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REVIEW

Terpenoids and breast cancer chemoprevention

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Abstract Cancer chemoprevention is defined as the use of natural or synthetic agents that reverse, suppress or arrest carcinogenic and/or malignant phenotype progression towards invasive cancer. Phytochemicals obtained from vegetables, fruits, spices, herbs and medicinal plants, such as terpenoids, carotenoids, flavanoids, phenolic compounds, and other groups of compounds have shown promise in suppressing experimental carcinogenesis in various organs. Recent studies have indicated that mechanisms underlying chemopreventive action may include combinations of anti-oxidant, anti-inflammatory, immune-enhancing, and anti-hormone effects. Further, modification of drug-metabolizing enzymes, and influences on cell cycling and differentiation, induction of apoptosis, and suppression of proliferation and angiogenesis that play a role in the initiation and secondary modification of neoplastic development, have also been under investigation as possible mechanisms. This review will highlight the biological effects of terpenoids as chemopreventive agents on breast epithelial carcinogenesis, and the utility of intermediate biomarkers as indicators of premalignancy. Selected breast chemoprevention trials are discussed with a focus on strategies for trial design, and clinical outcomes. Future directions in the field of chemoprevention are proposed based on recently acquired mechanistic insights into breast carcinogenesis.

Keywords Terpenoids · Cancer chemoprevention · Carcinogenesis · Biomarkers · Breast cancer

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Introduction

Breast cancer is the second most prevalent cancer worldwide. In the United States, breast cancer accounts for 26% of all cancers in women and is second only to lung cancer as a cause of cancer-related deaths. An estimated 182,460 new cases of invasive breast cancer will be diagnosed among women in the United States and an estimated 67,770 additional cases of in situ breast cancer will be added to the statistics in 2008. In addition to the diagnosis of new cases, approximately 40,480 women are expected to die from diagnosed breast cancer in 2008 [1]. Although still disconcertingly high, these numbers represent a downward trend that continued to decline by more than 2% per year since 1990. This trend has been credited to progress in the early detection and treatment of the disease [1]. Unfortunately, the severe morbidity of these cancers, reflected in the poor 5-year relative survival rate (only 14%), has not been improved by current treatments that include surgery, radiotherapy, hormone therapy and adjuvant chemotherapies [2]. The addition or withdrawal of estrogenic substances from a patient's milieu as part of the prevention or treatment of cancer has been a part of modern medicine for over 100 years. Although breast cancer research has developed at a rapid pace over the last decade, the curative potential of currently available therapies remains disappointing.

Primary cancer preventive strategies are those aimed at removing exposure to carcinogens, such as chemicals in the case of tobacco; electromagnetic-associated radiation such as protection from sun ultra violet (UV) exposure; or multifactorial in cases of poor diet and obesity. A variety of approaches have been employed in cancer chemoprevention. These include changes in diet, supplementation with specific vitamins and minerals, or administration of

pharmacologic compounds and identification and removal of preneoplastic lesions. More than 400 drugs, vitamins, hormones and other agents have been identified that might help in preventing cancer. Clinical trials are underway to investigate an increasing number of agents. Most of these trials involve healthy individuals with a higher-than-average risk of cancer [3, 4]. The development of cancer occurs over years and involves multiple genetic and phenotypic alterations. Chemoprevention is based on the premise that intervention is possible during the initiation, promotion and progression steps of carcinogenesis by the administration of one or more naturally occurring and/or synthetic compounds, as an alternative to treatment of cancer cases after clinical symptoms have appeared [5, 6]. For use as a chemopreventive agent among the general population, a compound must have minimal or no toxicity. Agents that show promise for this purpose include dietary constituents or their analogs, as well as medicinals, such as nonsteroidal anti-inflammatory drugs (NSAIDs) [7–9]. Fruits and vegetables contain an abundance of terpenoids, phenolic substances and other natural anti-oxidants that have been associated with protection from and treatment of chronic diseases such as cancer and heart disease. Terpenoids are a group of substances that occur in nearly every natural food. This class of compound has been shown to be beneficial to maintain and improve health, and include several subclasses such as monoterpenes (limonene, carvone and carveol), diterpenes (retinoids), triterpenes (oleanic acid and ursolic acid), and tetraterpenes (α- and β-carotene, lutein, lycopene, zeaxanthine and cryptoxanthine). These subclasses have been shown to possess an array of mechanisms of action that affect (among others) oxidative stress, carcinogenesis and cardiovascular diseases [10].

Chemopreventive agents

Cancer chemopreventive agents are divided into two principal categories: blocking agents that prevent the mutagenic initiation of the carcinogenic process and suppressing agents that prevent the further promotion or progression of lesions that have already been established [11]. Some agents are classified in both categories. A vast amount of information has been accumulated which demonstrates that chemical carcinogens act via common mechanisms. The ultimate carcinogenic forms of procarcinogens are often positively charged electrophilic species. Some carcinogens, termed "direct acting" exist in this form or assume it in solution. Others require metabolic activation. Blocking agents can be placed into three groups according to their mechanisms of action. One group acts simply by inhibiting the activation of a carcinogen to its ultimate carcinogenic form. An example of this type of inhibition is the prevention of symmetrical dimethylhydrazine-induced neoplasia of the large bowel by disulfiram [12]. A second group of blocking agents is effective by virtue of inducing increases in activity of enzyme systems having the capacity to enhance carcinogen detoxification. The third group of blocking agents has the capacity to act by scavenging the reactive forms of carcinogens. Physiological nucleophiles, such as glutathione (GSH) fall into this group. Since mutation continues as part of the entire chronic process of carcinogenesis, the distinction between the two categories, at least in part the dimension of time is artifactual. Extensive information is available that endogenous metabolism as well as exposure to exogenous agents can have major influences on the process of carcinogenesis [13]. Since chemoprevention is to have a practical impact on the control of cancer, it is necessary to develop a fundamentally pharmacologic approach to the problem. In the face of the intense mutagenic pressure that drives the process of carcinogenesis, it will be necessary to use agents that either are potent anti-mutagens or can significantly alter patterns of gene expression. α-Tocopherol and γ-tocopherol prevent formation of carcinogen from precursor compounds [14]. Diterpene kahweol palmitate is a naturally occurring compound which is a blocking agent, whereas retinoids, carotenoids, and sterols are suppressing agents [15, 16]. Large and diverse groups of naturally occurring terpenoids have demonstrated breast cancer chemopreventive effects (Table 1).

Terpenoids

Terpenoids, also referred to as terpenes, are the largest group of natural compounds that play a variety of roles in many different plants. All terpenoids are synthesized from two five-carbon building blocks. Based on the number of building blocks, terpenoids are commonly classified as monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes (C_{25}), triterpenes (C_{30}), tetraterpenes (C_{40}) and polyterpenes. Terpenoids, also known as isoprenoids, are perhaps the most diverse family of natural products synthesized from plants, serving a range of important physiological functions. Over 40,000 different terpenoids have been isolated from plant, animal and microbial species [17, 18]. A wide range of terpenoids has demonstrated pharmacological activity against human ailments such as cancer (taxanes from *Taxus brevifolia* and terpenoid indole alkaloids, including vincristine and vinblastine from *Catharanthus roseus*) [19, 20], human immunodeficiency virus (coumarins including calanolide A from *Calophyllum lanigerum*) and malaria (artemisinin from *Artemisia annua*) [21].

Table 1 Terpenoids tested in breast cancer

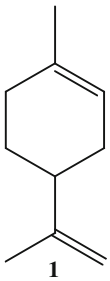
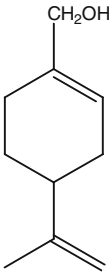
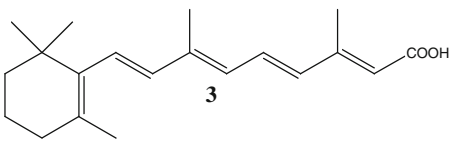
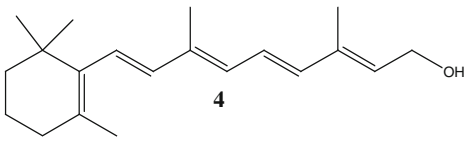
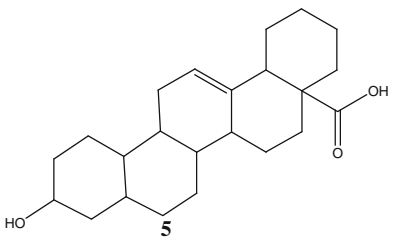
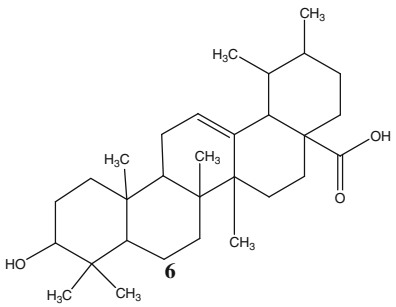
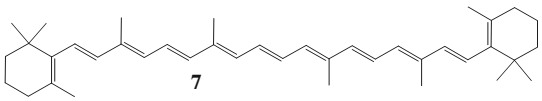
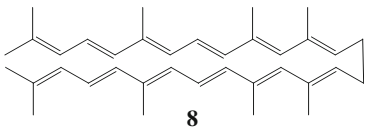
Terpenoids	Chemical structure	Source	References
Monoterpenes			
d-Limonene (**1**)	 **1**	Lemons, oranges, grapefruit, caraway, bergamot, dill, spearmint	[24]
Perillyl alcohol (**2**)	 **2**		
Diterpenes			
Retinol (**3**)	 **3**	Carrot, spinach, pumpkin, broccoli, mango, papaya, cherry, tomato, cabbage, corn, watermelon, lettuce	[46, 47]
Trans-retinoic acid (**4**)	 **4**		
Triterpenes			
Oleanic acid (**5**)	 **5**	Olives, figs, rosemary	[73, 75]

Table 1 continued

Terpenoids	Chemical structure	Source	References
Ursolic acid (**6**)			
Tetraterpenes			
Carotene (**7**)		Tomatoes, oranges, carrot, peas, sprouts, green beans, corn	[86, 88]
Lycopene (**8**)			

Monoterpenes

Monoterpenes are best known as secondary plant metabolites and constituents of essential oils, floral scents and defensive resins (both constitutive and induced) of aromatic plants [22, 23]. Monoterpenes are formed from geranyl diphosphate catalyzed by different terpene cyclases. Many monoterpenes are non-nutritive dietary components found in the essential oils of citrus fruits, cherry, mint, and herbs [24]. A number of dietary monoterpenes have anti-tumor activity, exhibiting not only the ability to prevent the formation or progression of cancer, but the ability to regress existing malignant tumors [25]. *d*-Limonene is the most abundant monocyclic monoterpene found in nature, and it occurs in a variety of trees and herbs. It is a major constituent of peel oil from oranges, citrus and lemons, and the essential oil of caraway. *d*-Limonene is a well-established chemopreventive and therapeutic agent against many tumor cells [10, 26] and has chemopreventive activity against rodent mammary cancer during the initiation phase as well as the promotion/progression phase [27] (Table 2).

