of Action

The possible mechanism by which *G. glabra* affords hepatoprotection is shown in Figure 36.8 (Anonymous, 2005; Wan et al., 2009).

Clinical Indications

Viral Hepatitis

In a double-blind study against viral hepatitis, intravenous administration of glycyrrhizin in physiologic saline along with cysteine and glycine [stronger neo minophagen-C, (SNMC)], stimulated endogenous interferon production in addition to strong antioxidant and detoxifying effects. SNMC treatment for 12 weeks resulted in an impressive 72.2 percent survival rate in patients with

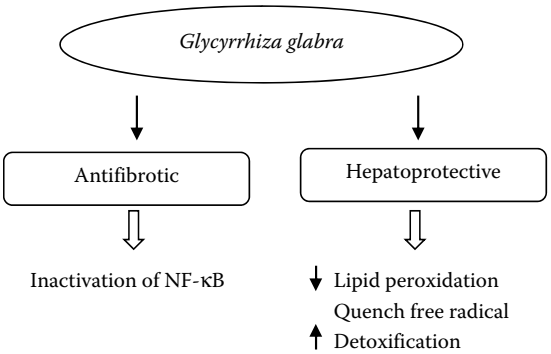

FIGURE 36.8 Mechanism of action of *Glycyrrhiza glabra*.

subacute hepatic failure due to viral hepatitis, compared to a survival rate of 31.1 percent in patients who received standard supportive therapy ($P < 0.01$) (Acharya et al., 1993). Most of the clinical trials of glycyrrhizin as a treatment for chronic viral hepatitis B and C were performed with SNMC. In some studies, glycyrrhizin was administered with interferon alpha or ursodeoxycholic acid, while in others glycyrrhizin monotherapy was carried out (Luper, 1999). Overall, significant differences between treatments were only observed with regard to biochemical responses while effects on viral infection were negligible. The only two European trials from a single group of researchers demonstrated a reduction in ALT levels during glycyrrhizin treatment which disappeared after the cessation of therapy. Various *in vitro* studies with Glycyrrhiza have shown its antiviral activity towards a number of viruses including hepatitis A, Varicella zoster, human immunodeficiency virus, herpes simplex type 1, Newcastle disease, and vesicular stomatitis viruses (Coon and Ernst, 2004). In a retrospective study of 193 patients positive for HCV antibodies demonstrated a significant 2.5-fold reduction of the relative risk of the evolution of HCC in a group of patients with chronic hepatitis C ($n = 84$) treated with glycyrrhizin compared with a control group that did not receive the infusion (Arase et al., 1997). But the mechanisms by which Glycyrrhiza treatment lead to low tumor incidence are unclear, and uneven randomization cannot be excluded.

Subacute Liver Failure

In an open-label trial including 56 patients with subacute liver failure, daily treatement with 100 mL glycyrrhizin for 30 days followed by an 8-week treatment every other day, showed a better survival in glycyrrhizin-treated group as compared to historical controls (Acharya et al., 1993). However, the design of this study does not allow any clear conclusions with regard to the usefulness of glycyrrhizin in the treatment of advanced liver disease.

Dosage and Toxicity

Glycyrrhiza is well tolerated by most patients at normal doses (1–4 g/day crude herb) but reported to show well-known pseudoaldosterone effect when increased the dose. Use of Glycyrrhiza is also not advisable in patients with a history of hypertension, renal failure, or current use of cardiac glycosides (Kageyama et al., 1991).

KAMPO TREATMENT OF HCC

Kampo medicines "TJ" originated in ancient China and uniquely developed in Japan, are widely used by surgeons and oncologists in regular practice for the treatment of cancer and cancer-related symptoms from the early stage to the terminal care. Presently, 148 Kampo-formula are officially approved for use and are numbered consecutively from TJ-1 to TJ-148. shosaikoto (TJ-9), a combination of seven herbals is widely used for the purpose of liver protection. In a five-year follow-up study of liver cirrhosis, 260 patients were randomly divided into two groups, one treated with TJ-9 and the other without, and the onsets of the HCC and survival rate were evaluated. The results revealed that in the group with TJ-9, onset of HCC decreased and longevity improved especially in the group of liver cirrhosis by non-B viruses. Though the mechanism is not obvious, it is speculated that TJ-9 inhibited the formation of 8-hydroxy-2-deoxyguanosine (8-OHDG) adduct, a genetic risk for hepatocarcinogenesis (Oka et al., 2002; Shiota et al., 2002; Watanabe et al., 2001a). Both *in vitro* and *in vivo* studies have shown that TJ-9 can also exert antifibrotic activities (Inoue and Jackson, 1999; Shimizu et al., 1999); however, well-designed and larger trials addressing the efficacy of TJ-9 or its major active ingredients baicalin/baicalein in the inhibition of the progression of chronic viral hepatitis B and C, or of metabolic liver diseases are needed. Another herbal combinations, termed "supplemental medicines" or "Hozai," are suggested to have immunostimulatory properties by activating macrophages or stimulating cytokines. TJ-I08 (Ninjin-yomei-to), TJ-41 (Hochu-ekki-to),

and TJ-48 (Juzen-daiho-to) have been extensively used in Japan, showed *in vitro* cytostatic properties (Li t al., 1999; Cyong et al., 2000). Besides, TJ-108 may have antiviral properties, because it contains Shisandrae Fructus, the active component being Gomisin A. In a study of 37 patients with hepatitis C, it was found to reduce HCV-RNA levels in 21% of patients (Cyong et al., 2000). In the United States, the effectiveness of Shosaikoto, Hochuekkito, and Ninjinyoueito as well as glycirrhizin are listed as the effective treatment for chronic hepatitis

TRADITIONAL CHINESE MEDICINE AND HCC

Traditional Chinese medicine (TCM) has been practiced for over two millennia, with comprehensive records of Chinese medical theories as early as 221 BC. Chinese medicine comprises over 100,000 recorded treatments, roughly 80% being herbal mixtures. Most Chinese medicines comprise four to five different herbs with one to two major pharmacologically active compounds (King herb), while the remaining herbs playing a "helper function," such as reducing toxicity, promoting delivery to the target site, or working synergistically with the "King" (Rosenberg, 1997). TCM herbs have been extensively investigated in the laboratory and are known to have multiple pharmacological effects (Boik, 1996). A literature search in a Traditional Oriental Medicine Database identified a number of herbal mixtures (approximately 76) for chronic liver diseases (Chen and Chen, 1998). A recent meta-analysis confirmed the utility of Chinese herbs in controlling the viral-induced HCC and reducing the side effects of chemotherapy (Shu et al., 2005). Among these, *Plantago asiatica* extract is a highest potent hepatoprotective extract with the lowest toxicity. Aucubin, the active component of *P. asiatica* inhibits hepatitis B virus (HBV) replication *in vitro* and in animals (100 mg/kg daily for 1 month). Results of a clinical trial suggested that 10 mg/kg i.v. administration of *P. asiatica* for four weeks, led to a 10–40% decrease in serum HBV-DNA levels that returned to pretreatment values after stopping therapy (Chang, 1998).

A second combination of 10 herbs, termed "Herbal Medicine 861 (HM861)" which contains *Salvia miltiorrhiza* (sage), *Astragalus membranaceus* and *Spatolobus suberectus* as the "King herbs" was tested for antifibrotic activity in three controlled clinical trials encompassing 107 patients with chronic hepatitis B. None of the patients cleared HBsAg, but ALT levels fell into the normal range in 73% of patients, while spleen size, portal pressure, and serum procollagen peptide and laminin levels decreased in 53% cases. Six months posttreatment, liver biopsies showed a reduction in fibrosis, inflammatory infiltrates and quantitative decreases in tissue hydroxyproline (Baoen, 1999). Since the formulations do not satisfy high quality criteria, further well-designed trials are needed. *In vitro* studies using human stellate cells and *in vivo* studies using animal models of fibrosis (CCl_4 and albumin induced) showed that HM861 inhibited stellate cell activation by blocking cyclin/cyclin-dependent kinase activity in the cell cycle, and that fibrotic tissues were remodeled, with revascularization of liver sinusoids. Transforming growth factor β and collagen type I, III, and IV gene transcripts were reduced while matrix metalloproteinase I was increased, suggesting a reversal of early stages of cirrhosis through the correction of imbalance in the dynamics of synthesis and degradation of the extracellular matrix (Jia et al., 1996a,b; Wang et al., 1998). In a double-blind, placebo-controlled trial involving patients with chronic hepatitis C, treatment with the CH-I00 (a formulation of 19 different herbs) was associated with a significant reduction in ALT levels, although no treated person cleared the virus (Batey et al., 1998). The National Center for Complementary and Alternative Medicine (NCCAM) is currently supporting a study of a 10-herb combination, referred to as 3AR. The trial will assess safety and adverse events, as well as symptoms of fatigue, quality of life, liver function, and HCV-RNA levels in patients who do not qualify for standard therapy of hepatitis C. In conclusion, several Chinese herbal combinations may be useful for the treatment of chronic liver diseases, especially viral hepatitis; further rigorous testing of candidate TCM compounds should be done (1) as alternatives to standard treatment, (2) to augment conventional treatments, or (3) to ameliorate the side effects of current therapies.

AFRICAN TRADITIONAL MEDICINE AND HCC

Several dietary plants, spices, and common herbs are used in the treatment of liver diseases in traditional system of African medicine. About 89% of the rural African population use traditional medicines. A unique feature of African medicine is that spices and herbs are determined to be food or medicines on the basis of distinct preparatory practices. *Garcinia kola* seeds have shown antiviral and anti-inflammatory properties while extracts of Combretum can prevent chemical-induced damage to the liver and exhibit *in vitro* antiviral activity. For the treatment of liver diseases of unknown etiology, a herbal formulation of *Garcinia kola* and the leaves of *Combretum micranthum* are used. Teas containing Utazi (*Gongronema latifolia*), bitter leaf (*Vernonia amygdalina*), or Nimbima (*Cryptolepis sanguinolenta*) are also used throughout West Africa for the management of diabetes and other metabolic diseases associated with the liver (Seeff et al., 2001).

FOOD BIOACTIVES AND HCC

Dietary agents consist of a number of bioactive dietary components which have been found to possess antimutagenic and anticarcinogenic properties. Further, the anticarcinogenic effect of phytochemicals may result from the combined or synergistic actions of a mixture of bioactives found in the diet (Surh, 2003). The purpose of this section is to discuss the potential effect of food bioactives in the prevention and treatment of HCC. Figure 36.9 illustrates the chemical structures and source of some common dietary agents that could benefit HCC patients.

Curcumin

Curcumin derived from the root of the plant *Curcuma longa* Linn. (Zingiberaceae) is widely used as a spice and has been used for centuries in Ayurvedic medicine for the treatment of a variety of inflammatory conditions and other diseases. It has a wide range of pharmacological activities including anti-inflammatory, anticancer, antioxidant, wound healing and antimicrobial effects. The molecular basis of anticarcinogenic and chemopreventive effects of curcumin is attributed to its effect on several targets including transcription factors, growth regulators, adhesion molecules, apoptotic genes, angiogenesis regulators, and cellular signaling molecules (Maheshwari et al., 2006; Sa and Das, 2008). In C3H/HeN mice, treatment with curcumin in diet caused reduction in multiplicity (81%) and incidence (62%) of development of HCC induced by *N*-nitrosodiethylamine (DEN) with reduction in the levels of p21/WAF1, PCNA, and cdc2 proteins in the hepatic tissues of mice (Sa and Das, 2008). While administration of curcumin to rats by gavage effectively suppressed DEN-induced liver inflammation and hyperplasia with inhibition of DEN-mediated increased expression of oncogenic p21/WAF1, p53, PCNA, cyclin E, and p34(cdc2) proteins and NF-κB in liver tissues of rats (Sa and Das, 2008). Dietary administration of curcumin for six consecutive days to rats bearing the highly cachectic Yoshida AH-130 ascites hepatoma resulted in an important inhibition of tumor growth (Busquets et al., 2001) and significantly reduced the number of gamma glutamyl transpeptidase-positive foci induced by AFB1 (Soni et al., 1997). Treatment with curcumin and embelin prevented the DEN and phenobarbital (PB) induced decrease in hepatic glutathione antioxidant defense, decreased lipid peroxidation, minimized the histological alterations induced by DEN/PB, but showed toxic effects on the hematopoietic cells (Sreepriya and Bali, 2006). Recently, it has been shown that curcumin significantly decreases hypoxia-induced HIF-1α protein levels in HepG2 and vascular endothelial cells. Treatment with curcumin can also suppress the transcriptional activity of HIF-1 under hypoxia, leading to a decrease in the expression of VEGF, a major HIF-1 target angiogenic factor (Bae et al., 2006). Curcumin treatment can also reduce the tumor-induced overexpression of COX-2 and serum VEGF in HepG2 groups significantly, indicating that curcumin could inhibit tumor angiogenesis (Yoysungnoen et al., 2006). Curcumin prevented methylglyoxal (MG)-induced cell death and apoptotic biochemical changes

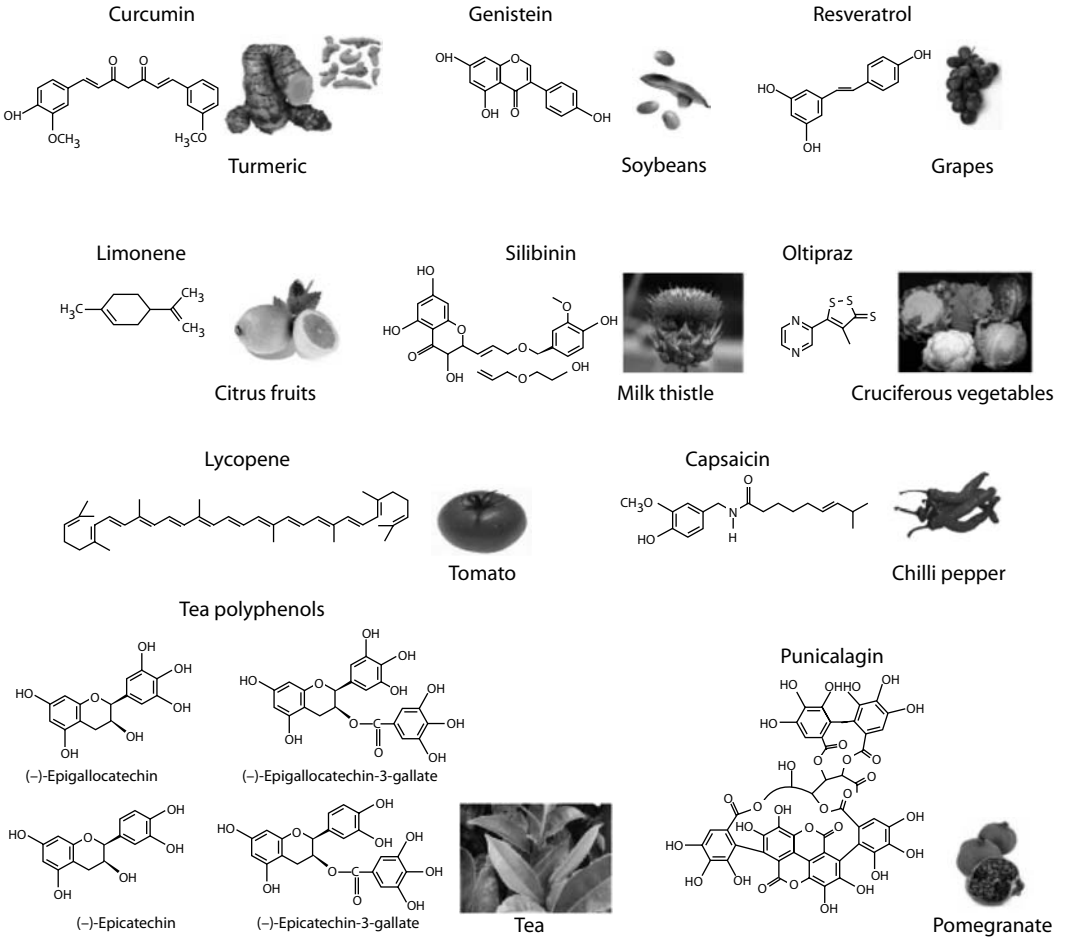

FIGURE 36.9 Food bioactives with their sources and structural formula that may have benefit HCC patients.

such as mitochondrial release of cytochrome *c*, caspase-3 activation, and cleavage of PARP with abolishing MG-stimulated intracellular oxidative stress (Chan et al., 2005). In highly invasive SK-Hep-1 cell line of HCC, curcumin inhibited cellular migration and invasion with its inhibitory action on MMP-9 secretion in a dose-dependent fashion (Lin et al., 1998) and suppressed the intrahepatic metastasis mediated by the inhibiton of several metastatic properties, in which the functional alteration of cytoskeletal organization could play an important role (Ohashi et al., 2003). Thus, curcumin appears to have the potential to be used as a chemopreventive as well as chemotherapeutic agent in HCC.