The mevalonate pathway, also known as the cholesterol pathway, produces cholesterol and a number of nonsterol products, and pools of farnesyl diphosphate and other phosphorylated products of the mevalonate pathway are essential to the post-translational processing and physiological function of small G-proteins, nuclear lamins, and growth factor receptors. Inhibitors of enzyme activities providing those pools, namely, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and mevalonic acid pyrophosphate decarboxylase, and of enzyme activities requiring substrates from the pools, the protein prenyltransferases, have potential for development as novel chemopreventive and chemotherapeutic agents [28]. *d*-Limonene inhibits the post-translational isoprenylation of cellular proteins with apparent selectivity that dislodge all Ras isoforms from the membrane and alter the interaction of Ras-guanosine-5′-triphosphate (GTP) with downstream targets, a class of proteins that includes a subset of cellular growth control-associated proteins that are active only after post-translational modification [29]. This provides a correlation between *d*-limonene-mediated inhibition of HMG-CoA reductase and protein prenyltransferases [29]. Mammary tumors that regressed following exposure of the hosts to a diet containing 10% *d*-limonene had increased levels of both mannose-6-phosphate (M-6-P)/insulin-like growth factor (IGF)-II receptors and transforming growth factor (TGF)-β1 and the increase in M-6-P/IGF-II receptor appeared to result from alterations at both transcriptional

Table 2 Effect of terpenoids on breast cancer chemoprevention and their possible mechanisms

Terpenoids	Biological effects	Mechanisms	References
Monoterpenes			
d-Limonene and Perillyl alcohol	Inhibit the growth of MCF-7, T47D and MDA-MB-231 cells	⊥G_0/G_1 phase; ↓cyclin D1	[26]
	Inhibit rat mammary tumors	↑M-6-P/IGF-II; ↑TGF-β1; ↓ras; ↑CYP-2B1; ↑CYP-2C; ↑apoptosis; ↑redifferentiation	[27, 30, 32]
Sesquiterpenes			
Farnesol	Inhibits the growth of MCF-7 cells	↓ER	[42]
Diterpenes			
Retinoic acid	Induces apoptosis in MCF-7 cells	⊥G_0/G_1 phase; ↑RAR-β; ↓ER; ↓PR; ↓pS2	[55, 56]
N-(4-hydroxyphenyl) retinamide and retinyl acetate	Inhibit rat mammary tumor; reduce cancer incidence, multiplicity	↓TEBH; ↓CIS	[57, 59]
Calcium glucarate	Inhibits the growth of MCF-7 cells	⊥G_0/G_1 phase; ↑TGF-β; ↓PKC	[60]
	Inhibits rat mammary tumors	↑differentiation	
Triterpenes			
Asciatic acid	Inhibits the growth of MCF-7 and MDA-MB-231 cells	⊥S/G_2 + M phase; ↑apoptosis	[80, 81]
Pristimerin	Inhibits the growth of MDA-MB-231 cells	↑apoptosis	[92]
Withaferin A	Inhibits the growth of MCF-7 cells	↓Cyclin D1; ↓NF-κB	[94]
	Inhibits rat mammary tumors	↑apoptosis	[95]
CDDO	Induces apoptosis in MCF-7 cells	⊥G_0/G_1-S phase; ↓cyclin D1; ↓HER2; ↑PPARγ; ↓COX-2; ↓NF-κB; ↑caveolin-1	[97]
CDDO-Me	Induces apoptosis in and inhibits the growth of 4T1 cells	⊥G_2/M phase; ↓STAT3; ↓Src; ↓Akt; ↓c-myc	[98]
Betulinic acid	Inhibits the growth of MCF-7 cells	↑Bax; ↓Bcl-2; ↓cyclinD1; ↑apoptosis	[104]
AMR	Induces apoptosis in and inhibits the growth of MCF-7 and MDA-468 cells	⊥G_2/M phase; ↑p53; ↑Bax; ↓Bcl-2; ↑caspases; ↑cytochrome *c*; ↑PARP cleavage; ↑DNA fragmentation	[113, 114]
AMR-Me	Induces apoptosis in and inhibits the growth of MCF-7 cells	⊥G_2/M phase; ↑p53; ↓Bax; ↓Bcl-2; ↑caspases; ↑JNK; ↑p38; ↑PARP cleavage; ↑DNA fragmentation	[115]
Tetraterpenes			
β-Carotene	Inhibits the growth of MCF-7 and MDA-MB-468 cells	↓PCNA; ↓Ki67	[122, 123]
Lycopene	Inhibits the growth of MCF-7 cells	⊥G_0/G_1 phase; ↓PCNA; ↓Ki67; ↑BRCA1, BRCA2 mRNA and protein; ↑RARalph; ↑Cx43; ↑GSTP1	[130]
	Induces apoptosis in MDA-MB-231 cells	⊥G_0/G_1 phase; ↑RARalph; ↑Cx43	[130]
Lutein	Inhibits mice mammary tumors	↑GJIC; ↑pim-1; ↑differentiation; ↑apoptosis; ↑T-cells	[131–140]
Vitamin E succinate	Induces apoptosis in MCF-7 and MDA-MB-435 cells	⊥G_0/G_1 phase; ↓DNA synthesis; ↓Ki67; ↑differentiation; ↑p21; ↑ERK1/2; ↓Her2/neu; ↑cytokeratin 18; ↑PARP cleavage	[146]

and post-transcriptional levels [30]. Subsequent studies confirmed the monoterpene-induced increase in M-6-P/IGF-II receptor mRNA in regressing mammary tumors [31]. Perillyl alcohol, a hydroxylated analog of limonene, exhibits chemopreventive activity against rat mammary tumors [32]. TGF-β type 1 and 2 receptors mRNAs in mammary carcinomas responding to perillyl alcohol were significantly increased when compared to levels in surrounding tissues [33]. Perillyl alcohol transiently induced the expression of growth associated genes, c-jun and c-fos, components of activator protein (AP)-1. The impact of perillyl alcohol on c-fos and c-jun expression and c-jun

phosphorylation was dose-dependent [34]. *d*-Limonene and perillyl alcohol suppressed the incorporation of radiolabeled mevalonate into small G-proteins and this action has been attributed to the inhibition of farnesyl protein transferase activity [35]. Phase I studies of *d*-limonene [36], and phase I [37] and II [38] studies of perillyl alcohol revealed dose-limiting toxicities, such as nausea, vomiting, anorexia, and eructation.

The monoterpenoids carveol, uroterpenol, and sobrerol have demonstrated chemopreventive activity against mammary cancer in rats when fed during the initiation phase [39]. The chemopreventive effects of monoterpenes during the initiation phase of mammary carcinogenesis are due to the induction of phase II carcinogen-metabolizing enzymes, resulting in carcinogen detoxification through a blocking mechanism. The post-initiation phase chemopreventive and chemotherapeutic activities of monoterpenes may be due to the induction of tumor cell apoptosis, tumor redifferentiation, and/or inhibition of the post-translational isoprenylation of cell growth-regulating proteins [39, 40].

Sesquiterpenes

The sesquiterpene farnesol found in lemongrass, chamomile, and lavender shows promise as a more potent compound than either *d*-limonene or perillyl alcohol in vivo, and is in development for clinical breast cancer prevention [41]. Farnesol has been selected for clinical development through the National Cancer Institute's Rapid Access to Preventive Intervention Development (RAPID) program. In MCF-7 cells stably transfected with an estrogen receptor (ER) reporter gene, farnesol induces a decrease of ER levels and increases progesterone receptor expression while stimulating ER-mediated gene transactivation [42]. Parathenolide (PTL) is a sesquiterpene lactone found as the major active component in Feverfew (*Tanacetum parthenium*), an herbal medicine that has been used to treat migraine and rheumatoid arthritis for centuries. PTL has been found to have anti-tumor activity, and inhibits DNA synthesis and cell proliferation in different cell lines [43, 44].

Diterpenes

The diterpenes represent a large group of terpenoids with a wide range of biological activities, isolated from a variety of organisms. One of the simplest and most important acyclic diterpenes is phytol, a reduced form of geranylgeraniol. Among diterpenes, vitamin A or retinol is the most important compound. Retinoids, a class of over 3,000 natural derivatives and synthetic analogs of vitamin A, are powerful modulators of epithelial carcinogenesis [45, 46]. About 1,500 different retinoids have been synthesized by modifying the ring structure, the side chain, or the terminal group of the molecule in attempts to obtain greater anticarcinogenic activity and less toxicity. The naturally occurring retinoids include: retinol, the alcohol of vitamin A; retinoic acid, the carboxylic acid; retinal, the aldehyde; and 13-*cis*-retinoic acid, an isomer of retinoic acid. Retinoids, including vitamin A (retinol) and its active metabolite, retinoic acid, play important roles in inhibiting cell proliferation, and promoting morphogenesis and differentiation [47, 48], and in cellular and humoral immunity [49, 50].

There have been many studies demonstrating chemoprevention and chemotherapy with retinoids and their derivatives in a variety of rodent mammary gland, prostate, bladder, skin and liver tumor models [51, 52]. Retinoid receptors are expressed in normal and malignant epithelial breast cells, which are critical for normal development. Although the mechanism underlying breast cell growth inhibition by retinoids has not yet been completely elucidated, experimental evidence suggests that it is likely to involve multiple signal transduction pathways and to result from direct and indirect effects on gene expression. Binding of retinoids to the nuclear receptors, namely retinoic acid receptor (RAR)-α, -β and -γ and retinoid X receptor (RXR)-α, -β and -γ, which are ligand-activated transcription factors, leads to regulation of several cellular processes, including growth, differentiation and apoptosis [53]. Several retinoids are able to inhibit the AP-1 transcription pathway, which is activated upon growth factor signaling [54] and is involved in breast cancer cell proliferation and transformation [55]. In addition, growth inhibition of breast cancer cells by retinoic acid has been associated with induction of the expression of RAR-β, which may act as a tumor suppressor and appears to be down-regulated in breast cancer tissue and cell lines and, conversely, upregulated in normal mammary epithelial cells [56].

The glucuronide derivative of *N*-(4-hydroxyphenyl)retinamide exhibited higher anti-tumor action in vivo against 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced mammary tumors in rats, and had lower toxicity than its parent compound [57]. This suggests that the conjugate may have an in vivo chemopreventive advantage over the parent retinamide. *N*-(4-hydroxyphenyl)retinamide inhibited *N*-methyl-*N*-nitrosourea (MNU)-induced mammary tumorigenesis in rats given grain-based diet but enhanced carcinogenesis in rats given a casein-based semipurified diet due to the interactions between *N*-(4-hydroxyphenyl)retinamide and the diet resulting in lower levels of circulating *N*-(4-hydroxyphenyl)retinamide [58]. Selenium with retinyl acetate augmented the chemopreventive effect of retinyl acetate, whereas selenium alone had no effect on mammary carcinogenesis [59]. Calcium glucarate, glucaric

acid and its derivatives exhibited chemopreventive activity in the mammary gland in mice and increased detoxification of carcinogens and tumor promoters/progressors by inhibiting β-glucuronidase and preventing hydrolysis of their glucuronides [60, 61]. They are present in low concentrations in the diet and showed no toxicity even at a concentration of 5% in the diet of rats [60, 61]. The synthetic retinoid, fenretinide, has been studied extensively as a chemopreventive agent against breast cancer and is less toxic than many other retinoids [62]. Clinical studies indicate that breast cancer patients aged over 55 years with a higher percentage of adipose tissue had higher plasma levels of the fenretinide metabolite, *N*-(4-methoxyphenyl)retinamide [63]. Retinoid provides resistance to chemical carcinogenic challenge, while vitamin A deficiency in humans has been associated with an increased incidence of cancer in the breast [64]. Some studies showed that vitamin A may have a protective effect [65], an adverse effect [66], or no effect [67] against breast cancer. The mechanisms of anti-carcinogenic action of retinoids are believed to lie at the level of gene expression [68]. Retinoids modulate cell differentiation by increasing the expression of some oncogenes and their elaborated growth factors [69]. Retinoic acid positively regulated c-myc expression during its growth inhibitory effects in MCF-7 human breast carcinoma cells [70].

Sesterterpenes

Terpenes having 25 carbons and five isoprene units are rare relative to the other classes. Extracts of the marine sponge Thorectandra sp. have been found to contain sesterterpenes, thorectandrols A, B, C, D, and E, luffarin R, luffarin V and palaolide. Thorectandrol A and B and palauolol have tested for in vitro cytotoxic activity against human breast cancer MCF-7 cells and all three compounds inhibited the growth of the MCF-7 cells [71].

Triterpenoids

Triterpenoids represent a group of natural substances, which include steroids and consequently sterols [72]. Squaline is the immediate biological precursor of all triterpenoids. The large groups of steroids including sterols are present in very small amounts in bacteria but in large amounts in plants and animals while hapanoids are very abundant in prokaryotes where they replace cholesterol [73]. Triterpenoid have shown to possess anti-inflammatory and anti-carcinogenic properties [74]. Phytosterols, especially sitosterol, are plant sterols that have been shown to exert protective effects against many types of cancer [75]. They have been reported to protect against cancer development. However, the mechanism of this protection remains unknown even though several have been proposed. Many triterpenoids have shown promising effects when applied as anti-neoplastic agents [76]. Asiatic acid, a plant-derived triterpenoid compound, was extracted from the tropical medicinal plant *Centella asiatica* [77]. It has been found to prevent UVA-mediated photoaging, inhibit β-amyloid-induced neurotoxicity, and possess anti-ulcer and anti-hepatofibric activities [78, 79]. It also has been reported to exhibit a cytotoxic effect against HepG2 cells by Ca^{2+} release and p53 up-regulation and inhibited the growth of human MCF-7 and MDA-MB-231 breast cancer cells, which were accumulated in the $S/G_2 + M$ phase of the cell cycle, and underwent apoptosis in a dose- and time-dependent manner [80, 81].

Celastrol, a quinone methide triterpene derived from the medicinal plant *Tripterygium wilfordii*, has been used to treat chronic inflammatory and autoimmune diseases and known to inhibit the proliferation of a variety of tumor cells, including those from leukemia, gliomas, and prostate cancer [82]. Celastrol is also known to modulate the expression of proinflammatory cytokines, MHC-II antigen, inducible nitric oxide synthase (iNOS), adhesion molecules in endothelial cells, proteasome activity, topoisomerase II, potassium channels and heat shock response [83–85]. Celastrol is significantly active against MCF-7 human breast cancer cells with ED_{50} value of 0.34 μg/ml [86]. Celastrol methyl ester derivative pristimerin is found in various species belonging to Celastraceae and Hippocrateaceae. Some of these plants, such as *Maytenus chuchuhuasca* and *Maytenus laevis*, have been used traditionally in the treatment of arthritis and skin cancer in South America [87, 88]. Pristimerin exhibited anti-microbial, anti-inflammatory, anti-peroxidation, and anti-tumor effects [89] and has been reported to be effective in preventing inflammatory responses in several animal models [90]. In addition, pristimerin inhibited the induction of iNOS in macrophages by suppressing nuclear factor (NF)-κB activation, an effect which may be responsible for its anti-inflammatory activity [91]. Pristimerin induced caspase-dependent apoptosis in the human breast cancer cell line MDA-MB-231 and the nontumorigenic human mammary epithelial cell line MCF-10A is less sensitive to pristimerin [92]. Withaferin A is a steroidal lactone major constituent of the medicinal plant *Withania somnifera*, consumed as a dietary supplement around the world and used in the treatment of tumors and inflammation in several Asian countries [93]. Withaferin A and its derivatives exhibited half maximal inhibitory concentration (IC_{50}) values ranging from 0.24 to 11.6 μg/ml against MCF-7 human breast cancer cells. Withaferin A inhibited human umbilical vein endothelial cell (HUVEC) proliferation ($IC_{50} = 12$ nM) at doses that are significantly lower than those required for tumor cell lines through a process

associated with inhibition of cyclin D1 expression which are relevant to NF-κB-inhibitory activity [94]. In addition, withaferin A has been shown to exert potent anti-angiogenic activity in vivo at doses that are 500-fold lower compared to one that exerted anti-tumor activity in vivo [95], which highlights the potential use of this natural product for breast cancer treatment or prevention.

Several hundreds of new synthetic triterpenoids based on oleanolic acid have been synthesized recently and 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO), its methyl ester (CDDO-Me) and 1-(2-cyano-3,12-dioxo-oleana-1,9-dien-28-oyl) imidazole (CDDO-imidazolide) have potent anti-inflammatory, anti-oxidative, and anti-proliferative activities. They also suppress induction of iNOS by inflammatory stimuli, suppress induction of cyclooxygenase-2 (COX-2), induce an entire set of anti-oxidative enzymes, inhibit activity of the transcription factor NF-κB by directly inhibiting its activating kinase, IκB kinase [96–98], inhibit phosphorylation of signal transducers and activators of transcription (STAT) factors, which is required for transcriptional activity of the STATs and they inhibit the ability of tumor necrosis factor (TNF)-α to induce expression of vascular endothelial growth factor [99]. Synthetic triterpenoid CDDO is a highly potent inhibitor of the proliferation of several ER-positive and ER-negative human breast cancer cell lines. Furthermore, CDDO at nanomolar levels blocks de novo synthesis of two inflammatory enzymes that have recently been implicated in the carcinogenic process, namely iNOS and inducible COX-2 [100]. Ursolic acid and oleanolic acid are pentacyclic triterpenoids, which naturally occur in many medicinal herbs and plants used for medicinal purposes in many Asian countries. Recent research revealed that several pharmacological effects could be attributed to ursolic acid and oleanolic acid, such as anti-tumor and anti-inflammatory activities [101]. Treatment with ursolic acid suppressed phorbol-12-myristate-13-acetate (PMA)-mediated induction of COX-2 protein and synthesis of prostaglandin E2 by inhibiting the protein kinase C (PKC) signal transduction pathway in human mammary epithelial cells [102]. Ursolic acid blocked PMA-induced translocation of PKC activity from cytosol to membrane and the activation of extracellular signal-regulated kinases (ERKs), C-jun N-terminal kinases (JNKs) and p38 mitogen-activated protein kinases (MAPKs) [97]. Ursolic acid also inhibited the in vivo formation of mammary DMBA-DNA adducts and the initiation of DMBA-induced mammary tumorigenesis in female rats [103].

Betulinic acid (BA), a pentacyclic triterpene isolated from birch bark and other plants, selectively inhibits the growth of human cancer cell lines and does not exhibit toxicity in animals at higher concentrations. BA derivatives that are markedly more potent than BA for inhibiting iNOS, activating phase II cytoprotective enzymes, and inducing apoptosis in human breast cancer cells and in Bax/Bak$^{-/-}$ fibroblasts, which lack two key proteins involved in the intrinsic mitochondrial-dependent apoptotic pathway. Higher plasma and tissue levels of 1-(2-cyano-3-oxolupa-1,20(29)-dien-28-oyl)imidazole (CBA-Im), a new BA analogue, were observed compared with the levels of BA at concentrations that were active in vitro [104]. These findings suggest that BA may be a useful platform for drug development, and the enhanced potency and varied biological activities of CBA-Im make it a promising candidate for further chemoprevention or chemotherapeutic studies.

Apple phytochemical extracts have been shown to have potent anti-oxidant property and anti-proliferative activity against human cancer cells and to prevent mammary cancers in rats in a dose-dependent manner [105, 106]. Triterpenoids, 2α-hydroxyursolic acid and 3β-trans-*p*-coumaroyloxy-2α-hydroxyolean-12-en-28-oic acid isolated from apple peels displayed potent anti-proliferative activity against MCF-7 cancer cells [107].

Legumes, especially black beans (*Phaseolus vulgaris* L) are widely consumed in the world, and are a staple in Central America as a major source of protein, energy, vitamins and minerals. Triterpenoids like 3-*O*-[(β-D-glucopyranosyl) (1 → 2)-β-D-galactopyranosyl(1 → 2)-β-D-glucuronopyranosyl]olean-12-en-3β, 22β,24-triolmethylester,3-*O*-[α-L-rhamnopyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 2)-β-D-glucuronopyranosyl]olean-12-en-3β,22β,24-triol methyl ester, 3-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-glucuronopyranosyl] olean-12-en-3β,22β,24-triol, 3-*O*-[β-D-glucopyranosyl (1 → 2)-β-D-galactopyranosyl(1 → 2)-β-D-glucuronopyranosyl]olean-12-en-22-oxo-3β,24diol, and 3-*O*-[α-L-rhamno pyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 2)-β-D-glucuronopyranosyl]olean-12-en-22-oxo-3β,24-diol methyl ester isolated from black beans demonstrated potent anti-tumor activity in MCF-7 cell culture [108]. Triterpenes 3-epi-sodwanone K, 10,11-dihydrosodwanone B isolated from Axinella sp. inhibited both hypoxia-induced and iron chelator (1,10-phenanthroline)-induced hypoxia-induced factor (HIF)-1 activation in T47D breast tumor cells [109]. Friedelin, friedelan-1,3-dione and lup-20(29)-en-3β-ol are triterpenoids isolated from the stem bark of *Mesua daphnifolia* showed strong inhibitory effects against human ER-negative breast cancer MDA-MB-231 cells [110].