Tea Polyphenols

Tea, a popular beverage all over the world, is processed in different ways in different parts of the world to give green, black, or oolong tea and is a rich source of large and diverse class of compounds with antioxidant properties. Green tea, produced by drying fresh tea leaves contains characteristic polyphenolic compounds, (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), and (–)-epicatechin (EC) commonly known as catechins. Green tea and its polyphenols (GTP) have been shown safe and effective as chemopreventive agents in various *in vitro* bioassays and *in vivo* animal models for inhibition of carcinogen-induced mutagenesis

and tumorigenesis at several target organ sites, including aflatoxin (AFB1)-induced liver tumors (Lambert and Yang, 2003) and have other bioactive properties. Several mechanisms have been proposed for the anticarcinogenic effect of GTP, with the well accepted one that GTP can capture and detoxify reactive oxygen species (ROS) produced in the process of carcinogen metabolism, inflammation, and aerobic respiration. All four tea catechins, black tea extract and oolong tea extract significantly decreased DEN and phenobarbital-induced number and area of preneoplastic glutathione-*S*-transferase placental form-positive foci in the liver (Lambert and Yang, 2003). EGCG inhibited the growth and secretion of α-fetoprotein by human hepatoma-derived PLC/PRF/5 cells without decreasing their viability and was also shown to reduce the incidence and average number of hepatomas per mouse indicating the preventive potential of EGCG against human hepatoma (Nishida et al., 1994). Administration of green tea is shown to prevent incidences and multiplicities of DEN-induced hepatocellular tumors and also arrest the progression of cholangiocellular tumors in mice (Umemura et al., 2003). A dose-related decrease was observed in oxidative DNA damage and cell proliferation in the liver by treatment with green tea in drinking water (Sai et al., 1998). Tea polyphenols and tea pigments significantly decreased the number and area of GST-Pi-positive foci cyclin D1, cdk4, and induction of p21/WAF1 in liver of rats (Jia et al., 2002). In randomized, phase IIa chemoprevention trial involving 124 individuals (seropositive for both HBsAg and aflatoxin-albumin adducts in a HCC high-risk population) GTP capsules were given daily at doses of 500 mg, 1000 mg, or a placebo for three months. Analysis of urine samples collected from this trial for excretion of green tea polyphenols (GTP) and oxidative DNA damage biomarker, 8-hydroxydeoxyguanosine (8-OHdG), suggested that chemoprevention with GTP can effectively reduce 8-OHdG levels (Luo et al., 2006). Recently, EGCG was found to inhibit hepatic glucose-6-phophatase system mediated through an elevated luminal glucose level (Csala et al., 2007). In summary, there is a considerable body of evidence to suggest that tea polyphenols may be beneficial in both prevention and treatment of HCC, justifying their further clinical trials.

Genistein

Genistein (4,5,7-trihydroxyisoflavone), a soy-derived isoflavone have shown preventive and therapeutic effects for cancers, osteoporosis, and cardiovascular diseases in animals and humans. It is a potent protein tyrosine kinase inhibitor that attenuates growth factor-stimulated and cytokine-stimulated proliferation of both normal and cancer cells (Polkowski and Mazurek, 2000). The role of genistein in the physiology and pathophysiology of liver has been studied in the last decade. More than a dozen reports regarding the effect of genistein on hepatic stellate cells have appeared. Genistein *in vitro* have been shown to have antifibrotic effects (Badria et al., 2005). It can cause inhibition of cell proliferation, induction of apoptosis, and activation of caspase-3 in DEN induced and phenobarbital promoted cancer-bearing rats and in human hepatoma cell lines (Gu et al., 2005; Chodon et al., 2007). The isoflavones caused tumor cell death by induction of apoptosis by activation of caspase-3, cleavage of PARP, downregulation of Bcl-2, and Bcl-xL expression. Genistein induced progressive and sustained accumulation of hepatoma cancer cells in the G2/M phase as a result of inhibition of cdc2 kinase activity (Su et al., 2003). In HepG2 cells, genistein was able to increase apo A-I secretion and transcriptional activity and inhibit EGF-induced EGF receptor degradation and tyrosine phosphorylation (Yang et al., 1996; Lamon–Fava, 2000).

Genistein have been shown to block cell cycle checkpoint through selective induction of cdk inhibitor p21/WAF1 in a p53-independent manner and abolishment of cdk2 phosphorylation (Park et al., 2001). It can also inhibit the growth of Bel 7402 HCC cells and cause G2/M cell cycle arrest leading to increased apoptosis. The expression of p125FAK is significantly lower in genistein-treated group as compared with the control group. Further, tumor growth in genistein-treated nude mice is significantly retarded in comparison to control mice and it also show less invasion of Bel 7402 cells into the renal parenchyma (Ye et al., 2001).

Resveratrol

Because of potential cancer chemopreventive properties resveratrol (3,5,4-trihydroxystilbene), a polyphenol found in peanuts, grapes, and red wine has gained considerable attention. Numerous biological activities have been ascribed to resveratrol, which may explain its anti-inflammatory, anticarcinogenic, or anticancer properties. Among its various actions, resveratrol has been shown to inhibit cellular survival signaling (Athar et al., 2007). There are a number of *in vitro* and *in vivo* studies showing that resveratrol possesses an ability to intervene in HCC. Administration of resveratrol alone or in combination with 5-FU show better tumor inhibition property in H22 bearing mice with decrease in expression of cyclin B1 and p34cdc2 protein (Yu et al., 2003; Wu et al., 2004). Administration of resveratrol to rats inoculated with a fast growing tumor, Yoshida AH-130 ascites hepatoma, has also shown a drammatic decrease in the tumor cell content (Carbo et al., 1999). In p53-positive HepG2 cells, resveratrol inhibit cell growth and induce apoptotic death by arresting cells in G1 phase and increasing in p21/WAF1 and Bax expression (Kuo et al., 2002). This apoptotic death is preceded by caspase activation, oligonucleosomal DNA fragmentation, and formation of apoptotic nuclei. Following DNA damage, resveratrol led to an activation of caspases-2, -3, -8, and -10 (Michels et al., 2006). It also prevented the binding of aryl hydrocarbon receptor (AHR) to promoter sequences that regulate CYP1A1 transcription and inhibit CYP1A1 expression in HepG2 cells (Ciolino et al., 1998). Resveratrol is known to significantly inhibit hypoxia-induced HIF-1α protein accumulation in cancer cells, without altering its mRNA level. In addition, resveratrol remarkably inhibits hypoxia-mediated ERK 1/2 and Akt activation, leading to downregulation of HIF-1α and transcriptional activation of VEGF (Zhang et al., 2005). Resveratrol treatment is known to cause induction of apoptosis as well as an increase in nuclear size and granularity in HL-60 and HepG2 cells (Stervbo et al., 2006). Furthermore, it can also modulate the NO/NOS system, by increasing iNOS and eNOS expression, NOS activity, and NO production. Inhibitions of NOS enzymes are associated with attenuation of its antiproliferative effect (Notas et al., 2006). All these results suggest a promising role for resveratrol as a chemotherapeutic agent for liver diseases.

Lycopene

Lycopene a carotenoid, is an acyclic isomer of β-carotene which is a highly unsaturated, straight chain hydrocarbon. Red fruits and vegetables like tomatoes, watermelons, pink grapefruit, apricots, and pink guavas are the most common sources of lycopene. Because of the presence of high number of conjugated double bonds in its structure, it exhibits higher singlet oxygen quenching ability compared with β-carotene or α-tocopherol (Arab and Steck, 2000). Antioxidative and free radical scavenging potential of lycopene has been suggested to prevent carcinogenesis and atherogenesis by protecting critical biomolecules including lipids, low-density lipoproteins (LDL), proteins, and DNA (Nakachi et al., 2000). Lycopene has show a remarkable 50% suppression in HCC in a five-year clinical study conducted in Japan in high-risk liver cancer patients. These high-risk patients included hepatitis C virus carriers, those with cirrhosis and/or those having a family history of liver cancer who consumed a daily combination of natural tomato extract providing 10 mg carotenes (30% α-carotene, 60% β-carotene) and 50 mg α-tocopherol as well as tocotrienols (Nishino, 2009). Feeding of rats with lycopene significantly decreased the size of gamma-glutamyl transpeptidase and glutathione-*S*-transferase-positive foci induced by DEN (by 64% and 65%, respectively), as well as the fraction of liver volume occupied by foci (84% and 79%, respectively), but did not significantly reduce their number (Astorg et al., 1997). However, long-term administration of lycopene or TJ-9 in Long-Evans Cinnamon (LEC) rats did not reduce the risk of HCC (Watanabe et al., 2001b). On the other hand, lutein and lycopene treatment in Wistar rats can lower the number of hepatic placental glutathione-*S*-transferase-positive preneoplastic lesions and DNA strand breakage (Toledo et al., 2003). Recently, the antimetastatic properties of

lycopene have been reported showing inhibition in the adhesion, invasion, and migration of SK-Hep1 human hepatoma cells through upregulation of nm23-H1, a metastasis suppressor gene (Huang et al., 2005; Huang and Lee, 2006).

Pomegranate

The Pomegranate (*Punica granatum* L.) is a delicious fruit used for centuries in ancient cultures for its medicinal purposes. Pomegranate has substances such as polyphenols that have antioxidant, antiviral, and antitumor activities. The most abundant of these polyphenols is punicalagin, responsible for 50% of the juice's potent antioxidant activity (Seeram et al., 2005). Various *in vitro* and *in vivo* studies have shown the anticancer properties of pomegranates against HCC. Delphinidin (an anthocyanidin found in pomegranate) is shown to induce apoptotic cell death, characterized by internucleosomal DNA fragmentation, induction of caspase-3, c-Jun and JNK phosphorylation, upregulation of Bax and downregulation of Bcl-2 protein in human hepatoma HepG2 cells (Yeh and Yen, 2005). In Fisher 344 male rats pomegaranate juice has been shown to inhibit azoxymethane-induced aberrant crypt foci with higher activity of total glutathione-*S*-transferase (GST) when compared to control groups (Boateng et al., 2006). Pretreatment with pomegranate flower extract show protection against ferric nitrilotriacetate (Fe-NTA) and carbon tetrachloride-induced oxidative stress as well as hepatic injury. The extract also accorded protection against hepatic lipid peroxidation and preserved glutathione (GSH) levels and activities of antioxidant enzymes as well as normalized liver functions (Chidambara Murthy et al., 2002; Kaur et al., 2006). From the above results it can be speculated that pomegranate has potential as preventive and therapeutic agent against HCC, but further clinical trials are needed to establish its role.

Oltipraz

Oltipraz, a synthetic derivative of dithiolthiones is found in cruciferous vegetables has been demonstrated as a potent inducer of phase II enzymes involved in the detoxification of several carcinogens including aflatoxin. Dietary administration of oltipraz has been shown to reduce AFB1-DNA adduct formation and the burden of serum aflatoxin–albumin adducts and urinary aflatoxin-N(7)-guanine following AFB1 exposure (Bammler et al., 2000). It also inhibited the development of both preneoplastic foci and hepatocellular neoplasms in rats (Maxuitenko et al., 1993). This evidence supports a promising preventative role for oltipraz in populations at high risk of HCC as a result of exposure to AFB1-contaminated foods. Based on these results, a phase II chemoprevention trial was initiated in the Qidong Province of China, an area at high risk of HCC owing to dietary aflatoxin exposure (Jacobson et al., 1997). This trial demonstrated intermittent high-dose oltipraz (500 mg once per week) to inhibit phase I activation of aflatoxins and sustained low-dose oltipraz (125 mg daily) to increase phase II conjugation of aflatoxin (Wang et al., 1999). In another clinical trial, a single dose of oltipraz markedly reduced CYP1A2 activity, an enzyme involved in carcinogen activation (Sofowora et al., 2001). Oltipraz has also been shown to have a dose-dependent inhibitory effect on hepatitis B virus replication, a significant aetiological factor for HCC (Chi et al., 1998).

Limonene

D-Limonene present in citrus fruits, is well tolerated in patients with advanced cancer (Vigushin et al., 1998). It is reported to have potential role in chemoprevention of HCC, as it reduces the number of GST-P-positive preneoplastic hepatic foci, the number of neoplastic nodules and HCC burden in a rat model of hepatocarcinogenesis (Kaji et al., 2001). It also inhibits murine DEN-induced hepatocarcinogenesis (Giri et al., 1999) and increases apoptosis in the neoplastic nodules and HCC (Kaji et al., 2001).

Capsaicin

Capsaicin (trans-8-methyl-*N*-vanillyl-6-non-enamide), a phenolic compound is present in black and chilli peppers and is responsible for their fiery taste. Recent evidence suggest that capsaicin may have chemopreventive and chemotherapeutic properties in HCC. In a rat model of diethylnitrosamine-induced hepatocarcinogenesis, capsaicin treatment significantly inhibited the formation of preneoplastic foci (Jang et al., 1991). It also inhibited proliferation and induced apoptosis of HCC cell lines in a dose-dependent manner (Kim et al., 2005). Further, HCC cells have been found to be more susceptible to capsaicin-induced cytotoxicity than normal hepatocytes, making its potential chemotherapeutic role more promising (Galati and O'Brien, 2003).

ADVERSE HEPATIC REACTIONS FROM HERBAL DRUGS

Several herbals have been identified as a cause of acute and chronic hepatitis, cholestasis, drug-induced autoimmunity, vascular lesions, and even hepatic failure. Recently an update of herbals as a cause of adverse hepatic reactions has been published (Stickel et al., 2005). Herbal preparations that have been reported to cause hepatotoxicity are shown in Table 36.2.

CONCLUSIONS AND FUTURE DIRECTIONS

HCC is considered silent killer disease that accounts for nearly one million deaths globally per annum. It is known to affect men more often than women (Jemal et al., 2005). Currently, there is no approved chemotherapy for HCC. Treatments for this disease include surgical resection, transcatheter arterial embolization, chemoembolization, and systemic chemotherapy. Liver transplantation may be considered as one option but again the recurrence of tumor or metastasis has limited its usefulness. The failure of conventional chemotherapy to effect major reduction in the mortality indicates the critical need for novel therapeutic approaches. The use of CAM against cancer is widespread. Conventional medical treatments have come a long way in recent years. There is also a common belief that herbs can do wonders. The benefit of herbal formula is that it can nourish the

TABLE 36.2
Herbs and Hepatotoxictiy

Herb/Plant Product	Types of Injuries
Jin Bu Huan (*Lypocodium serratum*)	Acute and chronic hepatitis, focal necrosis, portal fibrosis, microvesicular steatosis
Ma-Huang (*Ephedra* sp.)	Acute hepatitis
Qialbai Biyan Pian	Liver failure
Traditional Chinese herbal mixtures	Acute hepatitis, fulminant hepatic failure
Pyrrolizidine alkaloids	Veno-occlusive disease
Teucrium chamaedrys (Germander)	Acute, chronic and fulminant hepatitis, cirrhosis, liver failure
Chelidonium majus (Greater celandine)	Hepatotoxicity
Piper methysticum rhizome(Kava)	Hepatic necrosis, cholestatic hepatitis
Larrea tridentate (Chaparral)	Acute hepatocellular injury
Kombucha mushroom (tea)	Chronic hepatitis
Mistletoe	Chronic hepatitis
Margosa oil	Microvesicular steatosis
Valerian root and skullca	Hepatitis

whole body by supporting the vital organs and nourishing the liver and its function which makes this approach especially attractive (Mishra, 2004).

Safety of the potential agent is another major concern. Such agents should have minimal side effects, specific toxicity toward malignant cells, inexpensive, acceptable to patients, and sufficient bioavailable. Various herbal medicines cause adverse effects including hepatic damage, therefore, further rigorous scientific research should be done to develop agent delivery system which can result in safe pharmacological effects of phytochemicals. Since patient selection poses a serious problem, it would be more sensible to target chemopreventive agents to patients at high risk of developing HCC. In addition, patients undergoing hepatic resection should be identified for neo-adjunctive and adjunctive treatment including these agents. The levels of some specific molecular biomarkers monitored determine the outcome of such treatments.

In summary, recent research suggest that several herbs and phytochemicals have shown promise as candidates for prevention and therapy of HCC, but more scientific evidences and well-designed clinical trials are needed to include them for the routine treatment of any chronic liver disease. A clear understanding of the underlying molecular mechanisms of action of the phytochemicals will provide impetus for future development in basic and applied medicine in HCC treatment and, more importantly, it will also increase our understanding of the potential benefits of these compounds against liver disorders as they are part of our daily diet.

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37 New Zealand Christmas Tree
Historic Uses and Cancer Prevention

Felina Marie Cordova and Ronald Ross Watson

CONTENTS

INTRODUCTION

The *Metrosideros excelsa* naturally grows in New Zealand. It produces red flowers and contains anthocyanins and flavonoids, which as antioxidants may have a positive impact on human diseases and health such as in cancer prevention and treatment. In addition, it has had a prominent role in the history of New Zealand and continues to be a symbol for the culture of the country.

HABITAT AND DESCRIPTION

The New Zealand Christmas tree is also known by the Maori (Native New Zealander) name "Pohutukawa" or its scientific name *M. excelsa*. This tree is a species in the family Myrtaceae (3000 species in this family), genus Mertrosideros which has 50 species in this genus (Mustafa et al., 2004; Simpson, 1994). This genus is found in various parts all over the world such as in New Zealand, Australia, the southernmost part of Africa, and a few islands such as Hawaii, New Calcedonia, and Bonin, growing along coastlines in these locations (Andersen, 1988; Percy et al., 2008; Wright et al., 2000).