25-Hydroxy-3-oxoolean-12-en-28-oic acid (Fig. 1A), commonly known as amooranin (AMR), is a triterpene acid with a novel structure isolated by Rabi [111] from the stem bark of *Amoora rohituka*, a tropical tree growing wild in India. Recent studies by Rabi and colleagues [112–114] showed that multiple breast cancer cell lines respond to AMR in growth suppression assays. Mechanistic studies suggest that AMR suppresses growth factor signaling, induces cell cycle arrest, and promotes apoptosis [113,

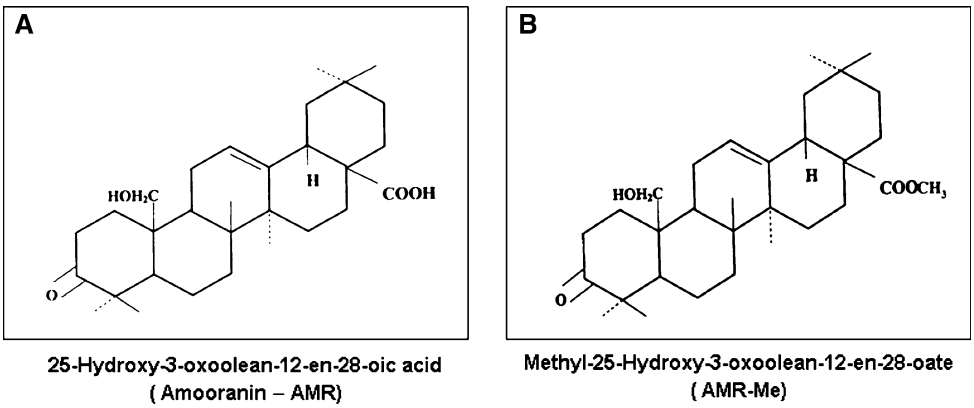


Fig. 1 Chemical structure of (**A**) AMR and (**B**) AMR-Me

114]. AMR-induced apoptosis in several human breast cancer cells are associated with the cleavage of caspase-8, -9, and -3; Bid and ER stress; release of cytochrome *c* from the mitochondria; cleavage of poly (ADP-ribose) polymerase (PARP); and DNA fragmentation with a concomitant upregulation of p53 and Bax, and down-regulation of Bcl-2 [113, 114]. Multiple tumor suppressors and oncogenes were identified as being regulated by AMR to mediate these tumor-suppressing activities [113]. In animal studies, intraperitoneal administration of AMR significantly reduced tumor size in MNU-induced mammary adenocarcinoma in rats with a concurrent prolongation of mean survival time in tumor-bearing animals [111]. Because the anti-neoplastic activity of the plant-derived compound AMR is relatively weak, new analogues of this molecule have been prepared by chemical transformations in an attempt to identify more potent agents. One of these analogues, AMR-Me (Fig. 1B), was found to inhibit proliferation of several breast cancer cells with greater potency than the parent compound AMR [112]. Preliminary screening of AMR-Me in in vitro experiments revealed an astonishing potency against breast cancer MCF-7 cells with concentrations down to the nanomolar range. Killing of MCF-7 cells proceeded more effectively ($IC_{50} = 0.5$ μM) than killing of normal breast epithelial cells, which required a 25-fold increase in the concentration of AMR-Me ($IC_{50} = 12.5$ μM). Moreover, AMR-Me has recently been reported by Rabi et al. [115] to be a potent inhibitor of cell growth by inducing MCF-7 cells to undergo apoptosis through a mitochondrial apoptotic pathway associated with DNA fragmentation and PARP degradation, preceded by changing the Bax:Bcl-2 ratios, cytochrome *c* release, and subsequent induction of caspases. AMR-Me also stimulated two different MAPK signaling pathways of p38 MAPK and JNK for amplifying the apoptosis cascade [115]. All these studies indicate that AMR-Me is a promising drug with potential to be used for human breast cancer prevention.

Tetraterpenoids

Carotenoids belong to the category of tetraterpenoids, derived from a 40-carbon polyene chain, which could be considered the backbone of the molecule. The hydrocarbon carotenoids are known as carotenes, while oxygenated derivatives of these hydrocarbons are known as xanthophylls. *β*-Carotene is a tetratepenoid distributed widely throughout the plant kingdom and is the predominant pigment in orange-flashed melan (Cucumismelo L) varieties [116]. Carotenoid group include α-carotene, *β*-carotene, lycopene, lutein, astaxanthin, cryptoxanthin and zeaxanthin [117]. Interest in *β*-carotene as a potential anticancer agent was established in the 1980s from the results of both case–control and cohort studies showing a consistent association for foods high in *β*-carotene and reduced risk of prostate cancer [118]. They possess anti-oxidant action as one of the presumed mechanisms of cancer preventive effects. Tomatoes are the major source of lycopene commercially. Although lycopene is the most abundant carotenoid in tomatoes, tomatoes also contain other potentially beneficial carotenoids such as α-carotene, *β*-carotene, lutein, phytoene, and phytofluene [119]. Carotenoids and vitamin E have been the focus of numerous studies because they may offer cellular protection against a variety of free radicals that can damage DNA. *β*-Carotene is the most commonly studied carotenoid with three studies reporting a non-significant inverse association with higher concentrations [120–122]. *β*-Carotene can also indirectly reduce the risk of breast cancer through conversion to retinol (pro-vitamin A) because retinol and related compounds are involved in the regulation of cell growth and differentiation. More recently, two studies evaluated additional carotenoids, namely *β*-cryptoxanthin, lutein, and lycopene [122, 123]. There was a significant dose response of reduced risk of breast cancer with higher lutein and *β*-cryptoxanthin concentrations and a threshold effect for lycopene [122, 123]. The overall influence of

β-cryptoxanthin, lutein, and lycopene on the enhancement of immune function, cellular protection against DNA damage, stimulation of gap junctional intercellular communication (GJIC), induction of detoxifying enzymes, and inhibition of cellular proliferation have been reported [124, 125]. α-Carotene may decrease the activity of cytochrome P450 1AA, an activator of procarcinogens, and it is effective in protecting lipid membranes from damage by free radicals and reactive species [126].

Lycopene is the most efficient quencher of singlet oxygen species, whereas lutein and zeaxanthin are scavengers of radical oxygen species [127]. Diet supplemented with lycopene at a concentration of 5.0×10^{-5} ppm significantly suppressed the mammary tumor development, which was associated with the decrease in the mammary gland activity of thymidylate synthetase, and serum levels of free fatty acid and prolactin. Body weight was little affected and no deleterious side effects of lycopene were detected. All results show that lycopene could be promising as a chemopreventive agent for mammary and other types of tumors [128]. Rats injected with lycopene-enriched tomato oleoresin or β-carotene (10 mg/kg, twice per week) for 2 weeks prior to tumor induction by DMBA and for an additional 16 weeks after carcinogen administration and high performance liquid chromatography analysis of carotenoids extracted from several tissues showed that both carotenoids were absorbed into blood, liver, mammary gland, and mammary tumors. The tomato oleoresin-treated rats developed significantly fewer tumors, and the tumor area was smaller than that of the unsupplemented rats. Rats receiving β-carotene showed no protection against the development of mammary cancer [129]. The antiproliferative properties of lycopene, the major tomato carotenoid, were compared with those of α- and β-carotene. Lycopene, delivered in cell culture medium from stock solutions in tetrahydrofuran, strongly inhibited proliferation of mammary MCF-7 human cancer cells with IC_{50} of 1–2 μM. α-Carotene and β-carotene were far less effective inhibitors and the inhibitory effect of lycopene was detected after 24 h of incubation, and it was maintained for at least 3 days. In contrast to cancer cells, human fibroblasts were less sensitive to lycopene, and the cells gradually escaped growth inhibition over time. In addition to its inhibitory effect, lycopene also suppressed IGF-I-stimulated growth. IGFs are major autocrine/paracrine regulators of mammary growth [130].

In animal models of breast cancers, lutein has been demonstrated to exhibit chemopreventive activity [131]. The mechanisms for a potential protective role of xanthophylls against carcinogenesis may include selective modulation of apoptosis, inhibition of angiogenesis, enhancement of GJIC, induction of cell differentiation, prevention of oxidative damage, and modulation of the immune system [132–135]. Oxidative metabolites of lutein, thought to arise from lutein's anti-oxidant mechanism of action, have been isolated and characterized from extracts of human serum and plasma [136]. However, lutein enhanced the recovery of cells from oxidative challenge by stimulating DNA strand break repair [137]. Protecting the immune system could enhance cell-mediated immune responses and consequently, resistance to tumor formation. In mice fed lutein-containing diets, lutein uptake by the spleen suggests a role for lutein in modulating immunity [138]. Lutein has been shown to enhance antibody production in response to T-dependent antigens in spleen cells in vitro, as well as in mice in vivo [139]. The numbers of immunoglobulin M- and G-secreting cells increased in vivo with lutein administration when mice were primed with T-dependent antigens [139]. Dose-related increases in the expression of the pim-1 gene, which is involved in early activation of T-cells, has been observed in splenic lymphocytes of mice fed lutein, but not β-carotene or astaxanthin [140].

Vitamin E is a general term used indiscriminately to refer to a group of eight different naturally occurring compounds known as tocopherols and tocotrienols, as well as synthetic vitamin E (a chemical mixture composed of 12.5% authentic *RRR*-α-tocopherol and 87.5% stereoisomers, namely, seven molecules produced during the manufacturing process that have the same number and types of atoms found in *RRR*-α-tocopherol linked in the same order but differing in their spatial arrangement) [141]. They are common in almonds, peanut oil and walnuts, which may explain why diets rich in these foods have consistently been shown to reduce the incidence of cancer [142, 143]. Much of the broad involvement of vitamin E in human metabolism is due to its role as the body's primary lipid soluble anti-oxidant. Tocopherols and tocotrienols are part of the body's highly effective anti-oxidant defense system, which consists of a network of anti-oxidants, interacting with and supporting each other. Anti-oxidants such as vitamin C, coenzyme Q10 and GSH are needed for effective recycling of tocopherols and tocotrienols. The unique power of both tocopherols and tocotrienols is their ability to break the chain reaction of lipid peroxidation by neutralizing peroxyl radicals to prevent the spread of free radical damage in cell membranes. Tocotrienols are more potent scavengers of the peroxy radical than α-tocopherol and provide far better protection against lipid peroxidation [144, 145]. Vitamin E succinate (VES) inhibits the growth of human breast cancers in culture by induction of DNA synthesis arrest, cellular differentiation, and apoptosis [146]. Inhibition of cell proliferation involves a G_0/G_1 cell-cycle block, mediated in part by MAP2K1 and ERK1 and upregulation of the key cell-cycle regulatory protein $p21^{waf1/cip1}$ [147]. Induction of differentiation is

characterized by morphological changes, elevated β-casein mRNA, expression of milk lipids, elevated cytokeratin 18 protein, and downregulation of Her2/neu protein expression [148]. Differentiation is mediated in part by activation of MAP2K1, ERK1/2, and phosphorylation of the transcription factor c-jun [149]. Of the multiple apoptotic signaling events modulated by VES, especially noteworthy are its ability to convert Fas/Fas ligand nonresponsive human breast cancer cells to Fas/Fas ligand responsiveness and to convert TGF-β nonresponsive breast cancer cells to TGF-β responsiveness. The restored signaling pathways converge on prolonged activation of JNK/c-jun, followed by translocation of Bax protein to the mitochondria, induction of mitochondria permeability transition, followed by cytochrome *c* release into the cytoplasm, activation of caspases-9 and -3, cleavage of PARP, and apoptosis [150]. Treatment of MDA-MB-435 breast cancer cells with α-tocopherol ether analog (TEA) restores both Fas/Fas ligand and TGF-β signaling pathways, which converge on JNK, followed by induction of apoptosis [151]. Of the vitamin E forms, δ-tocopherol; α-, γ-, and δ-tocotrienol; and derivatives VES and α-TEA selectively induce cancer cells to undergo apoptosis. The effect of palm tocotrienols and tocopherols on two human breast cancer cells lines, estrogen-responsive MCF-7 and estrogen-nonresponsive MDA-MB-435 was studied. It was found that tocotrienols inhibited cell growth strongly in both the presence and absence of estradiol. The γ- and δ-fractions of tocotrienols were most effective at inhibiting cell growth, while α-tocopherol was least effective [152]. In another study, δ-tocotrienol was shown to be the most potent inducer of apoptosis in both estrogen-responsive and estrogen-nonresponsive human breast cancer cells, and δ-tocopherol and α-tocotrienol were found to be least effective [153]. Although there are some agreement between inhibition of cell growth and induction of apoptosis in these studies, the differential results observed otherwise could be due to variations in two separate experimental conditions.

Breast cancer chemoprevention trials

The most promising research into breast cancer prevention was provided by four randomized placebo-controlled studies using the selective estrogen receptor modulator (SERM), tamoxifen [154]. Tamoxifen, a triphenylethylene, was introduced into clinical use on the basis of its now well-recognized estrogen antagonist activity in the breast by inhibiting the binding of estrogen-to-ERs. In addition to its effects in the breast, tamoxifen has an estrogen agonist effect in bone, liver, and uterus that may explain the favorable effects on inhibiting bone loss, improving serum lipid concentrations, and its effect of increasing the incidence of uterine cancer [154]. Tamoxifen was shown to induce regression of advanced breast malignancies. Complications of tamoxifen therapy include endometrial cancer and thromboembolic events, which are serious albeit rare. More common side effects include hot flashes, fluid retention, vaginal discharge, vaginal bleeding, and altered menses [155]. Estradiol induces the tumor-suppressor gene BRCA1 through an increase in DNA synthesis, which suggests that BRCA1 may serve as a negative modulator of estradiol-induced growth. Both prospective and retrospective genetic epidemiologic studies have demonstrated that women who carry mutations in either BRCA1 or BRCA2 genes are at very high risk for developing both breast and ovarian cancer. These women would seem to be ideal candidates for the use of tamoxifen as primary prevention of breast cancer, but there are no prospective data yet available that relate directly to these women [156]. The overall risk-to-benefit ratio for the use of tamoxifen in prevention remains unclear and longer follow-up of the current trials is required. Raloxifene is another SERM that has been shown clinically and experimentally to be anti-estrogenic in the breast and uterus. Raloxifene hydrochloride is a SERM that has anti-estrogenic effects on breast and endometrial tissue and estrogenic effects on bone, lipid metabolism, and blood clotting [157]. It is a benzothiophene with characteristics similar to but distinct from the triphenylethylene SERMs such as tamoxifen. During the past decade, a number of clinical trials have been conducted to assess the benefit of raloxifene on osteoporosis and fracture. After the publication of the results of the Breast Cancer Prevention Trial (BCPT) these osteoporosis trials also reported data related to the incidence of invasive breast cancer among women taking raloxifene compared to those taking placebo. The Multiple Outcomes of Raloxifene Evaluation (MORE) trial showed a reduction in breast cancer incidence of 76% in women treated for osteoporosis. Raloxifene seems to have a more favorable adverse effect profile than tamoxifen, especially regarding the uterus. These two SERMs are currently undergoing direct comparison in the Study of Tamoxifen and Raloxifene (STAR), which started in 1999.

Modulation of intermediate and endpoint biomarkers by terpenoids

Study of markers of risk and surrogate endpoint biomarkers (SEBs) holds great promise for cancer chemoprevention [158, 159]. The criteria for biomarker relevance are that they must be differentially expressed in normal and high-risk tissue, be closely linked to the causal pathway for cancer, be modified by the chemopreventive agent and with a shorter latency than cancer and finally, be assayed easily and with quantitative reliability. Studies reported in the literature have shown that

terpenoids have the potential to modify certain proteins and transcription factors, which could be used as intermediate and endpoint markers to evaluate the efficacy of the test compound. Accumulating evidence indicates that COX-2 inhibitors may be involved in breast cancer prevention [160]. Interest in breast cancer chemoprevention with COX-2 inhibitors has been stimulated by epidemiological observations that the use of aspirin and other NSAIDs is associated with the reduced incidence of breast cancer. Two isoforms of COX have been identified: COX-1, the constitutive isoform, and COX-2, the inducible form of the enzyme. COX-2 can undergo rapid induction in response to chemical carcinogens [161]. It has been suggested that COX-2 overexpression may lead to increased mutagenesis, mitogenesis, angiogenesis, inflammatory reaction and deregulation of apoptosis [162, 163]. Therefore the inhibition of COX-2 might have a general cancer preventive effect via anti-inflammatory activity and decrease angiogenesis. The triterpenoid CDDO-Me has already been proven effective in inhibiting COX-2 in breast cancer cells, and blocked the growth of breast cancer cells in mice.

In chemically induced mammary carcinogenesis models, especially those which are initiated by DMBA, investigations focused on pathogenic changes after DMBA administration to elucidate the mechanisms of carcinogenesis and DMBA-DNA adduct formation in mammary tissue. Most chemical carcinogens need activation by body enzymes to be transformed to a species that readily binds to genetic DNA to form DNA adducts [164]. Carcinogen-DNA adduct formation is an important DNA damage marker that predicts the possibility of cancer development. Carcinogen-DNA adducts can be repaired by body enzymes. The unrepaired adducts will be fixed after one cell cycle and the unrepaired, fixed DNA damage will be responsible for mutation and consequent breast cancer development. Therefore, preventing carcinogen-DNA adduct formation is a key step in breast cancer prevention at the initiation step of carcinogenesis [165].

Histology-based biomarkers are on the causal pathway to cancer and include preinvasive intraepithelial neoplasias such as carcinoma in situ of the breast, cervix, and prostate [166]. These lesions may be valid as SEBs for cancer incidence. Breast cancer initiates as the premalignant stage of atypical ductal hyperplasia (ADH), progresses into the preinvasive stage of ductal carcinoma in situ (DCIS), and culminates into the potentially lethal stage of invasive ductal carcinoma (IDC). COX-2 can undergo rapid induction in response to chemical carcinogens [166]. Histologic parameters defined by computer-assisted nuclear morphometry represent an extension of the pathologist in quantitating the nuclear morphologic characteristics of the cancer phenotype.

Cellular and molecular biomarkers are presumed to have biological relevance to carcinogenesis, including measures of proliferation, apoptosis, differentiation, and growth factor-mediated signal transduction. Some of these are proving to be closely correlated with changes in preinvasive lesions, telomerase activity and thus could serve as potential SEBs for breast cancer. Recent evidence suggests, however, that under certain circumstances, overexpression of the ornithine decarboxylase can function as an oncogene and contribute to the invasive potential of epithelial cancers [167]. Several lines of evidence support the biological role of the IGF family of ligands/receptors in the proliferation of breast cancer cells [168]. DNA microarray analysis shows that glutathione peroxidase (Gpx) 2 was commonly up-regulated in mammary carcinomas induced by the three carcinogens, MNU, DMBA and 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP) due to activation of ER-α via the Raf/Ras/MAPK cascade. In addition, it has been reported that the forced suppression of Gpx2 expression by siRNA resulted in significant growth inhibition in rat and human mammary carcinoma cell lines with wild type p53 cells indicating that Gpx2 may be a novel target for the prevention and therapy of breast cancer [169].

Conclusion

The future of terpenoid research remains open to innovation, with a specific need to emphasize important beneficial properties for human health. The biological role of terpenoids in the prevention and perhaps treatment of cancer and other chronic diseases is being studied and more information constantly added that improves our understanding of the mechanisms associated with these compounds. Although the anti-oxidant properties of some terpenoids have been extensively studied, their role as anti-cancer agents needs further investigation. The simple reason for this dearth of information could be that tumors have many molecular targets that function aberrantly in concert, and therefore requires extensive research. Cancer chemopreventive agents should be safe and non-toxic. It would be best if promising agents can be screened by first identifying biomarkers in breast cancer cells that will quickly tell researchers whether or not potential chemopreventive drugs are having any effect. Validation of SEBs for clinical cancer is essential to reduce the scope and duration of chemoprevention trials. This is important because long-term chemoprevention trials are expensive and take a long time to conduct. Tamoxifen is highly effective in preventing ER-positive breast cancer, but has no effect on the risk of ER-negative disease. Its use in patients, who develop ER-negative disease can, in fact, be harmful due to

its adverse effects. Identification of women most at risk of developing ER-positive disease could therefore lead to a more effective chemoprevention strategy. In a randomized trial of fenretinide to prevent a second breast malignancy in women with early breast cancer, the investigators observed no significant effect after five years of treatment. Research must be initiated in order to identify other agents that may be effective for patients at risk of developing ER-negative breast cancer.