The *M. excelsa* species is an evergreen found exclusively in Northern New Zealand, is native to this area and can grow to be 25 feet tall and live to be 1000 years old (Schmidt-Adam et al., 2000; Kubien et al., 2007; Simpson, 1994). This tree likes temperate climates and rarely naturally grows below 38 degrees latitude (Simpson, 1994). Hoputukawa is a hardy species and can flourish on lava rocks as well as in areas along rocky coastlines. Its leaves are made of a waxy material to prevent moisture from escaping (Simpson, 1994). Phenotypically the New Zealand Christmas tree is a very colorful tree giving rise to its name by having flowers that are bright red in color and bark of a deep red to brown color (Simpson, 1994). The roots of the tree run the spectrum from light colors (white) to deeper reds in the adventitious roots (Simpson, 1994; Solangaarachchi and Gould, 2001). *M. excelsa* flowers are produced between November and January during a 14-day span of time and everyday excreting 46 μL of nectar (Schmidt-Adam et al., 1999). This plant can self-pollinate but can also fertilize with the aid of nonnative birds, bees as well as lizards, and local bats (Schmidt-Adam et al., 2000).

The New Zealand Christmas tree can also produce plants by crossbreeding with the Northern Rata to create a hybrid (Simpson, 1994).

HISTORY OF THE TREE IN NEW ZEALAND

M. excelsa is a tree that is very significant to the history and people of New Zealand. Native New Zealanders (Maori) hold this tree in very high regard due to its significance in their culture. The word "Rakaurangatira" is what the Maoris call these trees, signifing a very special tree (Simpson, 1994). One of the reasons for the significance to the Maori was that this species was seen when they would leave and approach the island (Simpson, 1994). Additionally, the New Zealand Christmas Tree's Maori name of "Pohutukawa" translates closely to "head dress of red feathers" as the flowers according to legend were mistaken for feathers by a Maori chief (Simpson, 1994). The flowers have also served a religious purpose by linking the tree's red color (in the flowers) to the blood of a mythical Maoris death (Simpson, 1994). This myth provides the Maoris the religious link of their people being able to transition to the sky as the mythical character fell to his death after reaching the sky trying to find the ancestors (Simpson, 1994).

The Pohutukawa population has dwindled down to between 10% and 5% of their population prior to European colonization (Simpson, 1994; Schmidt-Adam et al., 1999). The population of trees has been declining since the 1800s due to settlers clearing the land for farming as well as using the tree in goods such as making sea vessels (Simpson, 1994). Modern deforestation and clearing of land for tourism has also led to the decrease of this species (Simpson, 1994). Contributing to the deforestation are also animals that are not endemic to New Zealand such as possums that feed on the leaves, suggesting safety of the plant materials (Schmidt-Adam et al., 1999). Due to the low percentage of plants left, there have been conservation efforts to halt the disappearance to this tree such as "Project Crimson" in New Zealand which raises awareness on the disappearance of this tree (Simpson, 1994). Adding to the numbers of Pohutukawa is the planting of trees further inland as part of landscaping (Simpson, 1994).

CONSTITUENTS AND HEALTH PROPERTIES

The phytonutrient, anthocyanin has been found in *M. excelsa*'s flowers and roots. In the tree's flowers are found malvidin-3-glucoside, dolphin-3-glucoside, petudin-3,5-diglucoside, delphnidin-3,5-diglucoside and malvidin-3,5-diglucoside (Andersen, 1988). High anthocyanin concentrations accumulate in areas of the plant that are exposed to sunlight, presumably to protect the plant from UV rays (Solangaarachchi and Gould, 2001). Anthocyanins are antioxidants (Solangaarachchi and Gould, 2001) with benefits seen in cancer inhibition. *In vitro* and in *vivo* animal research produce apoptosis of cancer cells, inhibit inflammation pathways via the suppression of COX-2 as well as inhibit cancer induction by being a reactive oxygen species scavenger (Wang and Stoner, 2008). Anthocyanins from any source have also shown positive effects on circulation problems and cholesterol accumulation that can produce atherosclerosis (Wang et al., 1997).

In addition, the pollen from the flowers of this plant contains tricetin and luteolin, both of which are flavonoid agycones (Campos et al., 2002). Through high performance liquid chromatography, flavonoids have also been found in the seeds of *M. excelsa* (Mustafa et al., 2004). Like anthocyanin, these flavonoids have high antioxidant properties. The flavonoid, luteolin may also be effective in cancer prevention and treatment as it can inhibit DNA Topoisomerase I, which is important to the DNA replication process (Chowdhury et al., 2002). Finally, this antioxidant induces apoptosis, as shown by Horinaka et al. (2005) in a study involving human tumor cells by increasing the activity of death receptor 5.

Chalcones and alcohols have also been found in the seeds of this plant (Mustafa et al., 2004). The level of concentration of chalcones was found to increase according to age, with the newest outgrowth having the highest levels (Mustafa et al., 2004). Chalcones like anthocyanins and flavonoids

may also play a part in being able to inhibit and control cancerous growth. The p53 tumor suppression gene activity has been found to be blocked by the binding of the Murine double Minute 2 gene (MDM2) whose human analog is HDM2 (Klein and Vassilev, 2004). Chalcones work to inhibit tumor creation by allowing the p53 tumor suppressor gene not to be inhibited by the MDM2 gene, therefore increasing the activity of p53 (Stroll et al., 2001). Lastly, *M. excelsa* produces sucrose via the nectar from the Hoputukawa flower (Schmidt-Adam et al., 1999).

CONCLUSION

The Hoputukawa links the natives of New Zealand to their religion and culture due to the tree's visual attributes as well as location along the coast. It contains numerous antioxidants such as anthocyanins, flavonoids, and chalcones which have been shown to produce positive effects on cancer. The New Zealand Christmas tree not only is important for cultural reasons but may also provide scientific research with a new resource.

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38 Nutrition and Colorectal Cancer

Mitra Rangarajan and Gerard E. Mullin

CONTENTS

INTRODUCTION

Colorectal cancer affects both men and women of all race and ethnicity. Most often it is found in people aged 50 years or more. For men, colorectal cancer is the third most common cancer. For women, colorectal cancer is the second most common cancer among Asian/Pacific Islander and Hispanic women. For Caucasian, African American and American Indian/Alaska Native women colon cancer is the third most common cancer. In the United States, death from colorectal cancer ranks number two. The National Cancer Institute estimates the incidence of colon and rectal cancer to be approximately 106,100 (colon); 40,870 (rectal) for 2009. The combined death from colon and rectal cancer is estimated to be 49,920 (http://www.cancer.gov/cancertopics/types/colonandrectal).

Despite improvements made in providing care for colon cancer patients, prevention may be the best way to avoid cancer therapy altogether. Screening for colon cancer has shown to be very effective; however, dietary modification or low-risk chemopreventive agents might prevent the development of colon cancer. Time and again, epidemiologic studies have shown a direct link between nutrition and colon cancer risk (Marshall, 2009). Prevention of colon cancer remains an important goal in the reducing the burden of colon cancer. While colonoscopy has remained the most promising means of detecting colon cancer, recent data suggests that approximately 30% of adenomatous polyps, especially those in the proximal colon are missed by screening. This leaves for improvement, particularly chemoprevention, using sound nutrition (Winawer et al., 1993).

COLON CANCER AND NUTRITIONAL EPIDEMIOLOGY

In recent years, there has been significant reference made to physical activity, exposure to electromagnetic fields, radiation, the use of various medications and fertility to be important in the development of cancer; however, nutrition continues to dominate this debate. Nutrients that we ingest comes into direct contact with the intestinal lumen and affect the bowel transit and stool characteristics (Wynder and Reddy, 1974). Interest in nutrition preceded population-based research. Wydner and Reddy showed that fecal bile acid composition differed greatly based on the individual's diet. Some of the earlier population-based research focused on particular food items. Graham et al. (1978) constructed indices for meat, cruciferous vegetables and total vegetables. They noted that increased intake of cruciferous vegetables consistently reduced the risk of cancer. In a later epidemiologic study that was more elaborate than previous studies, They failed to show a protective association between intake of cruciferous vegetables and colorectal cancer risk. There has a been a variety of studies in the literature that show varied association to food intake and colon cancer. The Nurses' Health Study identified red meat consumption to be a significant colon cancer risk (Willet, 1990).

One of the main challenges of nutritional epidemiology of cancer has been the importance of discovering biologic markers that can objectively indicate exposure to carcinogens. One such marker has been the isothiocynate (Moy, 2008) which are rapidly metabolized and excreted in the urine. Therefore, urinary levels of isothiocyanate can reflect recent intake. The limitation to measuring this marker is that, isothiocyanate has a short reference period. Hence it cannot be used as a marker for long-term diet. Moy et al. (2008) showed varying levels of risk of colon cancer that was associated with urinary isothiocyanate level. On the contrary, Ward et al. (2008) found no evidence that urinary markers of phytoestrogens were negatively associated with colon cancer risk. The European Prospective Investigation of Cancer and nutrition (EPIC) study was a large prospective study carried out in 10 different European countries to investigate the relation between food habits, nutritional status, different lifestyle and environmental factors, and the incidence of different forms of cancer (Margetts, 2009). The author concluded that higher consumption of fruits and vegetables may protect against the development of colorectal cancer, especially colon cancer. This association was found to be strong among subjects who never smoked or were former smokers. In this same study, the author showed a positive association between heterocyclic aromatic amines released from cooking meat and fish at high temperature, showing a positive association to colorectal adenoma risk.

There has been some debate as to whether there are significant disparities between colon cancer incidence and mortality rates between African Americans and Whites. In a recent study by Vinikoor et al. the authors concluded that consumption of trans fatty acid was not associated with colon cancer and did not contribute to the disparities in colon cancer rates between these two racial groups (Vinikoor et al., 2009). Hu et al. (2008) studied the relationship of meat and fish consumption and cancer (see Table 38.1). The authors of this study concluded that total meat and processed meat intake were directly related to the risk of cancers of colon, stomach, rectum, pancreas, lung, breast, prostate, testis, kidney, bladder, and leukemia. Red meat in particular was associated with colon, lung, and bladder cancer. They did not report excess risk from the consumption of fish and poultry. Fish and poultry were inversely related to the risk of cancer at different sites. It was therefore concluded that red meat played an unfavorable role toward the risk of cancer vs. fish and poultry (Hu et al., 2008).

In a population-based case-control association study, Kury et al. (2007) investigated possible association between colorectal cancers and environmental and genetic factors. In their study, they report that separate analysis of the single nucleotide polymorphosim did not show any effect on colorectal cancer. However, three allelic variant combinations with increased red meat intake were found to be associated with increased colorectal cancer risk. This is illustrated in Figure 38.1.

TABLE 38.1
Mean Intake of Total Meat, Processed Meat, Red Meat, Poultry, and Fish by Types of Cancer, National Enhanced Cancer Surveillance System of Canada, 1994–1997

			Mean (SD) for Food Groups (Servings per Week)					
Types of Cancer	Cases (*N*)	Total Meat[a]	Red Meat[b]	Processed Meat[c]	Poultry[d]	Total Fish[e]	Fresh Fish[f]	Smoked Fish[g]
Controls	5039	8.6 (8.7)	4.3 (4.0)	4.1 (6.2)	1.9 (2.1)	1.4 (2.4)	2.0 (0.6)	1.3 (0.5)
Stomach	1182	10.4 (9.1)	4.6 (3.9)	5.5 (7.0)	1.8 (1.7)	1.5 (2.2)	2.1 (0.6)	1.3 (0.5)
Colon	1727	9.6 (7.6)	4.8 (3.7)	4.5 (5.5)	1.7 (1.5)	1.5 (1.8)	2.1 (0.6)	1.3 (0.5)
Rectum	1447	9.5 (8.4)	4.8 (4.9)	4.5 (5.4)	1.7 (0.9)	1.5 (2.0)	2.1 (0.6)	1.3 (0.5)
Pancreas	628	9.9 (9.2)	4.6 (4.3)	5.0 (7.0)	1.8 (1.4)	1.4 (1.8)	2.1 (0.5)	1.2 (0.5)
Lung	3341	10.1 (9.1)	4.6 (3.9)	5.1 (7.0)	1.5 (1.6)	1.3 (2.4)	2.0 (0.6)	1.3 (0.5)
Breast (women)	2362	7.6 (6.5)	4.3 (4.4)	3.1 (3.8)	1.9 (1.9)	1.4 (1.9)	2.1 (0.6)	1.1 (0.4)
Premenopausal women	913	8.0 (6.6)	4.4 (3.9)	3.4 (4.4)	2.0 (1.6)	1.3 (1.8)	2.0 (0.6)	1.2 (0.4)
Postmenopausal women	1449	7.3 (6.4)	4.2 (4.6)	2.9 (3.4)	1.9 (2.1)	1.5 (2.0)	2.1 (0.6)	1.2 (0.5)
Ovarian	442	7.9 (6.2)	4.1 (3.4)	3.3 (4.0)	2.0 (2.3)	1.4 (2.2)	2.1 (0.6)	1.2 (0.5)
Prostate	1799	9.4 (10.9)	4.3 (4.6)	4.8 (6.9)	1.7 (1.7)	1.5 (2.7)	2.1 (0.6)	1.3 (0.5)
Testics	686	13.0 (12.5)	5.1 (4.7)	7.8 (9.6)	1.9 (2.0)	1.0 (1.1)	1.9 (0.6)	1.3 (0.5)
Kidney	1345	9.7 (12.0)	4.7 (4.8)	4.7 (7.7)	1.9 (2.2)	1.3 (3.0)	2.0 (0.6)	1.2 (0.5)
Bladder	1029	9.8 (8.4)	4.7 (3.6)	4.9 (6.5)	1.6 (1.5)	1.3 (1.9)	2.0 (0.6)	1.3 (0.5)
Brain	1009	9.9 (8.5)	4.6 (3.5)	5.1 (6.8)	1.9 (1.6)	1.2 (1.4)	2.0 (0.6)	1.2 (0.5)
Non-Hodgkin's lymphoma	1666	9.4 (9.4)	4.7 (5.2)	4.5 (6.3)	1.8 (1.6)	1.3 (2.4)	2.0 (0.6)	1.2 (0.5)
Leukemia	1069	9.5 (7.4)	4.5 (3.5)	4.8 (5.7)	1.7 (1.7)	1.1 (1.4)	2.0 (0.6)	1.2 (0.4)

Source: Adapted from Hu et al. *Nutrition and Cancer* 2008; 60(3):313–24.

[a] Beef, pork, lamb, hamburger, hotdogs, bacon, sausage, smoked meat or corned beef, luncheon meats, and liver.

[b] Beef, pork or lam as a main dish, beef and pork or lam as a mixed dish, and hamburger.

[c] Hotdogs, lunch meat, smoked meat or corned beef, bacon, and sausage.

[d] Chicken or turkey.

[e] Fresh, frozen, or canned fish or smoked, salted, or dried fish.

[f] Fresh, frozen, or canned fish.

[g] Smoked, salted, or dried fish.

NUTRITION INTERVENTION CLINICAL TRIALS

Nutritional intervention studies done in the early 1980s focused on adenomatous polyp as the study outcome. The reason for this was that interventional studies based on colon cancer had to be large and lengthy. Furthermore, since it was believed that colon cancer arose from adenomatous polyp it was a logical outcome measure on these interventional studies. Nutrition plays a major role in the development of colorectal cancer as shown in observational studies.

Pot et al. (2009) investigated the effects of six-month intervention with oil-rich or lean fish on apoptosis and mitosis within the colonic crypt. In this multicenter randomized, controlled interventional trial, patients with colorectal polyp, inactive ulcerative colitis, or no macroscopic sign of disease were recruited and randomly allocated to received dietary advice plus either 300 g oil-rich fish (salmon) or 300 g lean fish (cod) per week or dietary advice only.

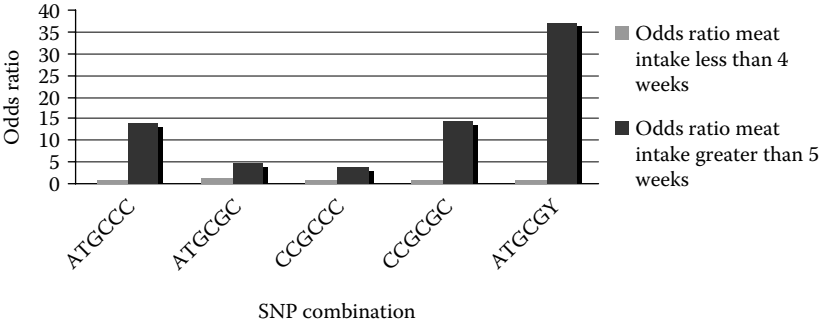

FIGURE 38.1 Analysis of association to colorectal cancer risk and multiple single nucleotide polypeptide (SNP) combination. Allelic variants composing the combinations of six SNPs are ordered as follows: first CYP1A2 c._163A > C, second CYP1A2 c.1548T > C, third CYP2E1g._1293G > C, fourth CYP2E1 g._1053C > T, fifth CYP1B1 c.1294C > G, and sixth CYP2C9 c.430C > T. Only the five more frequent combinations (>4%) are represented. (a) Cross-classification using a common reference category for both interacting variables. The group of moderate red meat consumers (V4 times/wk) exhibiting the most frequent combination of SNPs is selected as the reference category. ORs are estimated, together with 95%CI, for all other combinations distributed between the groups of moderate and great (z5 times/wk) red meat consumers, according to a log-additive model. Significant associations to modification of CRC risk are shown in bold characters. (b) The influence of SNP combination on modification of CRC risk is estimated by ORs calculation within each group of moderate and great red meat consumers. (c) Red meat consumption nested within SNP combinations: The influence of the frequency of red meat consumption is estimated by OR calculation within each combination of SNPs. (Adapted from Kury S, et al. *Cancer Epidemiology, Biomarkers & Prevention* 2007; (16):1460–1467.)