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References

1. American Cancer Society (2008) Cancer facts and figures. American Cancer Society, Atlanta
2. Wong JS, Harris JR (2001) Importance of local tumor control in breast cancer. Lancet Oncol 2:11–17. doi:10.1016/S1470-2045(00)00190-X
3. Kakizoe T (2003) Chemoprevention of cancer: focusing on clinical trials. Jpn J Clin Oncol 3:421–442. doi:10.1093/jjco/hyg090
4. National Cancer Institute (2007) Highlights of NCI's prevention and control programs. National Cancer Institute, Bethesda
5. Shukla S, Gupta S (2005) Dietary agents in the chemoprevention of prostate cancer. Nutr Cancer 53:18–32. doi:10.1207/s15327914nc5301_3
6. Gupta S (2007) Prostate cancer chemoprevention: current status and future prospects. Toxicol Appl Pharmacol 224:369–376. doi:10.1016/j.taap. 2006.11.008
7. Lippman SM, Benner SE, Hong WK (1994) Cancer chemoprevention. J Clin Oncol 12:851–873
8. Wattenberg LW (1996) Chemoprevention of cancer. Prev Med 25:44–45. doi:10.1006/pmed.1996.0015
9. Wattenberg LW (1993) Prevention, therapy, and basic science and the resolution of the cancer problem. Cancer Res 53:5890–5896
10. Wagner KH, Elmadfa I (2003) Biological relevance of terpenoids. Ann Nutr Metab 47:95–106. doi:10.1159/000070030
11. Mirvish SS (1981) Inhibition of the formation of carcinogenic *N*-nitroso compounds by ascorbic acid and other compounds. In: Burchenal JH, Oettgen HF (eds) Cancer achievements, challenges and prospects of the 1980s. Grune and Stratton, New York
12. Wattenberg LW (1975) Inhibition of dimethyl hydrazine-induced neoplasia of the large intestine by disulfiram. J Natl Cancer Inst 54:1005–1006
13. Miller EC (1978) Some current perceptive on chemical carcinogenesis in human and experimental animals. Cancer Res 38:1479–1496
14. Newmark H, Mergens W (1981) α-Tocopherol (vitamin E) and its relationship to tumor induction. In: Zedeck MS, Lipken M (eds) Inhibition of tumor induction and development. Plenum Publishing Corp, New York
15. Wattenberg LW (1983) Inhibition of neoplasia by minor dietary constituents. Cancer Res 43:2448–2453
16. Sporn MB (1983) Retinoids and suppression of carcinogenesis. Hosp Pract 18:83–98
17. Rohdich F, Bacher A, Eisenreich W (2005) Isoprenoid biosynthetic pathways as anti-infective drug targets. Biochem Soc Trans 33:785–791. doi:10.1042/BST0330785
18. Withers ST, Keasling JD (2007) Biosynthesis and engineering of isoprenoid small molecules. Appl Microbiol Biotechnol 73:980–990. doi:10.1007/s00253-006-0593-1
19. Cragg GM, Newman DJ (2005) Plants as a source of anti-cancer agents. J Ethnopharmacol 100:72–79. doi:10.1016/j.jep.2005.05.011
20. Srivastava V, Negi AS, Kumar JK, Gupta MM, Khanuja SPS (2005) Plant-based anticancer molecules: a chemical and biological profile of some important leads. Bioorg Med Chem 13:5892–5908. doi:10.1016/j.bmc.2005.05.066
21. Cragg GM, Newman DJ (2003) Plants as a source of anti-cancer and anti-HIV agents. Ann Appl Biol 143:127–133. doi:10.1111/j.1744-7348.2003.tb00278.x
22. Loza-Tavera H (1999) Monoterpenes in essential oils: biosynthesis and properties. Adv Exp Med Biol 464:49–62
23. Little DB, Croteau R (1999) Biochemistry of essential oil plants: a thirty year overview. In: Teranishi R, Wick EL, Hornstein I (eds) Flavor chemistry: thirty years of progress. Kluwer Academic, New York
24. Kris-Etherton PM, Kecker KD, Bonanoma A (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 113:71–88. doi:10.1016/S0002-9343(01)00995-0
25. Crowell PL, Gould MN (1994) Chemoprevention and therapy of cancer by *d*-limonene. CRC Crit Rev Oncol 5:1–22
26. Bardon S, Picard K, Martel P (1998) Monoterpenes inhibit cell growth, cell cycle progression, and cyclin D1 gene expression in human breast cancer cell lines. Nutr Cancer 32:1–7
27. Elegbede JA, Elson CE, Qureshi A, Tanner MA, Gould MN (1984) Inhibition of DMBA-induced mammary cancer by the monoterpene *d*-limonene. Carcinogenesis 5:661–664. doi:10.1093/carcin/5.5.661
28. Mo H, Elson CE (2004) Studies of the isoprenoid-mediated inhibition of mevalonate synthesis applied to cancer chemotherapy and chemoprevention. Exp Biol Med 229:567–585
29. Cox AD, Der CJ (1997) Farnesyltransferase inhibitors and cancer treatment: targeting simply ras? Biochim Biophys Acta 1333:51–71
30. Jirtle RL, Haag JD, Ariazi EA, Gould MN (1993) Increased mannose 6-phosphate/insulin-like growth factor II receptor and transforming growth factor β1 levels during monoterpene-induced regression of mammary tumors. Cancer Res 53:3849–3852
31. Rasmussen AA, Cullen KJ (1998) Paracrine/autocrine regulation of breast cancer by the insulin-like growth factors. Breast Cancer Res Treat 47:219–233. doi:10.1023/A:1005903000777
32. Haag JD, Gould MN (1994) Mammary carcinoma regression induced by perillyl alcohol, a hydroxylated analog of limonene. Cancer Chem Pharm 34:477–483. doi:10.1007/BF00685658
33. Dennis PA, Rifkin DB (1991) Cellular activation of latent transforming growth factor β requires binding to the cation-independent mannose-6-phosphate/insulin-like growth factor type II receptor. Proc Natl Acad Sci USA 88:580–584. doi:10.1073/pnas.88.2.580
34. Satomi Y, Miyamoto S, Gould MN (1999) Induction of AP-1 activity by perillyl alcohol in breast cancer cells. Carcinogenesis 20:1957–1966. doi:10.1093/carcin/20.10.1957
35. Hohl RJ, Lewis K (1995) Differential effects of monoterpenes and lovastatin on RAS processing. J Biol Chem 270:17508–17512. doi:10.1074/jbc.270.29.17508
36. Vigushin DM, Poon GK, Boddy A et al (1998) Phase I and pharmacokinetic study of *d*-limonene in patients with advanced cancer. Cancer research campaign phase I/II clinical trials

committee. Cancer Chemother Pharmacol 42:111–117. doi:10.1007/s002800050793
37. Ripple GH, Gould MN, Stewart JA et al (1998) Phase I clinical trial of perillyl alcohol administered daily. Clin Cancer Res 4:1159–1164
38. Liu G, Oettel K, Bailey H (2003) Phase II trial of perillyl alcohol (NSC 641066) administered daily in patients with metastatic androgen independent prostate cancer. Invest New Drugs 21:367–372. doi:10.1023/A:1025437115182
39. Crowell PL, Kennan WS, Vedejs E et al (1990) Chemoprevention of mammary carcinogenesis by hydroxylated metabolites of limonene. In: 81st annual meeting of the American association for cancer research. Proc Am Assoc Cancer Res, 23–26 May, Washington, DC, USA
40. Ariazi EA, Gould MN (1996) Identifying differential gene expression in monoterpene-treated mammary carcinomas using subtractive display. J Biol Chem 271:29286–29294. doi:10.1074/jbc.271.46.29286
41. Crowell PL (1999) Prevention and therapy of cancer by dietary monoterpenes. J Nutr 129:775–778
42. Journe F, Laurent G, Chaboteaux C et al (2008) Farnesol, a mevalonate pathway intermediate, stimulates MCF-7 breast cancer cell growth through farnesoid-X-receptor-mediated estrogen receptor activation. Breast Cancer Res Treat 107:49–61. doi:10.1007/s10549-007-9535-6
43. Zhang S, Ong CN, Shen HM (2004) Clinical roles of intracellular thiols and calcium in parthenolide-induced apoptosis in human colorectal cancer cells. Cancer Lett 208:143–153. doi:10.1016/j.canlet.2003.11.028
44. Wiedhopf RM, Young M, Bianchi E et al (1973) Tumor inhibitory agent from *Magnolia grandiflora* (Magnoliaceae) I: parthenolide. J Pharm Sci 62:345–350. doi:10.1002/jps.2600620244
45. Lippman SM, Kessler JF, Meyskens FL (1987) Retinoids as preventive and therapeutic anticancer agents (part I). Cancer Treat Rep 71:391–405
46. Tallman MS, Wiernik PH (1992) Retinoids in cancer treatment. J Clin Pharmacol 32:868–888
47. Lotan R (1980) Effects of vitamin A and its analogs (retinoids) on normal and neoplastic cells. Biochim Biophys Acta 605: 33–91
48. Sporn MB, Roberts AB (1983) The role of retinoids in differentiation and carcinogenesis. Cancer Res 43:3034–3040
49. Cai D, Webber MM, DeLuca LM (1991) Retinoids enhance lectin binding to gp130, a glycoprotein of NIH-3T3 cells: correlation with cell growth and adhesion. Exp Cell Res 192:366–372. doi:10.1016/0014-4827(91)90053-W
50. Kirven MJ, Wolf G (1991) Synthesis and glycosylation of fibronectin in hepatocytes from vitamin A-deficient rats. Mol Cell Biochem 101:101–114. doi:10.1007/BF00229528
51. Grubbs CJ, Moon RC, Sporn MB (1977) Inhibition of mammary cancer by retinyl methyl ether. Cancer Res 37:599–602
52. Thompson HJ, Becci PJ, Moon RC (1980) Inhibition of a methyl-1-nitrosurea-induced mammary carcinogenesis in the rat by the retinoid axerophthene. Arzneimittelforschung 30:1127–1129
53. Someone AM, Tari AM (2004) How retinoids regulate breast cancer cell proliferation and apoptosis? Cell Mol Life Sci 61:1475–1484
54. Fanjul A, Dawson MI, Hobbs PD et al (1994) A new class of retinoids with selective inhibition of AP-1 inhibits proliferation. Nature 372:107–111. doi:10.1038/372107a0
55. Darro F, Cohen P, Vianna A et al (1998) Growth inhibition of human in vitro and mouse in vitro and in vivo mammary tumor models by retinoids in comparison with tamoxifen and the RU-486 anti-progestagen. Breast Cancer Res Treat 51:39–55. doi:10.1023/A:1006098124087
56. Brtko J (2007) Role of retinoids and their cognate nuclear receptors in breast cancer chemoprevention. Cent Eur J Public Health 15:3–6
57. Abou-Issa H, Curley RW Jr, Panigot MJ, Wilcox KA, Webb TE (1993) In vivo use of *N*-(4-hydroxyphenyl retinamide)-*O*-glucuronidee as a breast cancer chemopreventive agent. Anticancer Res 13:1431–1436
58. Cohen LA, Epstein M, Saa-Pabon V (1994) Interactins between 4-HPR and diet in NMU-induced mammary tumorigenesis. Nutr Cancer 21:271–283
59. Thomson HJ, Meeker LD, Becci PJ (1981) Effect of combined selenium and retinyl acetate treatment on mammary carcinogenesis. Cancer Res 41:1413–1416
60. Walaszek Z, Hanausek-Walaszek M (1987) Dietary glucarate inhibits rat mammary tumorigenesis induced by *N*-methyl-*N*-nitrosourea. Proc Am Assoc Cancer Res 28:153–158
61. Bhatnagar RH, Abou-Issa RW, Curley A (1991) Growth suppression of human breast carcinoma cells in culture by *N*-(4-hydroxyphenyl) retinamide, its glucuronide and through synergism with glucarate. Biochem Pharmacol 41:1471–1477. doi:10.1016/0006-2952(91)90563-K
62. Graham S, Marshall J, Mettlin C (1982) Diet in the epidemiology of breast cancer. Am J Epidemiol 116:68–75
63. Torrisi RS, Parodi S, Fontana V (1994) Factors affecting plasma retinol decline during long-term administration of the synthetic retinoid fenretinide in breast cancer patients. Cancer Epidemiol Biomarkers Prev 3:507–510
64. Farias EF, Ong DE, Ghyselinck NB et al (2004) Cellular retinol-binding protein I, a regulator of breast epithelial retinoic acid receptor activity, cell differentiation and tumorigenesis. J Natl Cancer Inst 97:21–29
65. Katsouyanni KW, Willett D, Trichopoulos D (1988) Risk of breast cancer among Greek women in relation to nutrient intake. Cancer 61:181–185. doi:10.1002/1097-0142(19880101)61:1<181::AID-CNCR2820610130>3.0.CO;2-J
66. Richardson S, Gerber M, Cenee S (1991) The role of fat, animal protein and some vitamin consumption in breast cancer: a case–control study in Southern France. Int J Cancer 48:1–9
67. Toniolo P, Riboli E, Protta F (1989) Calorie-providing nutrients and risk of breast cancer. J Natl Cancer Inst 81:278–286. doi:10.1093/jnci/81.4.278
68. Miller RC (1981) Modification of sister chromatic exchanges and radiation-induced transformation in rodent cells by the tumor promoter 12-*O*-tetradecanoylphorbhol-13-acetate and two retinoids. Cancer Res 41:655–660
69. Sporn MB, Roberts AB, Roche NS (1986) Mechanism of action by retinoids. J Am Acad Dermatol 15:756–764
70. Sheikh MS, Shao ZM, Chen JC (1993) Retinoid modulation of c-myc and max gene expression in human breast carcinoma. Anticancer Res 13:1387–1392
71. Charan RD, McKee TC, Boyd MR (2002) Thorectandrrols C, D, and E: new sesterterpenes from the marine sponge Thorectandra sp. J Nat Prod 65:492–495. doi:10.1021/np010439k
72. Beveridge TH, Li TS, Drover JC (2002) Phytosterol content in American ginseng seed oil. J Agric Food Chem 50:744–750. doi:10.1021/jf010701v
73. Kakuda R, Iijima T, Yaolta Y (2002) Triterpenoids from *Gentiana scabra*. Phytochemistry 59:791–794. doi:10.1016/S0031-9422(02)00021-3
74. Manez S, Recio MC, Giner RM et al (1997) Effect of selected triterpenoids on chronic dermal inflammation. Eur J Pharmacol 334:103–105. doi:10.1016/S0014-2999(97)01187-4
75. Moghadasian MH (2000) Pharmacological properties of plant sterols: in vivo and in vitro observations. Life Sci 67:605–615. doi:10.1016/S0024-3205(00)00665-2

76. Kim KB, Lotan R, Yue P et al (2002) Identification of a novel synthetic triterpenoid, methyl-2-cyano-3, 12-dioxoole-ane-1, 9-dien-28-oate, that potently induces caspase-mediated apoptosis in human lung cancer cells. Mol Cancer Ther 1:177–184
77. Coldren CD, Hashim P, Ali J et al (2003) Gene expression changes in the human fibroblast induced by *Centella asiatica* triterpenoids. Planta Med 69:725–732. doi:10.1055/s-2003-42791
78. Jew SS, Yoo CH, Lim DY et al (2000) Structure–activity relationship study of asiatic acid derivatives against beta amyloid (A beta)-induced neurotoxicity. Bioorg Med Chem Lett 10:119–121. doi:10.1016/S0960-894X(99)00658-7
79. Lee MK, Kim SR, Sung SH et al (2000) Asiatic acid derivatives protect cultured cortical neurons from glutamate-induced cytotoxicity. Res Commun Mol Pathol Pharmacol 108:75–86
80. Lee YS, Jin DQ, Kwon EJ et al (2002) Asiatic acid, a triterpene, induces apoptosis through intracellular Ca^{2+} release and enhanced expression of p53 in HepG2 human hepatoma cells. Cancer Lett 186:83–91. doi:10.1016/S0304-3835(02)00260-4
81. Hsu YL, Kuo PL, Lin LT et al (2005) Asiatic acid, a triterpene, induces apoptosis and cell cycle arrest through activation of extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways in human breast cancer cells. J Pharmacol Exp Ther 313:333–344. doi:10.1124/jpet.104.078808
82. Nagase M, Oto J, Sugiyama S et al (2003) Apoptosis induction in HL-60 cells and inhibition of topoisomerase II by triterpene celastrol. Biosci Biotechnol Biochem 67:1883–1887. doi:10.1271/bbb.67.1883
83. Yang H, Chen D, Cui QC et al (2006) Celastrol, a triterpene extracted from the Chinese "Thunder of God Vine", is a potent proteasome inhibitor and suppresses human prostate cancer growth in nude mice. Cancer Res 66:4758–4765. doi:10.1158/0008-5472.CAN-05-4529
84. Huang FC, Chan WK, Moriarty KJ et al (1998) Novel cytokine release inhibitors Part I: triterpenes. Bioorg Med Chem Lett 8:1883–1886. doi:10.1016/S0960-894X(98)00331-X
85. Jin HZ, Hwang BY, Kim HS et al (2002) Antiinflammatory constituents of *Celastrus orbiculatus* inhibit the NF-kappaB activation and NO production. J Nat Prod 65:89–91. doi:10.1021/np010428r
86. Chang FR, Hayashi K, Chen IH et al (2003) Antitumor agents. 228. Five new agarofurans, reissantins A-E, and cytotoxic principles from *Reissantia buchananii*. J Nat Prod 66:1416–1420. doi:10.1021/np030241v
87. Gonzalez GJ, Monache DG, Monache DF et al (1982) Chuchuhuasha-a drug used in folk medicine in the Amazonian and Andean areas. A chemical study of *Maytenus laevis*. J Ethnopharmacol 5:73–77. doi:10.1016/0378-8741(82)90022-8
88. Shirota O, Morita H, Takeya K et al (1994) Cytotoxic aromatic triterpenes from *Maytenus ilicifolia* and *Maytenus chuchuhuasca*. J Nat Prod 57:1675–1681. doi:10.1021/np50114a009
89. Dirsch V, Wiemann W, Wagner H (1992) Antiinflammatory activity of triterpene quinine-methides and proanthocyanidines from the stem bark of *Heisteria pallida*. Engl Pharm Pharmacol Lett 2:184–186
90. Hui B, Wu YJ, Wang H et al (2003) Effect of pristimerin on experimental inflammation in mice and rats. Chin Pharm Bull 19:656–659
91. Dirsch VM, Kiemer AK, Wagner H et al (1997) The triterpenoid quinonemethide pristimerin inhibits induction of inducible nitric oxide synthase in murine macrophages. Eur J Pharmacol 336:211–217. doi:10.1016/S0014-2999(97)01245-4
92. Shao ZM, Dawson MI, Li XS et al (1995) P53 independent G_0/G_1 arrest and apoptosis induced by a novel retinoid in human breast cancer cells. Oncogene 11:493–504
93. Yang H, Shi G, Dou QP (2007) The tumor proteosome is a primary target for the natural anticancer compound Withaferin A isolated from 'Indian Winter Cherry'. Mol Pharmacol 71:426–437. doi:10.1124/mol.106.030015
94. Mohan R, Hammers HJ, Bargagna-Mohan P et al (2004) Withaferin A is a potent inhibitor of angiogenesis. Angiogenesis 7:115–122. doi:10.1007/s10456-004-1026-3
95. Jayaprakasam B, Zhang Y, Seeram NP et al (2003) Growth inhibition of human tumor cell lines by Withanolides from *Withania somnifera* leaves. Life Sci 74:125–132. doi:10.1016/j.lfs.2003.07.007
96. Liby KT, Yore MM, Sporn MB (2007) Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer. Nat Rev Cancer 7:357–369. doi:10.1038/nrc2129
97. Suh N, Wang Y, Honda T et al (1999) A novel synthetic oleanane triterpenoid, 2-cyano-3, 12-dioxoolean-1, 9-dien-28-oic acid, with potent differentiating, antiproliferative, and anti-inflammatory activity. Cancer Res 59:336–341
98. Ahmad R, Raina D, Meyer C (2006) Triterpenoid CDDO-Me blocks the NF-κB pathway by direct inhibition of IKKβ on cys-179. J Biol Chem 281:35764–35769. doi:10.1074/jbc.M607160200
99. Ling X, Konopleva M, Zeng Z (2007) The novel triterpenoid C-28 methyl ester of 2-cyano-3, 12-dioxoolen-1, 9-dien-28-oic acid inhibits metastatic murine breast tumor growth through inactivation of STAT3 signaling. Cancer Res 67:4210–4218. doi:10.1158/0008-5472.CAN-06-3629
100. Hyer ML, Croxton R, Krajewska M et al (2005) Synthetic triterpenoids cooperate with tumor necrosis factor-related apoptosis-inducing ligand to induce apoptosis of breast cancer cells. Cancer Res 65:4799–4808. doi:10.1158/0008-5472.CAN-04-3319
101. Furtado RA, Rodrigues EP, Araujo FR et al (2008) Ursolic acid and oleanolic acid suppress preneoplastic lesions induced by 1,2-dimethylhydrazine in rat colon. Toxicol Pathol (May):8. Epub ahead of print
102. Subbaramiah K, Michaluart P, Sporn MB et al (2000) Ursolic acid inhibits cyclooxygenase-2 transcription in human mammary epithelial cells. Cancer Res 60:2399–2404
103. Singletary K, MacDonald C, Wallig M (1996) Inhibition by rosemary and carnosol of 7, 12-dimethylbenz[a]anthracene (DMBA)-induced rat mammary tumorigenesis and in vivo DMBA-DNA adduct formation. Cancer Lett 104:43–48. doi:10.1016/0304-3835(96)04227-9
104. Liby K, Honda T, Williams CR et al (2007) Novel semisynthetic analogues of betulinic acid with diverse cytoprotective, antiproliferative, and proapoptotic activities. Mol Cancer Ther 6:2113–2119. doi:10.1158/1535-7163.MCT-07-0180
105. Yoon H, Liu RH (2007) Effect of selected phytochemicals and apple extracts on NF-κB activation in human breast cancer MCF-7 cells. J Agric Food Chem 55:3167–3174. doi:10.1021/jf0632379
106. Liu RH, Liu J, Chen B (2005) Apples prevent mammary tumors in rats. J Agric Food Chem 53:2341–2343. doi:10.1021/jf058010c
107. He X, Liu RH (2007) Triterpenoids isolated from apple peels have potent antiproliferative activity and may be partially responsible for apple's anticancer activity. J Agric Food Chem 55:4366–4370. doi:10.1021/jf063563o
108. Dong M, He X, Liu RH (2007) Phytochemicals of black bean seed coats: isolation, structure elucidation, and their antiproliferative and antioxidant activities. J Agric Food Chem 55:4366–4370. doi:10.1021/jf070706d
109. Dai J, Fishback JA, Zhou YD, Nagle DG (2006) Sodwanone and yardenone triterpenes from a South African species of the marine sponge Axinella inhibit hypoxia-inducible factor-1 (HIF-1)