The authors measured apoptosis and mitosis in colonic biopsy samples collected before and after intervention. The authors concluded that an increase in the consumption of either oil-rich or lean fish to two portions weekly over a 6-month period did not markedly change apoptotic and mitotic rates in the colonic mucosa. This was the first randomized control trial to have studied the effects of fish consumption on markers of colorectal cancer risk. The authors attribute their result partly to the fact that the subjects did not consume the salmon or cod in addition to their habitual fish consumption, as requested, but instead substituted the fish they would normally consume with the study fish. Therefore, the intended increase of two additional portions of fish per week actually only resulted in an increase of 1.4 and 1.3 extra portions per week of salmon and cod, respectively. As a result the consumption of fish increased by 0.99 g and 0.05 g of n-3 VLC-PUFAs/day for salmon and cod, respectively. Therefore, the contrast in their study between the intervention groups may not have been large enough to observe a beneficial effect of additional fish consumption. Additionally, the authors feel that an increase in 1.4 portions per week may be the maximally achievable dietary modification in a population of fish eaters.

Soy Isoflavones

Soy isoflavones have many biological properties suggestive of protection against colorectal cancer. Adams et al. (2005) tested the hypothesis that supplementation with soy protein-containing isoflavones decreases colorectal epithelial cell proliferation. This was a 12-month randomized controlled trial. The subjects included both men and women between the ages 50 and 80 with recently diagnosed adenomatous polyps. Total participants enrolled were 150. The participants were randomly assigned to the intervention group, which received 58 g of protein powder per day containing 83 mg of isoflavones per day and the control group which received ethanol extracted soy protein powder containing 3 mg isoflavones. Biopsy specimens were collected at the beginning of the study and at 12-month follow-up. One hundred and twenty-five subjects completed the study.

In the sigmoid colon, they noted cell proliferation by 0.9 labeled nuclei per crypt more in the +ISO group than in the –ISO group over the 12-month intervention which was quite the opposite of what the authors expected. The number of labeled nuclei per 100 μm crypt height also increased in the +ISO than in the –ISO group. In the cecum and sigmoid colon, but not in the rectum, the proliferation count increased as the serum genistein concentration increased. Proliferation distribution and crypt height were not changed by treatment at any site. The authors therefore concluded that supplementation with soy protein-containing isoflavones does not reduce colorectal epithelial cell proliferation or the average height of proliferating cells in the cecum, sigmoid colon, and rectum, and increases cell proliferation measures in the sigmoid colon (Adams et al., 2005).

Nonsteroidal Anti-Inflammatory Drugs

NSAIDs, cyclooxygenase-2 (COX-2) inhibitors, and aspirin have all been studied in prevention of colon cancer. Strong support for a chemopreventive effect of NSAIDs is provided by *in vitro* experiments, animal models, epidemiological studies, and clinical trials (Dubois et al., 1996). The mechanism of action of NSAIDs is inhibition of cyclooxygenase, which is a key enzyme in conversion of arachidonic acid to prostaglandins, prostacyclin, and thromboxanes. Prostaglandins affect cell proliferation and tumor growth through activation of second messengers in signal transduction pathways, and prostaglandin levels are increased in many cancers including colorectal adenomas and adenocarcinomas. COX-1 is present in most tissues, whereas COX-2 is expressed in response to growth factors, cytokines, mitogens, and tumor promoters. Much of the initial data on NSAIDs and chemoprevention of colon cancer in humans came from literature on familial adenomatous polyposis (FAP), which is an autosomal dominant disorder characterized by innumerable colorectal adenomas and eventual carcinoma. Multiple studies have evaluated sulindac (Clinoril®) in FAP, and all report either complete or partial regression of adenomas after three to six months of treatment with sulindac at doses of 300 to 400 mg per day (Dubois et al., 1996).

NSAIDs such as sulindac have gastrointestinal toxicity that may limit its use for prevention in colorectal cancer. This led to studies on COX-2 inhibitors. COX-2 is expressed in inflammatory states, premalignant lesions, and colorectal cancer. COX-2 inhibitors were speculated to have a role in chemoprevention of colon cancer. Celecoxib (Celebrex®) led to polyp regression in FAP and this led to investigation of NSAIDs in sporadic adenomas and colorectal neoplasia (Steinbach et al., 2000). Epidemiologic studies have shown a reduction in colorectal cancer in individuals taking NSAIDs (Dubois et al., 1996; Steinbach et al., 2000; Keller and Giardiello, 2003). Celecoxib led to polyp regression in FAP led to investigation of NSAIDs in sporadic adenomas and colorectal neoplasia (Dubois et al. 1996). Epidemiologic studies have shown a reduction in colorectal cancer in individuals taking NSAIDs (Dubois et al. 1996). A series of case-control studies revealed a 40 to 50% reduction in the risk of colonic adenomas or colorectal cancer among patients taking aspirin (Dubois et al., 1996). Giovannucci and associates measured cancer incidence in a cohort of women and found that regular aspirin use at doses similar to those recommended for cardiovascular disease prevention reduced risk of colorectal cancer, but this effect may require greater than a decade of use to occur (Steinbach et al., 2000). In a prospective cohort study involving 47,900 men in the United States, Giovannucci and associates (1992, 1998, 2002) showed that regular use of aspirin decreased the incidence of adenomas.

Calcium

Vitamins including calcium, vitamin D, folate, and selenium have also been implicated in chemoprevention of colon cancer. Dietary calcium has been hypothesized to neutralize fatty acids and free bile acids which have an irritative effect on the colonic epithelium. Numerous murine studies have shown that calcium decreases colonic mucosal hyperproliferation, decreases markers of colorectal mucosal proliferation and inhibits colonic tumor development. Though two early meta-analyses

showed little or no protection against colorectal cancer with calcium, numerous subsequent prospective cohort studies have reported modest decrease of colorectal cancer in high-intake calcium groups compared with low-intake groups (Martinez and Willett, 1998; Baron et al., 1999a; Baron et al., 1999b; Steinbach et al., 2000; Wu et al., 2002; McCullough et al., 2003; Flood et al., 2005) In their population-based prospective cohort of 61,433 women, Larsson et al. (2005) showed that increased intake of magnesium was associated with a decreased incidence of colorectal cancer.

In a pooled analysis of 10 prospective studies looking at intake of dairy foods, calcium and colorectal cancer, Wei et al. (2008) concluded that increased consumption of milk and calcium were related to a lower risk of colorectal cancer. This data, along with other previous experimental studies that demonstrate a beneficial effect of calcium supplementation on colonic epithelial cell turnover and colorectal adenoma recurrence, support the concept that moderate milk and calcium intake decreases the risk of colorectal cancer. A meta-analysis of three randomized controlled trials suggested that calcium prevents recurrent colorectal adenomas with an overall relative risk of 0.80 [95%CI (confidence interval) 0.68–0.93] (Shaukat et al., 2005). Calcium has recently been associated with higher ischemic cardiovascular events in postmenopausal women (Bolland et al., 2008). Many more studies are required to confirm and validate this observation (Flood et al., 2005; Freedman et al., 2007). Also, recent data showing that vitamin D deficiency appears to be a risk factor for developing cardiovascular disease, would contradict these findings as many women cosupplement calcium and vitamin D together.

Vitamin D

The possible effects of vitamin D in colon cancer prevention were first brought to light about 20 years ago. Cedric Garland and Frank Garland reported in 1980 that people who lived in sunny locations had a lower incidence of colon cancer compared with those who lived in colder regions. In 1989, the Garland brothers went on to provide further evidence that deficiency of vitamin D may pose a significant risk for the development of colon cancer. They analyzed air pollution data from 20 Canadian cities and found that cities where polluted air obscured vitamin D-producing sunlight had higher death rates from both colon and breast cancer. Furthermore, they pointed out that colon cancer rates were four to six times higher in North America and Northern Europe when compared with the incidence of colon cancer in countries close to the equator (Garland et al., 2006; Lance, 2008). Vitamin D has also been found to be inversely associated with the risk of colorectal cancer (Wu et al., 2002). In a prospective cross-sectional study of 3121 asymptomatic patients aged 50 to 75 years, a multivariate analysis found vitamin D to dominate calcium as a beneficial risk factor.

Freedman and colleagues (2007) studied a total of 16,818 participants in the Third National Health and Nutrition Examination Survey. Levels of serum 25(OH)D were measured at baseline by radioimmunoassay. Cox proportional hazards regression models were used to examine the relationship between serum 25(OH)D levels and mortality from specific cancers. Colorectal cancer mortality was inversely related to serum 25(OH)D level, with levels 80 nmol/L or higher associated with a 72% risk reduction (95%CI = 32% to 89%) compared with lower than 50 nmol/L, p(trend) = 0.02, supporting an inverse relationship between 25(OH)D levels and colorectal cancer mortality. Overall, a meta-analysis showed that in all five studies, a significant protective effect against developing colorectal cancer was conferred by vitamin D sufficiency (Freedman et al., 2007). Finally, the most "natural" way to enhance vitamin D status is to soak in the rays of sunshine. The body can convert pre-vitamin D3 in the skin to vitamin D in the presence of ultraviolet light (UVB) from the sun, depending upon the latitude, time of day, presence of sun block, skin color, and skin concentration of 7-dehydro-cholesterol (Martinez and Willet, 1998). Figure 38.2 illustrates the variation of colorectal cancer mortality according to sunshine exposure and latitude is shown below (Grant, 2007). The influence of vitamin D on several cancer processes is shown in Figure 38.3.

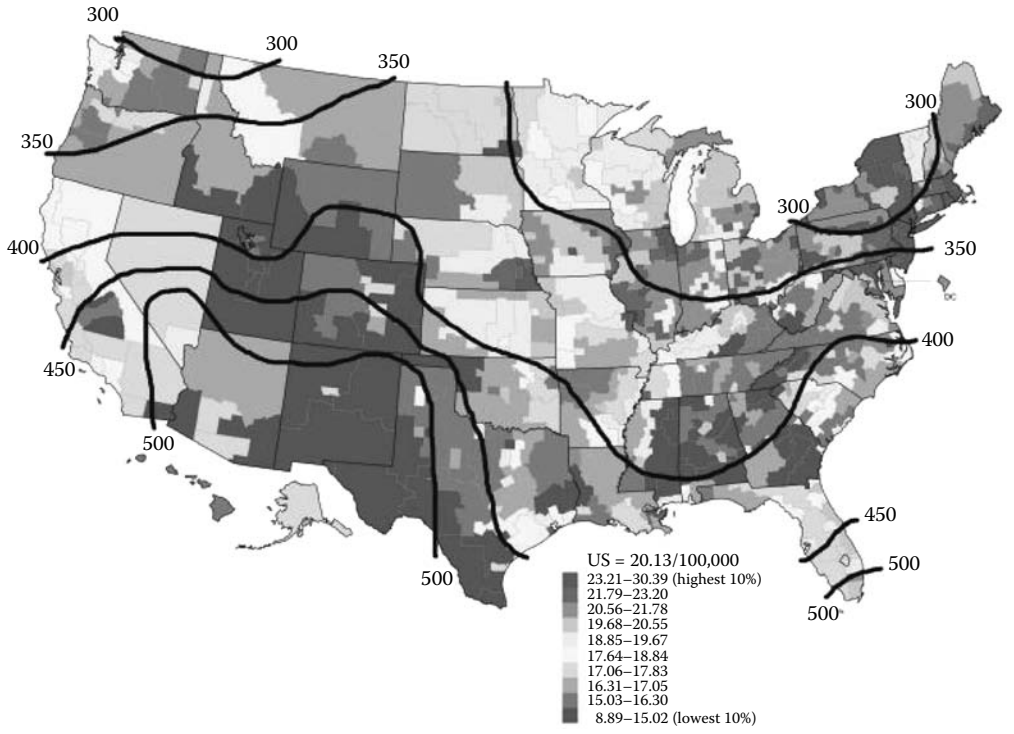

FIGURE 38.2 Age-adjusted colon cancer mortality rates, by county area, and contours of annual mean dally solar irradiance in Langleys (calories/cm^2), United States, 1970–1994. (Adapted from Garland et al. *American Journal of Public Health* 2006; 96(2):252–61.)

Folate

Several cohort and case-control studies have suggested a reduced risk of colorectal cancer with a higher consumption of vegetables and fruits (Grant, 2007). Folate is a B vitamin found in leafy green vegetables, citrus fruits, and dried beans, and peas. Folic acid is involved in DNA synthesis and repair, DNA methylation and modulation of cell proliferation shown in both *in vitro* and animal

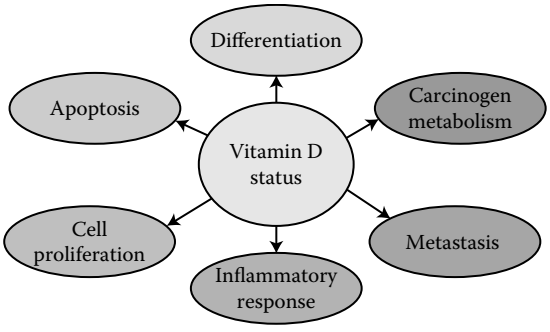

FIGURE 38.3 Vitamin D may influence genetic events associated with several cancer processes. Vitamin D can diminish the risk of colorectal cancer development via enhancing the programmed cell-killing (apoptosis) of cancerous cells, downregulating the inflammatory response by attenuating the expression of nuclear factor kappa B (NFκB), controlling the spread of established cancer (metastasis), limiting the proliferation and differentiation of malignant and pre-malignant cells. (Adapted from Mullin GE, Dobs A. *Nutr Clin Pract*. 2007; 22(3):305–22.)

studies (Mason and Levesque, 1996). Murine models of colorectal cancer report that folate administration is associated with decreased risk of colonic neoplasm, and epidemiologic studies show an inverse relationship between dietary folate and colorectal cancer incidence (Kim, 2007). Two large prospective cohort studies show an association between folate intake and reduced colorectal cancer incidence (Su and Arab, 2001; Giovannucci, 2002). In the Nurses' Health Study, 88,756 women provided diet assessments including multivitamin use. This investigation reported that long-term use of folate at doses greater than 400 μm daily was associated with a substantial decreased risk for colon cancer (Giovannucci et al., 1998).

In the National Health and Nutrition Epidemiologic Follow-Up Study subjects were followed for 20 years and an inverse relationship of folate intake and colon cancer was found for men (RR 0.40; 95%CI 0.18–0.88) (Su and Arab, 2001). Just as folate, vitamin B6 also appears to be inversely associated with risk of colorectal neoplasia (Wei et al., 2005). Despite the beneficial effects of folic acid for the prevention of colorectal cancer, future studies are needed to determine the exact amount to be consumed for optimal benefit.

Selenium

Selenium is an essential trace element in the human diet and is incorporated into proteins. Selenoproteins are involved in the neutralization of reactive oxygen species and are important for the antioxidant defense of cells (Al-Taie et al., 2003). Selenium is found in plant foods, and the concentration of selenium in food correlates with the content of the soil where plants are grown. In ecologic studies, selenium intake was found to have an inverse relationship to colon cancer risk (Shamberger, 1985). In colon cancer cell lines, selenomethionine inhibits growth and decreased levels of cyclooxygenase proteins in the cells (Baines et al., 2002), and animal studies have consistently shown selenium has activity against colorectal cancer (Duffield-Lillico et al., 2004). Though *in vitro* and animal studies show activity of selenium against colorectal cancer, epidemiologic studies have been inconsistent (Normura et al., 1982; van der Brandt et al., 1993; Early et al., 2002). However, several investigations reveal a protective relationship for selenium and colorectal adenomas and cancer (Willett et al., 1983; Clark et al., 1993). A pooled analysis of three randomized trials of 1763 individuals found an inverse association between higher blood selenium concentration and adenoma risk (Jacobs et al., 2004). Other vitamins have been studied as well. In a prospective, randomized trial, beta carotene and vitamins C and E failed to prevent colorectal adenomas (Greenberg et al., 1994).