activation in both breast and prostate tumor cells. J Nat Prod 69:1715–1720. doi:10.1021/np060278q
110. Ee GC, Lim CK, Rahmat A, Lee HL (2005) Cytotoxic activities of chemical constituents from *Mesua daphnifolia*. Trop Biomed 22:99–102
111. Rabi T (1996) Antitumor activity of amooranin from *Amoora rohituka* stem bark. Curr Sci 70:80–81
112. Rabi T, Karunagaran D, Krishnan Nair M, Bhattathiri VN (2002) Cytotoxic activity of amooranin and its derivatives. Phytother Res 16:S84–S86. doi:10.1002/ptr.803
113. Rabi T, Ramachandran C, Fonseca HB, Alomo A, Melnick SJ, Escalon E (2003) Novel drug amooranin induces apoptosis through caspase activity in human breast carcinoma cell lines. Breast Cancer Res Treat 80:321–330. doi:10.1023/A:1024911925623
114. Rabi T, Wang L, Banerjee S (2007) Novel triterpenoid 25-hydroxy-3-oxoolean-12-en-28-oic acid induces growth arrest and apoptosis in breast cancer cells. Breast Cancer Res Treat 101:27–36. doi:10.1007/s10549-006-9275-z
115. Rabi T, Banerjee S (2007) Novel synthetic triterpenoid AMR-Me induces apoptosis through JNK and p38 MAPK pathways in MCF-7 human breast cancer cells. Mol Carcinog 47:415–423. doi:10.1002/mc.20399
116. Keijer J, Bunschoten A, Palou A et al (2005) Beta-carotene and the application of transcriptomics in risk-benefit evaluation of natural dietary components. Biochim Biophys Acta 1740: 139–146
117. Krinsky I, Johnson EJ (2005) Carotenoid actions and their relation to health and disease. Mol Aspects Med 26:459–516. doi:10.1016/j.mam.2005.10.001
118. Peters U, Leitzmann ME, Chatterjee N (1997) Serum lycopene, other carotenoids and prostate cancer risk: a nested case–control study in the prostate, lung, colorectal and ovarian cancer screening trial. Cancer Epidemiol Biomarkers Prev 16:109–126
119. Gerster H (1997) The potential role of lycopene for human health. J Am Coll Nutr 16:109–126
120. Wald NJ, Boreham J, Hayward JL (1984) Plasma retinol, β-carotene and vitamin E levels in relation to the future risk of breast cancer. Br J Cancer 49:321–324
121. Knekt P, Aromaa A, Maatela J (1990) Serum vitamin A and subsequent risk of cancer: cancer incidence follow-up of the Finnish mobile clinic health examination survey. Am J Epidemiol 132:857–870
122. Toniolo P, Van Kappel AL, Akhmedkhanov A (2001) Serum carotenoids and breast cancer. Am J Epidemiol 153:1142–1147. doi:10.1093/aje/153.12.1142
123. Dorgan JF, Sowell A, Swanson CA (1998) Relationships of serum carotenoids, retinol, α-tocopherol, and selenium with breast cancer risk: results from a prospective study in Columbia. Missouri (United States). Cancer Causes Control 9:89–97. doi:10.1023/A:1008857521992
124. Panayiotidis M, Collins AR (1998) *Ex vivo* assessment of lymphocyte antioxidant status using the comet assay. Free Radic Res 27:533–537. doi:10.3109/10715769709065793
125. Shklar G (1998) Mechanism of cancer inhibition by anti-oxidant nutrients. Oral Oncol 34:24–29. doi:10.1016/S1368-8375(97)00060-2
126. Cui Y, Lu Z, Bai L (2007) Beta-carotene induces apoptosis and up-regulates peroxisome proliferator-activated receptor gamma expression and reactive oxygen species production in MCF-7 cancer cells. Eur J Cancer 43:2590–2601. doi:10.1016/j.ejca.2007.08.015
127. Arab L, Steck S (2000) Lycopene and cardiovascular disease. Am J Clin Nutr 71:1691–1695
128. Nagasawa H, Mitamura T, Sakamoto S et al (1995) Effects of lycopene on spontaneous mammary tumour development in SHN virgin mice. Anticancer Res 15:1173–1178
129. Sharoni Y, Giron E, Rise M (1997) Effects of lycopene-enriched tomato oleoresin on 7, 12-dimethylbenz[a]anthracene-induced rat mammary tumors. Cancer Detect Prev 21:118–123
130. Levy J, Bosin E, Feldman B et al (1995) Lycopene is a more potent inhibitor of human cancer cell proliferation than either alpha-carotene or beta-carotene. Nutr Cancer 24:257–266
131. Park JS, Chew BP, Wong TS (1998) Dietary lutein from marigold extract inhibits mammary tumor development in BALB/c mice. J Nutr 128:1650–1656
132. Sumantran VN, Zhang R, Lee DS et al (2000) Differential regulation of apoptosis in normal versus transformed mammary epithelium by lutein and retinoic acid. Cancer Epidemiol Biomarkers Prev 9:257–263
133. Chew BP, Brown CM, Park JS (2003) Dietary lutein inhibits mouse mammary tumor growth by regulating angiogenesis and apoptosis. Anticancer Res 23:3333–3339
134. Zhang LX, Cooney RV, Bertram JS (1991) Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer chemopreventive action. Carcinogenesis 12:2109–2114. doi:10.1093/carcin/12.11.2109
135. Haegele AD, Gillette C, O'Neill C et al (2000) Plasma xanthophyll carotenoids correlate inversely with indices of oxidative DNA damage and lipid peroxidation. Cancer Epidemiol Biomarkers Prev 9:421–425
136. Khachik F, Beecher GR, Smith JC Jr (1995) Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. J Cell Biochem 22:236–246. doi:10.1002/jcb.240590830
137. Astley SB, Elliott RM, Archer DB et al (2002) Increased cellular carotenoid levels reduce the persistence of DNA single-strand breaks after oxidative challenge. Nutr Cancer 43:202–213. doi:10.1207/S15327914NC432_11
138. Park JS, Chew BP, Wong TS (1998) Dietary lutein absorption from marigold extract is rapid in BALB/c mice. J Nutr 128:1802–1806
139. Jyonouchi H, Zhang L, Gross MD (1994) Immunomodulating actions of carotenoids: enhancement of in vivo and in vitro antibody production to T-dependent antigens. Nutr Cancer 21:47–58
140. Park JS, Chew BP, Wong TS (1999) Dietary lutein but not astaxanthin or beta-carotene increases *pim-1* gene expression in murine lymphocytes. Nutr Cancer 33:206–212. doi:10.1207/S15327914NC330214
141. Kline K, Lawson KA, Yu W (2003) Vitamin E and breast cancer prevention: current status and future potential. J Mammary Gland Biol Neoplasia 8:91–102. doi:10.1023/A:1025787422466
142. Kline K, Yu W, Sanders BG (2001) Vitamin E: mechanisms of action as tumor cell growth inhibitors. J Nutr 131:161–163
143. Yu W, Liao QY, Hantash FM (2001) Activation of extracellular signal-regulated kinase and c-Jun-NH_2-terminal kinase but not p38 mitogen-activated protein kinases is required for *RRR*-α-tocopheryl succinate-induced apoptosis of human breast cancer cells. Cancer Res 61:6569–6576
144. Meydani M (1995) Vitamin E. Lancet 345:170–175. doi:10.1016/S0140-6736(95)90172-8
145. Eicholzer M, Stalielin HB, Gey KF (1996) Prediction of male cancer mortality by plasma levels of interacting vitamins: 17-year old follow-up of the prospective basal study. Int J Cancer 66:145–150. doi:10.1002/(SICI)1097-0215(19960410)66:2<145::AID-IJC1>3.0.CO;2-2
146. You H, Yu W, Sanders BG et al (2001) RRR-α-tocopheryl succinate induces MDA-MB-435 and MCF-7 human breast cancer cells to undergo differentiation. Cell Growth Differ 12:471–480
147. Yu W, Sanders BG, Kline K (2002) RRR-α-tocopheryl succinate-induction of DNA synthesis arrest of human MDA-MB-435

cells involves TGF-β independent activation of p21 (Waf1/Cip1). Nutr Cancer 43:227–236. doi:10.1207/S15327914NC432_13
148. Prasad KN, Edwards-Prasad J (1992) Vitamin E and cancer prevention: recent advances and future potentials. J Am Coll Nutr 11:487–500
149. You H, Yu W, Munoz-Medellin D (2002) Role of extracellular signal-regulated kinase pathway in RRR-alpha-tocopheryl succinate-induced differentiation of human MDA-MB-435 breast cancer cells. Mol Carcinog 33:228–236. doi:10.1002/mc.10040
150. Bob WY, Sanders G, Kline K (2003) RRR-α-tocopheryl succinate-induced apoptosis of human breast cancer cells involves bax translocation to mitochondria. Cancer Res 63:2483–2491
151. Charpentier A, Simmons-Menchaca M, Yu W (1996) RRR-α-tocopheryl succinate enhances TGF-β1, -β2, and -β3 and TGF-βR-II expression by human MDA-MB-435 breast cancer cells. Nutr Cancer 26:237–250
152. Guthrie N, Gapor A, Chambers AF, Carroll KK (1997) Inhibition of proliferation of estrogen receptor-negative MDA-MB-435 and -positive MCF-7 human breast cancer cells by palm oil tocotrienols and tamoxifen, alone and in combination. J Nutr 127:544S–548S
153. Yu W, Simmons-Menchaca M, Gapor A et al (1999) Induction of apoptosis in human breast cancer cells by tocopherols and tocotrienols. Nutr Cancer 33:26–32
154. Vogel VG (2007) Chemoprevention strategies. Curr Treat Options Oncol 8:74–88. doi:10.1007/s11864-007-0019-z
155. Day R, Ganz PA, Costantino JP et al (1999) Health-related quality of life and tamoxifen in breast cancer prevention: a report from the national surgical adjuvant breast and bowel project P–I Study. J Clin Oncol 17:2659–2669
156. Vogel G (2001) Reducing the risk of breast cancer with tamoxifen in women at increased risk. J Clin Oncol 19:87–92
157. Vogel VG (2007) Raloxifene: a second generation selective estrogen receptor modulator for reducing the risk of invasive breast cancer in postmenopausal women. Womens Health 3:139–153. doi:10.2217/17455057.3.2.139
158. Bostwick DG, Burke HB, Wheeler TM (1994) The most promising surrogate endpoint biomarkers for screening candidate chemopreventive compounds for prostate adenocarcinoma in short-term phase II clinical trials. J Cell Biochem 19:283–289
159. Stadtzkin LS, Freedman LS, Stern HR (1996) Surrogate end points in cancer research: a critique. Cancer Epidemiol Biomarkers Prev 5:947–953
160. Merk JB, Rottey S, Olaussen K et al (2006) Cyclooxygenase-2 as a target for anticancer drug development. Crit Rev Oncol Hematol 59:51–64. doi:10.1016/j.critrevonc.2006.01.003
161. Harris RE, Beebe-Donk J, Doss H (2004) Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective cox-2 blockade. Oncol Rep 13:559–583
162. Parrett ML, Harris RE, Joarder FS (1997) Cyclooxygenase-2 gene expression in human breast cancer. Int J Oncol 10:503–507
163. Masferrer JL, Leahy KM, Koki AT et al (2000) Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res 60:1306–1311
164. Abbadessa G, Spaccamiglio A, Sartori ML et al (2006) The aspirin metabolite, salicylate, inhibits 7, 12-dimethylbenz[a]anthracene-DNA adduct formation in breast cancer cells. Int J Oncol 28:1131–1140
165. Dipple A, Moschel RC, Bigger CAH (1984) Polynuclear aromatic carcinogens. In: Searle CE (ed) Chemical carcinogens. ACS monographs. American Chemical Society, Washington, DC
166. Allred DC, Mohsin SK, Fuqua SA (2001) Histological and biological evolution of human premalignant breast tissue. Endocr Relat Cancer 8:47–61. doi:10.1677/erc.0.0080047
167. Matsuoka Y, Fukamachi K, Uehara N et al (2008) Induction of a novel histone deacetylase 1/c-Myc/Mnt/Max complex formation is implicated in parity-induced refractoriness to mammary carcinogenesis. Cancer Sci 99:309–315. doi:10.1111/j.1349-7006.2007.00689.x
168. Thordarson G, Slusher N, Leong H et al (2004) Insulin-like growth factor (IGF)-I obliterates the pregnancy-associated protection against mammary carcinogenesis in rats: evidence that IGF-I enhances cancer progression through estrogen receptor-alpha activation via the mitogen-activated protein kinase pathway. Breast Cancer Res 6:423–436. doi:10.1186/bcr812
169. Naili-Ito A, Asamoto M, Hokaiwado N et al (2007) Gpx2 is an overexpressed gene in rat breast cancer induced by three different chemical carcinogens. Cancer Res 67:11353–11358. doi:10.1158/0008-5472.CAN-07-2226