Curcumin

Chemoprevention has emerged as a promising and pragmatic medical approach to reduce the risk of cancer. Numerous components of edible plants, collectively termed "phytochemicals," have been reported to possess substantial chemopreventive properties. Curcumin, a diferuloylmethane derived from the plant *Curcuma longa*, is a potent antioxidant with anti-inflammatory and antitumor activities (Lev-Ari et al., 2005). Curcumin is commonly consumed as turmeric spice. Both *in vitro* studies and animal models have demonstrated a chemopreventive effect of curcumin (Kwon et al., 2004; Li et al., 2007). Curcumin inhibits COX-2 expression, inhibits growth in human colon cancer cell models, and suppresses nuclear factor κB, a transcription factor that controls expression of cytokines (Johnson and Mukhtar, 2007). Curcumin is one of the most extensively investigated and well-defined chemopreventive phytochemicals. Curcumin has been shown to protect against skin, oral, intestina,l and colon carcinogenesis and also to suppress angiogenesis and metastasis in a variety animal tumor models (Surh et al., 2007). Curcumin inhibits the proliferation of cancer cells by arresting them in the various phases of the cell cycle and by inducing apoptosis. Curcumin has a capability to inhibit carcinogen bioactivation via suppression of specific cytochrome P450 isozymes, as well as to induce the activity or expression of phase II carcinogen detoxifying enzymes. In patients with advanced colorectal cancer refractory to standard chemotherapy, 5 of 15

individuals given daily oral curcumin had stable disease after two to four months of treatment (Sharma et al., 2001). Furthermore, a recent study of 5 FAP patients with prior colectomy treated with oral curcumin and quercetin (flavonoid found in green tea, onions, and red wine) had a reduction in number and size of ileal and rectal adenomas after 6 months of treatment (Prochaska et al., 1992). Well-designed intervention studies are necessary to assess the chemopreventive efficacy of curcumin in normal individuals as well as high-risk groups. Sufficient data from pharmacodynamic as well as mechanistic studies are necessary to advocate clinical evaluation of curcumin for its chemopreventive potential.

Glucosinolates [Sulforaphane and Indole-3-Carbinol (I3C)]

In 1992, Johns Hopkins researcher Paul Talalay and colleagues found that an antioxidant called sulforaphane, produced in the body from a compound in broccoli, triggered the production of enzymes that helped detoxify cancer-causing chemicals. The discovery, published in the *Proceedings of the National Academy of Sciences*, attracted worldwide attention and was hailed as a major breakthrough in our understanding of the link between increased fresh vegetable consumption and reduced cancer risk (Prochaska et al., 1992). Subsequent studies found that sulforaphane prevented the development of breast and colon cancer, as well as other tumors, in mice, exhibiting a powerful role in cancer prevention and protection. Talalay's team found that the key protective compound in broccoli (a chemical called glucoraphanin, which the body turns into sulforaphane) was 20 times more concentrated in young, three-day-old broccoli sprouts than it is in more mature broccoli plants. More recently, the team launched a line of teas enhanced with the antioxidant SGS (sulforaphane glucosinolate).

Cruciferous vegetables are a rich source of glucosinolates and their hydrolysis products, including indoles and isothiocyanates, and high intake of cruciferous vegetables has been associated with lower risk of lung and colorectal cancer in some epidemiological studies (Higdon et al., 2007). Glucosinolate hydrolysis products alter the metabolism or activity of sex hormones in ways that could inhibit the development of hormone-sensitive cancers, but evidence of an inverse association between cruciferous vegetable intake and breast or prostate cancer in humans is limited and inconsistent. Organizations such as the National Cancer Institute recommend the consumption of five to nine servings of fruits and vegetables daily, but separate recommendations for cruciferous vegetables have not been established. Isothiocyanates and indoles derived from the hydrolysis of glucosinolates, such as sulforaphane and indole-3-carbinol (I3C), have been implicated in a variety of anticarcinogenic mechanisms, but deleterious effects also have been reported in some experimental protocols, including tumor promotion over prolonged periods of exposure.

Epidemiological studies indicate that human exposure to isothiocyanates and indoles through cruciferous vegetable consumption may decrease cancer risk, but the protective effects may be influenced by individual genetic variation (polymorphisms) in the metabolism and elimination of isothiocyanates from the body (Higdon et al., 2007). Cooking procedures also affect the bioavailability and intake of glucosinolates and their derivatives. Supplementation with I3C or the related dimer 3,3′-diindolylmethane (DIM) alters urinary estrogen metabolite profiles in women, but the effects of I3C and DIM on breast cancer risk are not known. Small preliminary trials in humans suggest that I3C supplementation may be beneficial in treating conditions related to human papilloma virus infection, such as cervical intraepithelial neoplasia and recurrent respiratory papillomatosis, but larger randomized controlled trials are needed. List of potential chemopreventive agents are listed in Table 38.2.

Diet and Exercise

Diet has been implicated in colon cancer, including meats, vegetables, fruit, and fiber. Prospective cohort studies have demonstrated an increase in risk of colorectal cancer in patients who consume red

TABLE 38.2
Potential Chemopreventive Agents

NSAIDs
Sulindac
Celecoxib
Aspirin
Ursodeoxycholic acid
Hormones
Estrogen
Medroxyprogesterone acetate
Vitamins/Antioxidants
Calcium
Folate
Selenium
Curcumin
Vitamin D
Supplements/Sunshine
Curcumin
Ginger
Glucosinolates
Sulforaphane and indole-3-carbinol (I3C)

meat (Willett et al., 1990; Giovannucci et al., 1992). Red meat is associated with increased risk of colorectal cancer and increases the endogenous formation of *N*-nitroso compounds (NOC), DNA, and protein oxidation products along with inhibition of apoptosis of cancer cells (Lih-Brody et al., 1996; Cross et al., 2002; Lewin et al., 2006; de Vogel et al., 2008). There does appear to be convincing evidence for a correlation between high vegetable and fruit intake and low rates of colorectal neoplasia. The majority of case-control and cohort studies published show a relative risk of less than 0.8 for colorectal neoplasia in subjects with the highest intake of fruits and vegetables (Gastof and Ahnen, 2002). The role of fiber in the risk of colorectal cancer is less clear. About 60% of epidemiologic studies have linked high-fiber diets with a decreased risk of colorectal adenomas and cancer, and a large European prospective study showed similar results (Bingham et al., 2003; Bingham, 2006). Increase the amount of wheat bran in the diet. As an insoluble fiber, wheat bran increases the fecal bulk and weight as well as increasing the frequency of eliminating the fecal matter, thereby reducing the colon's exposure to toxins, for example, carcinogens. Two large prospective, randomized controlled studies showed that adopting a diet low in fat and high in fiber, fruits, and vegetables did not influence the risk of recurrence of colorectal adenomas. Along with diet, body habitus, and physical activity have been studied in relation to colon cancer. A meta-analysis of the association of physical activity and colon cancer shows an inverse association between physical activity and colon cancer. The author of this study concluded that if individuals participate in physical activity, they will have a 24% reduction in the risk of developing colon cancer. Two large studies investigated dose-related response of exercise on colon cancer reduction. The Harvard Alumni Study followed 17,148 men for a maximum of 26 years. During the study period, 225 men developed colon cancer. Those men who participated in physical activity equivalent to at least 30 minutes per day, 5 days per week, had a 50% reduction in colon cancer rates compared with men who were sedentary. Physical activity was defined in this study as both the time set aside for daily exercise, as well as other daily physical activities such as climbing stairs (Alberts et al., 2000; Schatzkin et al., 2000, 2007; Lanza et al., 2007). The actual

TABLE 38.3
Consensus Public Health Recommendations on Physical Activity and Colon Cancer Risk

Physical activity recommendation should be included in primary intervention for cancer prevention.
All messages for physical activity should be in the context of reducing the risk of colon cancer rather than preventing cancer.
Physical activity should compromise at least 30–45 minutes of moderate to vigorous activity on most days of the week.
Physical activity should be encouraged at all ages.

Source: From Kury S, et al. *Cancer Epidemiology, Biomarkers & Prevention* 2007; 16:1460–7. With permission.

mechanism of how exercise helps prevents colon cancer is not fully understood. However, it is well known that exercise decreases intestinal transit time, therefore decreasing the exposure of dietary procarcinogens, which are activated by intestinal bacteria and transformed into carcinogens within the intestinal lining. This theory is not well supported by data, but nevertheless a mechanism that we must entertain in understanding the role of exercise in prevention of colon cancer. Public health recommendation on physical activity and colon cancer risk is shown below in Table 38.3.

A revolutionary theory of colorectal cancer chemoprevention is that exercise strengthens the immune system by promoting the function of many types of immune cells that prevent and fight cancer, including natural killer cells. Another possible mechanism is that exercise imparts protection from colorectal cancer by improving insulin responses to meals. Insulin and insulin-like growth factors appear to promote the growth of colorectal cancer in laboratory and animal studies. Thus, the hyperinsulinism that is associated with obesity, type 2 diabetes and the metabolic syndrome (syndrome X) may be the means by which these conditions predispose toward the development of colorectal cancer. Finally, exercise is able to ward off unwanted cellular proliferation as well as decrease some factors such as prostaglandin E2, which is thought to promote colon cell proliferation. In order to make concrete recommendations with regard to the type, duration, and intensity of exercise, additional research is deemed necessary. Overall, case-control and cohort studies have demonstrated a positive association between obesity, hyperinsulinism and cancer risk, whereas the majority of studies have shown an inverse relationship between physical activity and colorectal cancer risk. The Mediterranean diet includes omega-3 fatty acids and olive oil, which appear to be chemopreventive. A pooled analysis of the small but ever-growing body of science of omega-3 and colorectal cancer indicates that fish oil does protect against the cancer (Willett et al., 1990; Lewin et al., 2006; de Vogel et al., 2008). For each extra 100 g of fish consumed per week the risk of colorectal cancer incidence was reduced by 3%. The Mediterranean diet is rich in extra-virgin olive oil (EVOO) and is associated with a lower incidence of colorectal cancer. EVOO contains phenolic extracts with potential antioxidant effect. Pinoresinol-rich EVOO extracts have potent chemopreventive properties and specifically upregulate the tumor suppressor p53 cascade *in vitro*. This result was achieved at substantially lower concentrations in EVOO than with purified pinoresinol, indicating a possible synergic effect between the various polyphenols in olive oil. Combination of phytonutrients (e.g., curcumin, green tea extract) attenuates proinflammatory and carcinogenic cascades.

CONCLUSIONS

Prevention, as English clergyman Thomas Adams observed in 1618, "is so much better than healing because it saves the labor of being sick." Preventive tools were much more sparse during the time of Thomas Adams than they are today. Despite the availability of vaccines to prevent infectious disease and medicines to lower cholesterol and blood pressure, we are at a greater quest to discover agents that can lower the risk of developing cancer and other noncommunicable diseases. Colon cancer is ranked as the third leading cause of cancer-related death. Cancer is a leading cause of death globally. Seventy percent of the cancer deaths occur among low- and middle-class income

population in countries where the resources available for prevention, diagnosis, and treatment are limited. In industrialized nations such as the United States, with rising healthcare costs and the present economic crisis leaving millions without jobs and health insurance, the disease burden from cancer, especially colorectal cancer, is bound to show an upward trend. In 2008, WHO projected the global burden of death from noncommunicable disease to rise by 17% by the year 2018. Tackling this burden is a challenge to every nation. Preventive strategies for cancer must be considered in the context of activities that prevent other chronic diseases, especially those with which cancer shares common risk factors, such as coronary artery disease, diabetes mellitus, substance abuse, and respiratory illness. The common risk factors for the chronic disease listed above include a sedentary lifestyle, a diet low in fiber and antioxidants but high in fat and simple sugars, tobacco and alcohol use, and overweight and obesity. Other risk factors include environmental exposure to toxins, which can be endogenous or exogenous. Cancer, especially colon cancer, is largely preventable. For primary prevention of colorectal cancer, our strategy should aim at reducing the level of the risk factors in the population as a whole. When we reduce the risk factors associated with cancer, we not only prevent the incidence of cancer, we also prevent incidence of other chronic conditions. Overweight, obesity, and sedentary lifestyle accounts for at least 274,000 deaths worldwide. As healthcare providers, the first step we must undertake in preventing colorectal cancer is to have a systematic approach to the assessment of risk factors. Next, using the risk assessment to prioritize our action, for example, how many of the risk factors are modifiable; how many of the risk factors are attributable to avoidable exposure to carcinogens. Decision-making in prevention strategies should consider social and economic factors. Incorporating a daily exercise routine of 30 minutes of walking does not require going to a gym. Likewise, including fiber-rich seasonal fruits and vegetables and adequate calcium and vitamin D is of the utmost importance. With regard to folic acid, though the benefits are certainly noted, the exact amount and timing of supplementation need to be determined by further studies. In conclusion, eating healthfully, engaging in regular exercise, and taking nutritional supplements tailored to meet individual needs is likely to help keep colorectal cancer away.

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39 *Feijoa* (Pineapple Guava) Fruit
A Role in Health Promotion?

Mercedes C. Argüelles and Ronald Ross Watson

CONTENTS

INTRODUCTION

Feijoa sellowiana Berg. syn. *Acca sellowiana* is a fruit-bearing evergreen shrub relative of the tropical guava, commonly known as pineapple guava. The *Feijoa* plant is indigenous of South America but can be found in tropical and subtropical dry areas. The *Feijoa* plant is mainly grown for its fruit, and used primarily for the production of juice that has a sweet and acidic flavor. Beyond the interest in its fruits, this species is used as an ornamental plant for its hardiness and eye-catching flowers and leaves. Recently, the species has assumed some medicinal relevance due to its content of biologically and nutritionally interesting compounds.

BOTANICAL DATA

Feijoa sellowiana (O. Berg) Burret belongs to the *Myrtaceae* family which includes many tropical and subtropical plants of great economic importance (USDA/NRCS, 2009). In more recent times, *F. sellowiana* has been renamed *Acca sellowiana*, but most sources still use the older name. Well-known species such as clove, cinnamon, and nutmeg also belong to the *Myrtaceae* family as well as ornamental shrubs and trees as *Myrtus callistemon* and *Eucalyptus*. In addition to *Feijoa*, the number of *Myrtaceae* species producing edible fruits is quite large, including the common guava (*Psidium guajava*), pará-guava (*Britoa acida* Berg), and jaboticaba (*Myrciaria caulifola* Berg) to mention a few (Ruehle, 1948).

The *Myrtaceae* fruit-bearing trees and shrubs are an interesting and varied group offering definite possibilities for future commercial development of excellent fruits. From this group

F. sellowiana appears to offer the best possibilities. *Feijoa*'s are grown commercially on a limited scale primarily in California and New Zealand. In California, *Feijoa* is grown for its fruits especially in cool coastal locations. There has also been a major effort in New Zealand to commercialize the *Feijoa*, but development of the industry is hindered by low fruit set and a high degree of variability in the size and shape of the fruits.

Origin and Distribution

The *Feijoa* is indigenous to South America (Argentina, Brazil, Uruguay, and Paraguay) but has been distributed to practically all tropical and subtropical areas throughout the world (Pescador et al., 2009). *Feijoa* is grown in New Zealand, Australia, Asia, Africa, Europe, and in California and Florida in the United States.

The *Feijoa* was first collected in southern Brazil by the German explorer Freidrich Sellow around 1815 and introduced into Europe by the French botanist and horticulturist, Dr. Edouard Andre circa 1890. *Feijoa* is named after Brazilian naturalist João da Silva Feijó.

Description and Habitat

Feijoa plants are well adapted to subtropical dry areas and can withstand a few degrees of frost. Plants grow well in a wide variety of soil types, but prefer slightly acidic conditions. *Feijoa*s are grown for their edible fruit with a very distinctive aromatic flavor with tropical overtones including pineapple and guava, hence the common name of "pineapple guava." Beyond the interest in its fruits, *Feijoa* is also used as an ornamental plant for its attractive flowers and leaves, and because it is relatively easy to grow resulting in an outstanding container plant, hardy in the landscape in the United States Department of Agriculture (USDA) zones 9–10 (Fact Sheet, 1993).

The *Feijoa* is a slow-growing evergreen shrub that can reach 15 feet high and 15 feet wide (Fact Sheet, 1993). The bark is pale gray and the spreading branches are swollen at the nodes and white-hairy when young. In addition to the fruit it provides, the shrub also doubles handsomely as a landscape specimen. When planted close together, the shrubs make a nice hedge, screen, or windbreak. *Feijoa*s can also be espaliered or trained as a small tree (20–25 feet tall) with one or more trunks. The wood is dense, hard, and brittle.

Flowers are about 1 inch, borne singly or in a cluster, and have long, dark red stamens topped with large grains of yellow pollen. Flowers appear late, from May through June. Each flower contains four to six fleshy flower petals that are white tinged with purple on the inside. These petals are mildly sweet and edible and can make a refreshing addition to spring salads. Birds eating the petals pollinate the flower (Fact Sheet, 1993; California Rare Fruits Growers, Inc., 2009).

The fruits range from three-fourths to three and half inches long and vary in shape from round to ovoid, with the persistent calyx segments adhering to the apex. The waxy skin is dull blue-green to blue or grayish green, sometimes with a red or orange blush. Skin texture varies from smooth to rough and pebbly and is 3/16 to 5/8 inch thick. The fruit emits a strong long-lasting perfume, even before it is fully ripe. The thick, white, granular, watery flesh, and the translucent central pulp enclosing the seeds are sweet or low-acid, suggesting a combination of pineapple and guava or pineapple and strawberry, often with overtones of winter green or spearmint. There are usually 20–40, occasionally more, very small, oblong seeds hardly noticeable when the fruit is eaten.

FOOD USES AND NUTRIENT AND CHEMICAL CONTENT

Food Uses

The fruit is usually eaten by cutting it in half, then scooping out the pulp with a spoon (California Rare Fruits Growers, Inc., 2009, Specialty Produce Online, 2009).

The fruits have a juicy sweet seed pulp, and slightly gritty flesh nearer the skin. The flavor is aromatic and sweet. The skin is sour and can be bitter, but provides a nice balance to the sweet pulp. A *Feijoa* can also be used as an interesting addition to a fruit smoothie, and can be used to make *Feijoa* wine or cider and *Feijoa* infused vodka. It is also possible to buy *Feijoa* yogurt, fruit drinks, jam, and ice cream in New Zealand. The *Feijoa* can also be cooked and used in dishes where one would use stewed fruit. It is a popular ingredient in chutney (Sharpe et al., 1993).

Fruit maturity is not always apparent from the outside as the fruits remain green until they are overmature or rotting. Generally the fruit is at its optimum ripeness the day it drops from the tree. While still hanging it may well prove bitter. Once fallen, the fruit very quickly become overripe, so daily collection of fallen fruit is advisable during the season. When the fruits are immature the seed pulp is white and opaque, becoming clear and jelly-like when ripe. Fruits are at their optimum maturity when the seed pulp has turned into a clear jelly with no hint of browning. Once the seed pulp and surrounding flesh start to brown, the fruit is overmature and should not be eaten. However, these overmature but not rotten fruits can be used to make a delicious juice very popular in places like the Colombian Highlands (Purdue Agriculture, 2009).

Nutrient and Chemical Content

Some nutrient and chemical components of *Feijoa* have been identified and measured (Romero-Rodriguez et al. 1994; USDA 2008). *F. sellowiana* fruit contains vitamins, with ascorbic acid and folate present in significant amounts. Six average sized fruits fill an adult's daily ascorbic acid needs. They are also a good source of folate with one fruit delivering about 10% of an adult's minimum daily needs. The main minerals found in *Feijoa* are potassium and phosphorus. It also has a significant amount of iodine: 3 mg/100 g (Ferrara and Montesano, 2001).

The lipids content in *Feijoa* fruit has also been identified, and it contains some interesting groups (Kolesnik et al., 1991). The main group of lipids found is neutral lipids, with carotenoids and tocopherols being among the most notable. In addition to neutral lipids, glycolipids and phospholipids were also found. Recently, the essential oil of *F. sellowiana* has been extracted and its chemical constituents determined. The major constituents present in the oil were limonene, beta-caryophyllene, alpha-pinene, beta-pinene, isocaryophyllene, and estragole (Saj et al., 2008).

Other nutrients present in *F. sellowiana* extracts are amino acids such as tryptophan, lysine, methionine, and asparagines (Nakashima, 2001). *Feijoa* is known to contain high amounts of polyphenols such as catechin, leucoanthocyanins, flavonols, ellagic acid pentoside, quercitin, hyperin, and proanthocyanidins (Nakashima, 2001; Ruberto and Tringali, 2004).

BIOLOGICAL ACTIVITIES

Irrespective of the presence of a large variety of phyto-constituents in the genus *Feijoa*, a limited number of publications regarding the pharmacological investigations on its fruit, peel, and leaves are available. Most of the pharmacological work has been carried out on its antimicrobial and antioxidant biological effects.

Antimicrobial Activity

Feijoa may be an important tool as an antimicrobial and antioxidant agent. A number of *Feijoa* extracts have been found to greatly inhibit the growth of Gram-positive and Gram-negative bacteria and to have a strong antioxidant capacity (Basile et al., 1997; Vuotto et al., 2000; Ebrahimzadeh and Hosseinimehr, 2008).

A group of Italian researchers have performed a number of antimicrobial and antioxidant studies from *Feijoa* leaves, fruit peel and pulp, and stem (Basile et al., 1997; Vuotto et al., 2000). In one study (Basile et al., 1997), this group studied acetone extracts from three plants, *Actinidia chinensis*, *F. sellowiana* and *Aberia caffra* and tested them against eight Gram-positive and Gram-negative

bacterial strains. The Gram-positive bacteria were: *Staphylococcus aureus*, *Streptococcus pyogenes*, and *S. faecalis*, and the Gram-negative bacteria were: *Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi*, and *Klebsiella pneumoniae*. Of the plants tested *Feijoa* was the most active. *F. sellowiana* fruit peel and pulp extracts showed inhibition against all bacterial strains employed (minimum inhibiting concentration (MIC) between 1 and 8 μg/mL). The strongest inhibition activity was observed against *P. aeruginosa, P. mirabilis,* and *E. coli*. The leaves and stem extracts did not show activity against *S. faecalis* and *K. pneumoniae*.

In a more recent study the same Italian group evaluated *Feijoa* fruit antimicrobial activity and antioxidant activity but this time using an aqueous extract (Vuotto et al., 2000). The extract was analyzed against the Gram-positive bacterial strains *S. aureus* and *S. faecalis*, and Gram-negative strains *P. mirabilis, P. vulgaris, K. pneumoniae, Enterobacter cloacae, P. aeruginosa, E. coli, S. typhi*, and *Enterobacter aerogenes*. The effect of the extract on the oxidative burst of human whole blood phagocytes as well as on isolated polymorphonuclear leukocytes (PMNs) was evaluated. To measure the metabolic activation of these cells, the authors analyzed the light emitted during the oxidative reactions after amplifying photon emission with luminol, a chemilumigenic (CL) probe. Data collected from this study showed that the antimicrobial activity of the extract was mainly against Gram-negative bacteria particularly *P. aeruginosa, E. aerogenes*, and *E. Enterobacter aerogenes*. No activity was observed against Gram-positive bacteria. The data concerning the chemilumigenic probe displayed a significant decrease in CL emission from human whole blood phagocytes and isolated PMNs. The authors concluded that the decrease in CL emission is most likely due to the fruit extract scavenger effect on free radicals.

Antioxidant Activity

A different group investigated the antioxidant capability of aqueous and methanol extracts obtained from the leaves and fruit peel of *Feijoa* using different *in vitro* assay systems (Ebrahimzadeh and Hosseinimehr, 2008). The extracts and its antioxidant activity were evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical-scavenging activity, reducing power determination, nitric oxide-scavenging activity, metal chelating activity, and ferric thiocyanate method (FTC) which measures the antioxidant activity by the inhibition of lipid oxidation. The aqueous and methanol extracts of *F. sellowiana* fruits and leaves displayed different levels of antioxidant activity in all the systems studied. The DPPH radical-scavenging activities of all the extracts were in the order: leaf aqueous extract > leaf methanol extract > fruit peels aqueous extract > fruit methanol extract. All the extracts showed weak oxide-scavenging activity, whereas nearly all exhibited good iron-chelating ability. Further, the extracts had good reductive capability for reducing Fe^{3+} to Fe^{2+}, and all extracts exhibited lipid peroxidation inhibition activity ranging from around 92% to 98% at 72 hours.

The antioxidant activity of polyphenols from *Feijoa* ethanol extract was compared with those of other fruits (kiwano, cherimoya, papaya, and mango) (Yuka et al., 2003). Suppression of the peroxidation of linoleic acid was measured by the FTC method, and the DPPH radical and OH radical scavenging activities were measured by ESR spectrometry. Interestingly, the antioxidant activity of the polyphenols in the extract was equivalent to or higher than that in the other fruits. The total polyphenol content in *Feijoa* measured by the Folin Ciocalteu method was 59.2 mg per 100 g of edible matter; chose to that of cherimoya. The authors also observed a high correlation between the total polyphenol content and the DPPH radical-scavenging activity.

Anti-Inflammatory Activity

Feijoa, specifically acetone fruit extracts, have been found to have anti-inflammatory activity (Rossi et al., 2007). The study was aimed to evaluate the anti-inflammatory activity of an acetone extract from *F. sellowiana* Berg. fruits on the nitric oxide (NO) pathway, which plays an important role in inflammation. To this intent, the research group used an ingenious *in vitro* model of inflammation.

The model consisted of murine macrophages J774 cell line, which expresses inducible nitric oxide synthase (iNOS) following stimulation with lipopolysaccharide (LPS) and evaluated the effects of the extract and its fractions on NO production, protein expression, and signal pathways involved in its regulation. The authors reported that *F. sellowiana* acetone extract exhibits anti-inflammatory activity because it inhibited in J774 cells the LPS-induced NO production and iNOS expression probably at the transcriptional level as demonstrated by the decrease in LPS-induced IκBα degradation and ERK-1/2 phosphorilation. Further, the authors observed that the mechanism of this inhibition seems to be related to an action on the expression of the enzyme iNOS through the attenuation of nuclear factor κB (NF-κB) and/or mitogen-activated protein kinase (MAPK) activation.

Immunomodulating Activity

The effects of dietary *Feijoa* on the immune responses of the intestinal epithelial cells have also been studied (Manabe and Isobe, 2005). The experimental design used an aqueous extract from *Feijoa* and *in vitro* digested *Feijoa* on the secretion of IL-7 and TGF-β from Caco-2 cells. Although IL-7 secretion was not suppressed by the extract, significantly less ($p < 0.001$) TGF-β was secreted by Caco-2 cells when compared with the control. Equally, the *in vitro* digested *Feijoa* suppressed TGF-β secretion ($p < 0.05$) but showed no effect on IL-7 secretion. Based on the results, the authors suggested that continued intake of *Feijoa* may induce a decrease in TGF-β concentrations in the intestinal epithelium, which may in turn cause suppressions of oral tolerance and disorders of mucosal homeostasis.

Anticancer *In Vitro* Activity

Some anticancer activities of *Feijoa* extract have been reported (Nakashima, 2001; Bontempo et al., 2007). A Japanese study fractionated extracts of *Feijoa* peels and studied the antitumor activity, 50% cell cytotoxic activity (CC_{50}), and antihuman immunodeficiency virus (HIV) activity (Nakashima, 2001). *Feijoa* peel fractions were extracted with hexane, acetone, methanol, and 70% methanol. For the antitumor and cytotoxic activity human oral squamous cell carcinoma cells (HSC-2), human oral salivary tumor cells (HSG) and human oral gingival fibroblast (HGF) were used. The suppression of HIV-induced cytopathic effects was studied by infecting human T cell leukemia virus 1 (HTLV-1)-bearing CD4 positive human T cell lines and MT-4 cells with HIV-1 I IIB. *Feijoa* fractions only showed some, not significant, anti-HIV activity; likewise, most fractions exhibited only low cytotoxicity (IC_{50} values > 100 μg/mL). However, one fraction (A3) displayed significant potency and was relatively cytotoxic to two tumor cell lines (HSC-2 and HSG) and the healthy cell line (HGF).

An Italian investigation showed that the *Feijoa* fruit acetone extract exerted antiproliferative effects on solid and hematological cancer cells (Bontempo et al., 2007). In addition, the group identified the flavone content in the fruit as the active component. In human myeloid leukemia cells flavone induced apoptosis, which was accompanied by caspase activation and p16, p21, and TRAIL (TNF-related apoptosis-inducing ligand) overexpression. In *ex vivo* myeloid leukemia patients blasts, both the acetone *Feijoa* extract and its derived flavone were able to induce apoptosis too. In both cell lines and in myeloid leukemia patients blasts, the *Feijoa* extract and flavone apoptotic activity was accompanied by an increase of histone and nonhistone acetylation levels and by HDAC (histone deacetylase inhibitors) inhibition.

CONCLUSION

Several experimental studies have demonstrated that *F. sellowiana* has a broad antimicrobial and antioxidant activity, as well as anti-inflammatory activity, and can inhibit cancer cell proliferation, in addition to immunomodulatory activity. *Feijoa* contains a wide variety of phytochemicals, many of which have been found to have strong antioxidant and anticancer activity. The documented

biological activities and the phytochemicals content of *Feijoa* merit more study to further determine its potential health benefits.

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40 Indian Bael (*Aegle marmelos*) for the Prevention/ Treatment of Cancer

Weighing Cost versus Benefit

Manjeshwar Shrinath Baliga, Harshith P. Bhat, Ponemone Venkatesh, R.K. Sharma, and Rajesh Arora

CONTENTS

CANCER AND NATURAL PRODUCTS

The costs of healthcare are increasing world over and medical spending in developed (Kennedy et al., 2009) and developing nations is increasingly consuming a major share of the gross domestic product (GDP); unfortunately, the underprivileged classes, which constitute a major chunk of the population, continue to have limited or no access to modern healthcare facilities. The cost-benefit and cost-effectiveness issues in herbal medicine for the treatment of disease in general (Herman et al., 2005; MacLennan et al., 2006; Kennedy et al., 2009), and cancer in particular, are important from the perspective of patients, physicians, and policymakers alike.

Cancer, today is the second leading cause of death after cardiovascular diseases, and predictions are that by the year 2010 it would be the leading cause of death worldwide (Aggarwal et al., 2009). The cost of treatment of cancer by the modern conventional methods, viz., chemotherapy, radiotherapy, and surgery is enormous. Consequently, for the general population in most parts of the world, affordable and efficient healthcare remains beyond the reach of the common man due to paucity of funds. The problem is more acute since even general healthcare is far beyond the reach of the general population, and for cancer patients the hospital-related costs are beyond reach. Consequently, they resort to complementary and alternative medicine, mainly herbal medicine (Arora, 2010).

The characteristic feature of cancer is the unregulated proliferation of cells. Depending on the stage and localization, cancer may be treated with surgery, radiation, chemotherapy, or a

combination of these modalities. The use of radiotherapy and chemotherapy are associated with serious side effects as they also affect the normal tissues (Dorr and Fritz, 1980; Hall, 2000).

The deleterious responses of immediate concern are those involving the hemopoetic and gastrointestinal cells. These organs are highly sensitive and have life-supporting functions. At times, the reactions can be very severe and may inevitably compel the physician to discontinue or reduce the dose of treatment, which will invariably affect the cure and ultimately the survival of the patient. The other major concern is the consequential late effect or the delayed side effects, which critically affect most long-term cancer survivors and appear months or years after therapy (Dorr and Fritz, 1980).

Natural products, especially plants have been used to treat various diseases for thousands of years. Studies have shown that nearly 60% of the commercially available drugs are from natural origin, mostly from plants (Cragg and Newman, 2005). Ethnobotanical guided studies have lead to the discovery of important molecules and phytochemicals like taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan, and irinotecan. Etoposide derived from epipodophyllotoxin are of use in clinics. Further, a number of promising agents such as flavopiridol, roscovitine, combretastatin A-4, betulinic acid, and silvestrol are in clinical or preclinical development (Cragg and Newman, 2005). Recent studies have also shown that more than 60% of cancer patients use herbs as therapeutic agents, thereby indicating the continual reliance of humans on plants (Madhuri and Pandey, 2008).

Ayurveda (Sanskrit Ayur = life, Veda = knowledge) meaning science of life is the traditional system of Indian medicine. It is one of the ancient healthcare systems developed in India between 2500 BC and 500 BC. Today Ayurveda is accepted and practiced even in countries such as Europe, United States, and Japan. The Ayurvedic treatment is mostly based on the use of medicinal plant formulations, and the ancient texts report more than 2000 plant species for their therapeutic potentials. Many of the plants used as remedies have been scientifically evaluated for their beneficial effects and reports occur in numerous scientific journals (Balachandran and Govindarajan, 2005).

THE INDIAN BAEL (*AEGLE MARMELOS*)

Aegle marmelos (Rutaceae family) is one of the most important medicinal plants in Ayurveda (Figure 40.1). It is commonly known as the stone apple or golden yellow apple tree in English; Bilva or Sriphal or Shivadruma (the tree of Shiva) in Sanskrit and Bael in Hindi (Kritikar and Basu, 1984; Kulkarni, 1997). It is a slow growing, spinous, tough subtropical tree. It is the only plant belonging to the genus Aegle and is found widely throughout India, Ceylon, Burma, Thailand, and Indo-China (Kritikar and Basu, 1984).

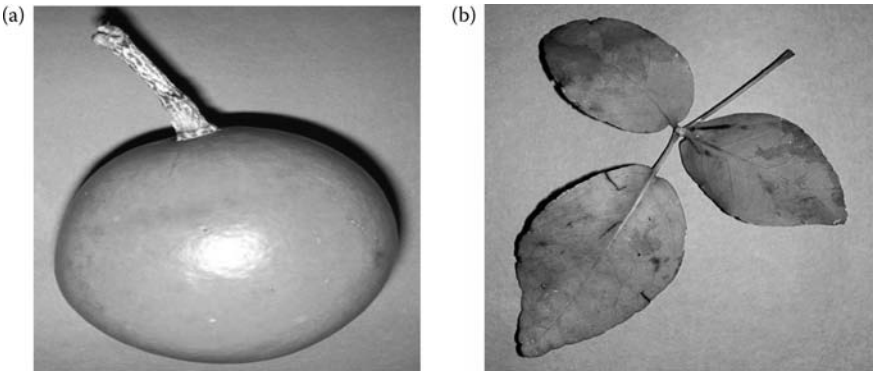

FIGURE 40.1 The Bael fruit (a) and leaf (b).

Bael has been used for the management of various illnesses in the Ayurvedic, Unani, and Siddha systems of medicine in India, as well as in Bangladesh and Sri Lanka. They are also of use in the folk and local traditional systems of medicine (Kritikar and Basu, 1984; Kulkarni, 1997). In Ayurveda, Bael is referred to as an emblem of fertility and is considered a healing tree that strengthens the body. This accreditation seems largely due to its diverse medicinal properties as all parts of this tree, viz., root, leaf, trunk, fruit, and seed, are used to cure various human ailments and diseases (Kritikar and Basu, 1984; Sharma and Dash, 1998). In fact, as per Charaka (1500 BC), the author of the ancient Ayurvedic text *Charaka Samhita*, no drug has been longer or better known or appreciated by the inhabitants of India than Bael (Sharma and Dash, 1998; Jagetia et al., 2004a,b).

The unripe fruits of Bael are bitter, acrid, sour, and astringent. In folk medicine, its consumption is reported to aid digestion and relieve stomach irritation. It is also useful in treating diarrhea, dysentery with spells of constipation and stomachalgia (Shoba and Thomas, 2001). A sweet drink (sherbet) prepared from the fruit pulp produces a soothing effect on the patients who have just recovered from bacillary dysentery. Recently, Abdullakasim et al. (2007) have reported that Bael fruit drink had the highest total phenolic compounds among Thai health beverages and possessed good antioxidant affects and confirms the previous observations (Kamalakkannan and Stanley, 2003).

Decoction prepared from the bark of the tree is used in the treatment of fever. The roots are also used to, cure the fevers, stop pain in the abdomen, the palpitation of the heart, and allay urinary troubles. The roots are also one of the important ingredients of the Ayurvedic traditional drug Dashamula, a panacea for colitis, dysentery, diarrhea, flatulence, and fever (Kirtikar and Basu, 1984). The leaves have astringent, febrifuge, and expectorant properties and are useful as a remedy for fevers associated with catarrhal symptoms. They are good for bowel complaints, bleeding piles, dropsy, diarrhea, and dysentery (Kirtikar and Basu, 1984).

Studies with experimental animals and cell-free assays have shown that Bael possess diverse pharmacological properties. The leaf extract is shown to possess good antimicrobial activity on enteric pathogens. The best activity was observed in *Salmonella typhimurium*, *Vibrio cholera*, *Shigella dysenteriae* and the least in *Pseudomonas aeruginosa* and *Klebsiella aerogenes* (Shobha and Thomas, 2001; Rani and Khullar, 2004).

The aqueous decoction of the leaf is reported to possess significant hypoglycemic effect, help in the regeneration of damaged pancreas (β-cells) in diabetic rats and to be as effective as insulin in restoring the levels of blood glucose and body weight (Karunanayake et al., 1984; Das et al., 1996; Seema et al., 1996). The alcoholic and aqueous extracts of the leaves are shown to have similar effects as digoxin in amplitude and contractions of the frog heart. Further, the methanolic extracts of the roots inhibited the beating rate (Seema et al., 1996).

Phytochemical studies have shown that Bael contains aegeline, aegelenine, marmelosine, marmelin, *o*-methyl halfordinol, alloimperatorin methyl ether, *o*-isopentenyl halfordinol, linoleic acid, cineole, *p*-cymene, citronella, citral, cuminaldehyde, D-limonene, eugenol, tannins, phlobatannins, flavon-3-ols, leucoanthocyanins, anthocyanins, and flavonoid glycosides (Rastogi and Mehrotra, 1990). Most of these chemicals possess beneficial health effects, and some of them are reported to possess diverse pharmacological effects, including anticancer, antidiabetic, and cardioprotective properties.

Bael as an Anticancer Agent

Most of the chemotherapeutic agents used in the treatment of cancer have been reported to be accompanied with undesirable side effects, the most important being the nausea, vomiting, diarrhea, fatigue, ulcerations, infection, myelosuppression, thrombocytopenia, and anemia. Further, most of these drugs are highly expensive, mutagenic, genotoxic, gonadotoxic, teratogenic, and carcinogenic (Dorr and Fritz, 1980). Therefore, studies are on for discovering substitutes for the conventional chemotherapeutic agents which are effective in controlling the cancer at nontoxic doses and are

inexpensive. Preclinical studies with *in vitro* and *in vivo* systems of studies have shown that Bael and some of its constituents possess antineoplastic effects.

The extracts of Bael leaf has been observed to inhibit the proliferation of human tumor cell lines of different histological origins; the leukemic K562, T-lymphoid Jurkat, B-lymphoid Raji, erythroleukemic HEL, melanoma Colo38, and breast cancer MCF7 and MDA-MB-231 cell lines in the *in vitro* assays (Khan et al., 2002; Lampronti et al., 2003). Fractionated phytochemical studies showed that three molecules butyl-*p*-tolyl sulfide, 6-methyl-4-chromanone, and butylated hydroxyanisole exhibited strong activity in inhibiting *in vitro* cell growth of human K562 cells. The antiproliferative activities of these compounds were equivalent to that of clinically used antineoplastic agents cisplatin, chromomycin, 5-fluorouracil, and cytosine arabinoside. Further, the antiproliferative activity of butyl-*p*-tolyl sulfide, 6-methyl-4-chromanone, and 5-methoxypsolaren was observed to be associated with the activation of the differentiation pattern of K562 cells (Lampronti et al., 2003).

Studies have shown that the treatment with the Bael extract did not increase ERalpha mRNA levels in MCF7 cells and MDA-MB-231 (Lambertini et al., 2003). However, when added in combination with the decoy molecule, bael brought about significant effects. Detailed phytochemical isolation and studies with the compounds showed that lupeol was the active compound, and like the Bael extract, stimulated the decoy effect of RA4 DNA sequence, increased ERalpha gene expression in MDA-MB-231 ERalpha-negative breast cancer cells, and inhibited cell proliferation (Lambertini et al., 2005).

In vivo studies have also confirmed the *in vitro* observations of antineoplastic effects of the hydroalcoholic extract of *Aegle marmelos*. Treatment of Ehrlich ascites carcinoma-bearing Swiss albino mice, once daily for six consecutive days caused a concentration-dependent effect till 400 mg/kg body weight (BW), beyond which toxic manifestations were seen; the effect was optimal when the extract was administered through intraperitoneal route rather than the oral route and the effective dose of 400 mg/kg BW was 20% of the LD_{10} dose of 2000 mg/kg BW (Jagetia et al., 2005).

Citral (3,7-dimethyl-2,6-octadien-1-al) (Figure 40.2), a key component of Bael has been recently shown to induce apoptosis in several hematopoietic cancer cell lines. The apoptotic activity (22.25 μM) was compared to a reference compound like staurosporine (0.7 μM), with respect to DNA fragmentation and caspase-3 enzymatic activity (Dudai et al., 2005). Recently Chaouki et al. (2009) have reported that citral had antiproliferative effects, inhibited cell cycle progression in G2/M phase, induced apoptosis of the human breast cancer cell line MCF-7, and decreased prostaglandin E(2) synthesis (Chaouki et al., 2009).

Moteki et al. (2002) have reported that 1,8-cineole (Figure 40.2), induced apoptosis in the human leukemia cell lines Molt 4B and HL-60 cells, but not in human stomach cancer KATO III cells. A concentration and time-dependent DNA fragments were also observed in both Molt 4B and HL-60 cells, but not in KATO III cells, confirming that the antineoplastic effects of 1,8-cineole is mediated through induction of apoptosis (Moteki et al., 2002).

The monoterpene, D-limonene (Figure 40.2), also present in the Bael is reported to possess chemotherapeutic activity against pancreatic, mammary, and prostatic tumors; and that several mechanisms are responsible for this. However, the most important observation is that monoterpenes inhibit the post-translational isoprenylation of cell growth-regulatory proteins such as Ras. Monoterperpenes are also observed to regress rat mammary tumors by increasing the expression of transforming growth factor beta with concomitant tumor remodeling/redifferentiation to a more benign phenotype (Crowell et al., 1996).

Atsumi et al. (2000) have observed that eugenol (Figure 40.2), has cytotoxic effects against salivary gland tumor cell line (HSG) and normal human gingival fibroblast (HGF) *in vitro* (Atsumi et al., 2000). Eugenol is also observed to be cytotoxic to the malignant HepG2 hepatoma cells, malignant Caco-2 colon cells, and the nonmalignant human VH10 fibroblasts (Slamenová et al., 2009). Studies with human malignant melanoma cell line, WM1205Lu, have shown that eugenol arrested cells in the S phase of the cell cycle, induced apoptosis, and that the deregulation of E2F1

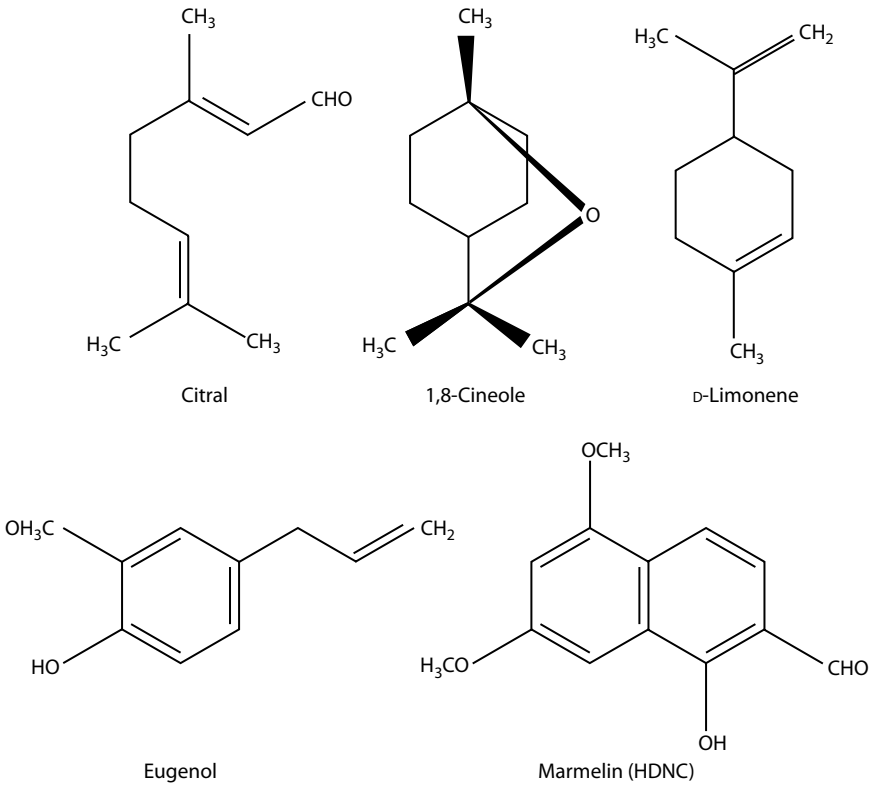

FIGURE 40.2 Bael phytochemicals reported to possess antineoplastic effects.

may be a key factor in eugenol-mediated melanoma growth inhibition both *in vitro* and *in vivo* (Ghosh et al., 2005).

Studies with the B16 melanoma-bearing mice showed that eugenol (4-allyl-2-methoxyphenol) treatment inhibited cell proliferation, retarded tumor growth kinetics, reduced the tumor size by nearly 40%, increased the median survival time by nearly 20%, and inhibited invasion and metastasis in nearly 50% of the animals when compared with the control group which was never administered eugenol. Further, eugenol was observed to be well tolerated as the body weights of the mice treated were not affected (Ghosh et al., 2005).

Subramaniam et al. (2005) investigated the antineoplastic effects of various fractions (hexane, dichloromethane, ethyl acetate, and *n*-butanol soluble fractions) of the ethanol extract in HEp-2 cells. All fractions exhibited potent cytotoxic activities, but the best effect was observed in the ethyl acetate fraction; a further fraction of which showed that the observed cytotoxic effects were primarily due to the molecule 1-hydroxy-5,7-dimethoxy-2-naphthalene-carboxaldehyde (HDNC, marmelin) (Figure 40.2). Detailed studies showed that marmelin was a potent antiproliferative molecule with activity only against epithelial cancer cells of different origin (HCT-116 colon and HEp-2, alveolar epithelial carcinoma cells) but not on the normal cells (normal mouse embryo fibroblasts), suggesting its possible efficacy as a selective antineoplastic agent (Subramaniam et al., 2008).

Marmelin activated apoptosis through tumor necrosis factor-alpha (TNF-alpha), TNF receptor (TNFR)-associated death domain (TRADD), and caspases (Subramaniam et al., 2008). It induced G (1) cell cycle arrest and mediated apoptosis through activated caspase-3, which was abrogated when pretreated with caspase-3 inhibitors. Activation of caspase-8 and Bid, with release of cytochrome *c* was also observed, suggesting the existence of a crosstalk between death receptor and the mitochondrial pathways (Subramaniam et al., 2008). Studies showed that marmelin significantly

suppressed TNF-alpha-mediated activation and translocation of nuclear factor-kappaB (NF-kappaB) (Subramaniam et al., 2008).

Marmelin treatment inhibited the growth of HCT-116 colon cancer tumor xenografts *in vivo*. Immunostaining for CD31 showed that there was a significant reduction in microvessels in the marmelin-treated animals, coupled with decreased cyclooxygenase-2, interleukin-8, and vascular endothelial growth factor mRNA. It also inhibited AKT and extracellular signal-regulated kinase phosphorylation both in cells in culture and in tumor xenografts (Subramaniam et al., 2008).

Bael as a Chemopreventive Agent

Carcinogenesis is a multistep process in which accumulation of genetic events within a single cell line leads to a progressively dysplastic cellular appearance, deregulated cell growth, and, finally, carcinoma (Aggarwal et al., 2009). Cancer chemoprevention, as first defined by Sporn in 1976, is the use of natural, synthetic, or biologic chemical agents to reverse, suppress, or prevent carcinogenic progression to invasive cancer. It is based on the concepts of multifocal field carcinogenesis and multistep carcinogenesis and arresting one or several of the steps, which may impede or delay the development of cancer (Aggarwal et al., 2009).

The concept on prevention and the success of several recent clinical trials in preventing cancer in high-risk populations suggests that it is a practical and interesting strategy in delaying/preventing carcinogenesis (Aggarwal et al., 2009). With regard to the Bael plant there are no scientific data suggesting their chemopreventive effects. However, the individual phytochemicals limonene, citral, anthocyanins, monoterpenes, tannins, and eugenol have all been reported to possess chemopreventive effects. The methanol and acetone extract of the fruits is also observed to be effective in reducing the hydrogen peroxide and aflatoxin B1-induced SOS response in chromotest, suggesting it possess antimutagenic effects and prevents the mutagenesis, which is the antecedent for carcinogenesis (Kaur et al., 2009).

Studies have shown that pretreatment with eugenol inhibited two-stage skin carcinogenesis initiated by the application of 7,12-dimethylbenz[*a*]anthracene as initiator and croton oil as tumor promoter in mice. The tumor multiplicities were also decreased per mouse indicating its effectiveness (Sukumaran et al., 1994). Biochemical studies indicate that eugenol inhibited the formation of superoxide and lipid peroxidation and that the radical scavenging activity may be responsible for its chemopreventive action (Sukumaran et al., 1994).

Feeding a diet containing 3% eugenol (w/w) for 13 weeks is observed to have enhanced the levels of the drug-detoxifying enzymes UDP-glucuronyltransferase (GT), UDP-glucose dehydrogenase (DH) and glutathione S-transferase (GST), while the levels of cytochrome P-450 were unaffected. Eugenol enhanced the activity of DH, GST and GT in the liver microsomes in a concentration dependent manner toward the various xenobiotic substances such as 4-nitrophenol, 1-naphthol, 4-hydroxybiphenyl and 4-methylumbelliferone (Yokota et al., 1988). These results suggest that the intracellular content of the active intermediates of various drugs or carcinogens would be reduced by the specific enhancement of drug-detoxifying enzymes in the liver of rats by eugenol (Yokota et al., 1988).

Citral is also reported to be effective in preventing the Dimethylbenzanthracene-initiated and tetradecanoylphorbol-13-acetate promoted skin carcinogenesis in mice. The tumor incidence were observed to be 88% and 72%, 60% and 96%, and 96% and 84% at the end of weeks 10 and 15, respectively, for the animals treated with 0, 1, and 10 µmol citral. At the end of the experiment, a dose-dependent decrease in the tumor multiplicity (number of tumors per mice) was also observed (Conner, 1991).

Russin et al. (1989) have seen that D-limonene prevented the 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced mammary carcinogenesis in the rat model (Russin et al., 1989). The chemopreventive activity of limonene during initiation is credited to the induction of phase I and phase II enzymes, which results in the detoxification of the carcinogen, while that during promotion/progression

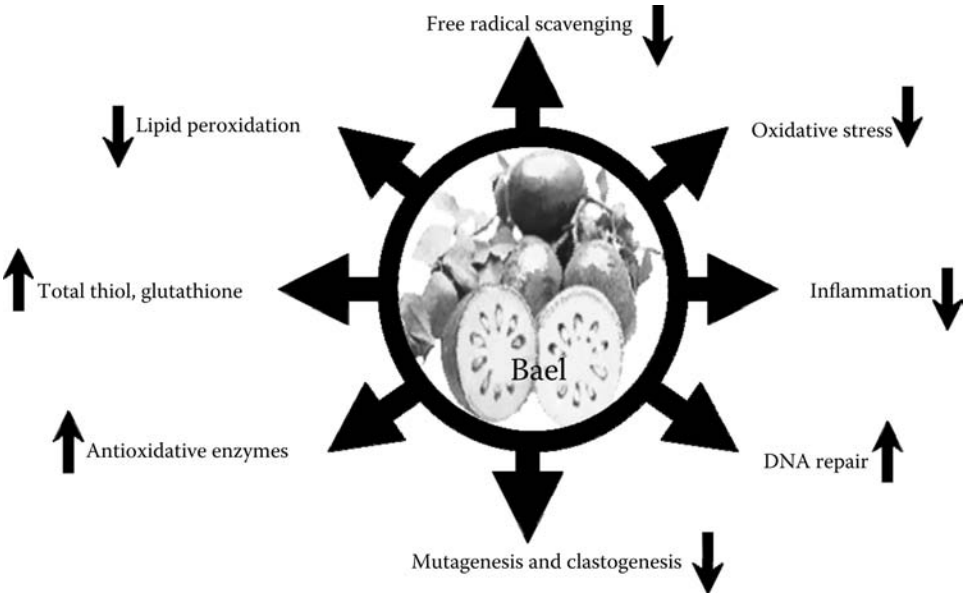

FIGURE 40.3 Representation of the influence of Bael in various pathways in preventing cancer.

may be due in part to inhibition of the posttranslational isoprenylation of growth-controlling small G proteins, such as p21ras. The complete regression of mammary carcinomas by limonene appears to involve tissue redifferentiation (Crowell and Gould, 1994).

An increasing body of evidence also suggest that the pigments anthocyanins and anthocyanidins, their aglycons, especially cyanidin and delphinidin and tannins present in the ripe Bael fruits delay cancer development in rodent models of carcinogenesis by its antioxidant properties, preventing DNA strand scission, stimulating cell differentiation, inducing apoptosis, inhibiting aberrant cell proliferation, inhibiting the activities of oncogenic transcription factors and protein tyrosine kinases and antiangiogenic properties (Hou, 2003; Li et al., 2003). All these reports suggest that the constituents of Bael influence myriad pathways in preventing cancer (Figure 40.3).

Bael Protects against Doxorubicin-Induced Cardiotoxicity and Mutagenecity

The anthracycline antibiotic adriamycin (doxorubicin), a topoisomerase II inhibitor is one of the most effective chemotherapeutic agents against ovarian, breast, lung, uterine and cervical cancers, Hodgkin's disease, soft tissue and primary bone sarcomas, as well as against several other cancer types. However, its use is seriously limited by the development of myelosupression, anemia, nausea, alopecia, gastrointestinal toxicity, and dose-dependent acute and chronic cardiotoxicity (Dorr and Fritz, 1980).

Although anthracycline-induced injury appears to be multifactorial, a common denominator among most of the proposed mechanisms is cellular damage mediated by reactive oxygen species. Experimental studies suggests that an increase in oxidative stress brought about by increased generation of free radical production and decreased myocardial endogenous antioxidants in the heart, plays an important role in the pathogenesis of doxorubicin-induced cardiac failure. As doxorubicin is a useful chemotherapeutic agent, several strategies have been tried to prevent/attenuate their side effects (Dorr and Fritz, 1980).

Recently, Panda and Kar (2009) have reported that cardenolide isolated from the leaves of *Aegle marmelos* protected mice against the doxorubicin-induced cardiotoxicity and lipid peroxidation. Treatment of mice with doxorubicin alone caused both cardiotoxicity and hepatotoxicity. There was

a marked change in the levels of serum creatine kinase-MB, glutamate-pyruvate transaminase, and tissue lipid peroxidation with a concomitant decrease in superoxide dismutase, catalase, and glutathione. It also increased the levels of different serum lipids, but decreased the amount of high-density lipoprotein (HDL). Cotherapy of the test cardenolide and doxorubicin for four weeks reversed all these adverse effects. The best effect was seen for 25 mg/kg BW of periplogenin and was better than vitamin E (alpha-tocopherol) (Panda and Kar, 2009).

As with most anticancer agents, doxorubicin can cause physiological side effects and induce mutations and other genotoxic effects in nontumor cells. Administering leaf extract orally once for five consecutive days reduced the frequency of doxorubicin-induced micronuclei formation at all time points studied, indicating it possesses antigenotoxic effects. Bael also increased the polychromatic erythrocytes to normochromatic erythrocytes ratio, an indicator of the protection to the hemopoetic system. The greatest protection against doxorubicin-induced genotoxicity was observed at 350 mg/kg BW of Bael and was safe as the extract was nontoxic up to 6 g, the highest dose tested (Venkatesh et al., 2007).

Bael as a Radioprotective Agent

Radiation is an important modality in the treatment of cancer, and in some instances it may be the single best agent for treatment. However, a major problem associated with cancer radiotherapy is the severe side effects resulting from the normal tissue damage. Accordingly, agents (referred to as radioprotectors) which protect normal tissues against the radiation-induced damage are of preference in treatment (Hall, 2000; Arora et al., 2005).

Since the discovery by Patt et al. (1949) that cysteine protects against radiation sickness and mortality, several compounds with varied chemical structures and pharmacologic properties have been screened for their radioprotective ability in mammals. However, these compounds appear to produce serious side effects and are considered to be toxic at the doses required for radioprotection (Uma Devi, 1998). Amifostine the only FDA-approved (U.S. Food and Drug Administration) radioprotector in use, is currently employed in the clinics for reducing the incidence and severity of xerostomia in head and neck cancer patients undergoing radiotherapy (Uma Devi, 1998; Arora et al., 2005). Unfortunately, application of this drug has so far been less than hoped for, owing to untoward toxicity often being evidenced at optimal radioprotective doses (Uma Devi, 1998; Arora et al., 2005).

An ideal radioprotector should be relatively nontoxic to normal cells irrespective of the type of cell/organ, administrable through the oral route and should not compromise the therapeutic effects of radiation treatment. Unfortunately, none of the investigated drugs satisfy these criteria, and therefore the search to identify or develop less toxic or nontoxic agents to counter the effects of ionizing radiation remains an area of intense focus (Arora et al., 2005).

The hydroalcoholic extract of both fruit and leaf were effective in ameliorating radiation-induced sickness and mortality in the Swiss albino mice when administered intraperitonealy (Jagetia et al., 2004a,b). The optimal radioprotective doses were 15 mg/kg BW and 20 mg/kg BW and the dose modification factor (DMF) was observed to be 1.15 and 1.1 for leaf and fruit extracts, respectively (Jagetia et al., 2004a,b). Comparatively, the leaf extract was observed to be better than fruit extract, which was comparable to that of the positive control 2-mercaptopropionyl glycine, a synthetic radioprotective thiol compound (Jagetia et al., 2004a,b).

The leaf extract was also effective when administered orally and the optimal dose of 250 mg/kg BW resulted in a DMF of 1.2 (Jagetia and Venkatesh, 2005). Altering the administration schedule by reducing to single or three and increasing the schedule to seven days was not as effective as when administered for five days. Further, administering the extract after irradiation was also not effective, indicating its use only when the application of radiation is planned. The extract was nontoxic even at a high concentration of 6 g/kg BW, suggesting its nontoxic nature (Jagetia and Venkatesh, 2005).

These observations have immense clinical significance as the oral route is the most preferred, as it does not need medical intervention, and is convenient to administer to patients and people at risk to the deleterious effects of radiation when planned. Accordingly, detailed studies with the leaf extract showed it to be effective in protecting mice against the radiation-induced damage to the gastrointestinal epithelium and hemopoetic progenitor cells in the bone marrow, the two most radiosensitive organs essential for sustenance of life (Hall, 2000).

Pretreatment with leaf extract offered protection to the hemopoetic cells against the deleterious effects of ionizing radiations, when evaluated on days 1 and 7 postirradiation. It increased the RBC, hemoglobin levels, the total leukocyte counts, and lymphocytes count, when compared with the irradiation group only. The leaf extract elevated the leucocytes and lymphocytes counts in both sham and irradiation groups (Jagetia et al., 2007). A significant increase in the number of spleen colony forming units (CFU-S) was also observed (Jagetia et al., 2007).

The leaf extract was also observed to prevent radiation-clastogenesis in the cultured human peripheral blood lymphocytes and the bone marrow cells of mice (Jagetia et al., 2003; Jagetia and Venkatesh, 2007). Oral administration of eugenol, a constituent of the Bael also caused a dose-dependent reduction in the frequencies of micronucleated polychromatic erythrocytes (Tiku et al., 2004). All these reports clearly indicate that the leaf extract was protective to the hemopoetic system and prevented DNA against the radiation clastogenesis (Figure 40.4).

In survival studies it was observed that Bael extracts delayed the radiation-induced mortality and thereby indicated that Bael protected against the gastrointestinal damage. To validate these observations, mice were orally administered with the leaf extract or the placebo (saline) and exposed to radiation. The animals were sacrificed on day 1 and 7 postirradiation and the crypt microcolony

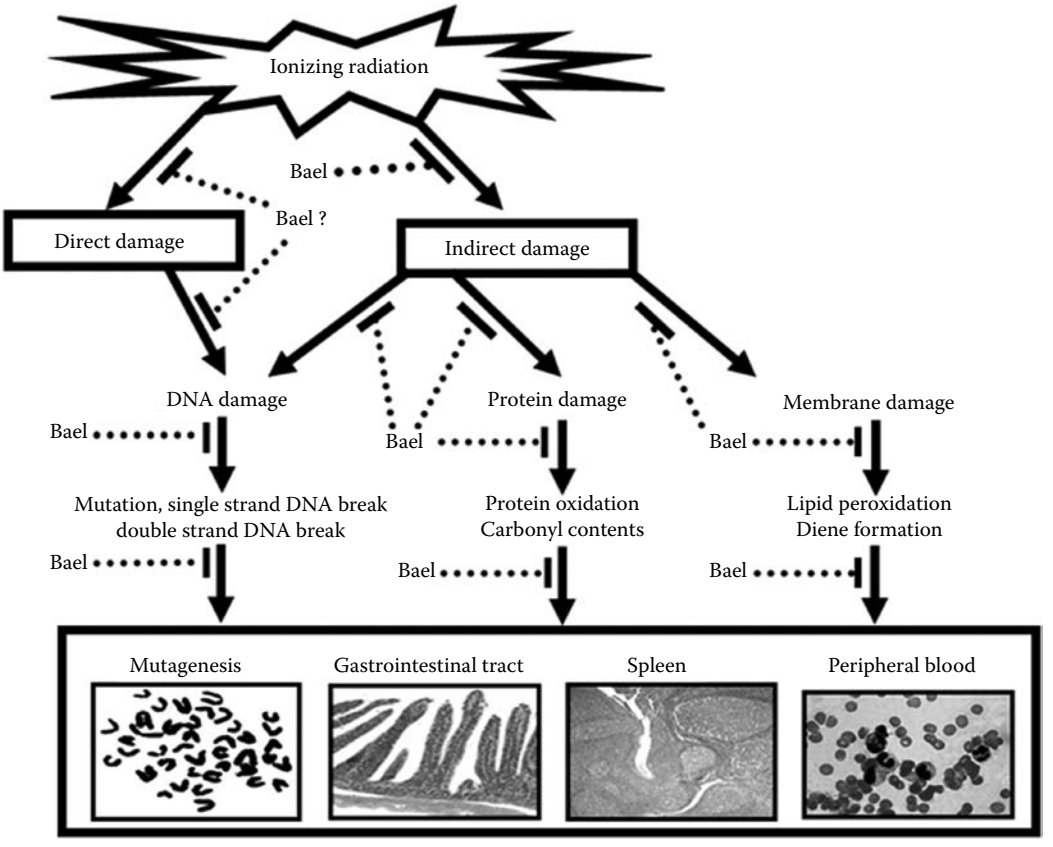

FIGURE 40.4 Mechanistic representation of the radioprotective effects of Bael.

assay was performed to obtain information on the protective effect of Bael on the GI tract (Jagetia et al., 2006).

Treatment of mice with leaf extract increased the crypt cell number, elevated the villus height, restored the crypt architecture; decreased edema in the jejunum, reduced the number of dead and goblet cells when compared with the irradiation group. These results clearly show that the leaf extract is effective in protecting the clonogenic stem cells in the crypts of small intestine, ensuring that more stem cells survive, which can then contribute to epithelial regeneration (Jagetia et al., 2006).

Mechanistic studies suggest that the radioprotective effects of Bael are multifactorial due to the free radical scavenging, antioxidant, immunomodulatory properties along with increasing the glutathione levels and decreasing the lipid peroxidation activities. The leaf extract is observed to be a potent scavenger of both reactive oxygen species (ROS) and reactive nitrogen species (RNS) and a good iron chelator in the *in vitro* systems of studies (Jagetia et al., 2003; Jagetia and Baliga, 2004; Venkatesh, 2006). Bael leaf has been reported increase the activities of the antioxidant enzymes: superoxide dismutase, catalase, and glutathione peroxidase in normal mice (Singh et al., 2000), irradiated mice (Venkatesh, 2006), and in alloxan-treated diabetic rats (Sabu and Kuttan, 2004).

The leaf and fruit extracts also prevented the radiation-induced lipid peroxidation in the liver, kidney, intestine, and spleen of mice with a concomitant increase in the levels of glutathione (Jagetia et al., 2004a,b; Jagetia and Venkatesh, 2005; Venkatesh, 2006). Eugenol protected against the peroxidative damage and decreased the specific activities of lactate dehydrogenase (LDH) and concurrently increased the levels of methylglyoxalase I (Gly I) in mice liver (Tiku et al., 2004).

The leaf extract stimulated the immune response by acting on macrophages from the systemic immune compartment, in particular with the peritoneal cavity. The effect that has been observed played a significant role in view of the fact that macrophages form the first line against microbial invasion and neoplastic diseases (Venkatesh, 2006).

CONCLUSION

Considerable information from preclinical studies in the recent past suggest the usefulness of Bael in prevention and treatment of cancer suggesting its utility. Further, the acute administration of the hydroalcoholic extracts of the Bael leaf and fruit extracts were nontoxic up to a dose of 2.5 and 6 g/kg BW mice (Jagetia et al., 2004a,b). The subacute toxicity studies have also shown that the administration of various Bael leaf extracts intraperitoneally for 14 consecutive days to both male and female Wistar rats did not induce any short-term toxicity, gross abnormalities, or pathological changes at histopathological levels (Veerappan et al., 2007). Together, these observations suggest the possibility of Bael as a safe cancer preventive and radioprotective agent that may be taken on a daily basis.

Feeding Bael before mating to female rats did not cause any abnormalities in any of the pregnant rats and did not affect the number of corpus lutea, implanted, and dead fetuses, as well as the sizes of the fetuses, suggesting its safety (Saenphet et al., 2006). The leaf extract was not genotoxic in both human peripheral blood and mice bone marrow micronuclei studies (Jagetia et al., 2003; Jagetia and Venkatesh, 2007). Collectively, these data demonstrate that the leaf extracts have a high margin of drug safety and the protective doses were nontoxic and nonclastogenic at their optimal protective effects in both *in vitro* and *in vivo* systems. In total, all these results clearly suggests that the Bael extract is safe and devoid of any adverse effects.

Further studies on determining the chemopreventive and radioprotective activity of Bael and its active components should ideally include human intervention trials as its effectiveness against human cancers and as a selective radioprotective agent in treating cancer in the clinics can be investigated. Apart from applications in the clinics, studies should also be planned with human volunteers to evaluate the chemopreventive and radioprotective effects in people at risk due to their genetic factors and occupations or both.

As there is considerable variation in the chemical composition among various samples of Bael, it is imperative that a quality control be established for the authenticity of the plant and the presence of active phytochemicals in the required levels. In this regard the availability of authentic metabolite standards for quantification of the secondary metabolite will make the scientific observations more reliable and reproducible. Due to its abundance, low cost and safety in consumption, Bael remains a species with tremendous potential and countless possibilities for further investigation. Bael has the potential to develop as a nontoxic chemopreventive, chemoprotective, and radioprotective agent when existing lacunas in the information are bridged.

The innumerable benefits the patient(s) can expect by the use of Bael include, but are not limited to, the overall reduction of the risk of mortality or morbidity. The usefulness of Bael as a cancer preventive and therapeutic drug is particularly promising in low financial resource milieu. For a thorough cost-benefit analysis it is essential to identify the impact of prophylactic/therapeutic intervention with the herbal drug, in this case Bael, to estimate which impacts are economically relevant, to calculate the increase in life expectancy (which is most precious), to carry out physical quantification of relevant factors, the monetary valuation and the accruing nominal and real benefits. However, from a cost-benefit/effectiveness analysis point of view, several issues need to be taken into account before arriving at a final decision when utilizing herbal medicine, for example, issues of macro and microcosting, discounted lifetime costs and life expectancy, estimates of time spent traveling, waiting, and receiving cancer care, particularly from far flung rural/urban settings will have to be taken into consideration. Besides, the humanitarian issues too need to be taken into consideration.

From the scientific data available thus far, it appears that benefits derived from the use of Bael would far outweigh the nominal costs associated with its use, particularly as a chemopreventive agent. There is a need for manufacturing isolated bioactive components from Bael under strict good manufacturing practice (GMP) norms. Nonetheless, the promise of using Bael particularly as a cancer preventive agent definitely has only benefits and virtually very little cost associated with it!

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Nutrition

Bioactive Foods and Extracts

Cancer Treatment and Prevention

Though there is considerable historical and anecdotal record for the use and efficacy of the cancer preventative properties of vegetables, fruits, and herbs, modern healthcare professionals require scientific evidence and verifiable results to make defensible decisions on the benefits, risks, and value of botanicals and their extracts in the prevention and treatment of cancers.

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