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REVIEWS

Molecular Targets for Nutrients Involved With Cancer Prevention

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Abstract: *Dietary nutrients can influence cancer risk by inhibiting or enhancing carcinogenesis through diverse mechanisms of action. The identification and elucidation of their sites of action have been a focus of nutrition and cancer research for more than four decades. Transforming nutrition and cancer research from a predominantly observational to a molecular approach offers exciting opportunities for truly identifying those who will and will not benefit from dietary intervention strategies. The emerging field of nutritional genomics, defined here as the study of any genetic or epigenetic interaction with a nutrient, will be key to this evolution. Unraveling which genetic upregulation or downregulation leads to subsequent phenotype changes will not be easy. There is evidence that genetic polymorphisms can influence the dynamics between nutrients and molecular targets and, thus, contribute to variation in response among individuals. Because many molecular targets will likely be identified, it may be necessary to credential nutrients, that is, to determine which specific nutrient-related genetic and epigenetic changes bring about phenotypic changes, to establish which interactions are the most important and under what circumstances. Vitamin D, calcium, folate, selenium, genistein, and resveratrol are highlighted, because they represent specific classes of nutrients and illustrate the need to credential various nutrients to understand their physiological significance in cancer prevention. As the science of nutrition unfolds, a clearer understanding will emerge about how nutrients can modulate cancer risk through molecular interactions and how foods might be changed by agronomic approaches and/or biotechnology. Undeniably, embracing new genomic technologies offers exciting opportunities for advances in the broad area of nutrition, especially those related to cancer prevention.*

Introduction

There is little doubt that nutrition is intimately involved in cancer prevention. Optimizing nutrition by the use of foods and their bioactive components represents a noninvasive and cost-effective strategy for reducing risk. The melding of information from epidemiological, preclinical, and clinical studies has provided fundamental insights about the dynamic relationships among nutrients, defined here as any substance in the diet that brings about a physiological effect, and cancer. Unfortunately, far too often, data across studies are inconclusive or even diametrically opposed. Developing a better understanding of nutrient interactions at the molecular level offers one avenue for traversing this conundrum, although significant challenges, both scientific and technological, must be overcome before realistic applications for cancer prevention can be realized (1). DellaPenna (2) coined the term nutritional genomics to describe work at the interface of plant biochemistry, genomics, and human nutrition aimed at understanding and manipulating nutrient reactions and interactions at the molecular or genomic level. For purposes of this review, nutritional genomics refers to the study of any genetic or epigenetic interaction with a nutrient that leads to phenotypic changes. Genetic interactions involve direct alteration of the DNA coding sequence, whereas epigenetic changes are mechanisms exclusive of direct modification or damage to DNA. The basis for nutritional genomics arises from rather compelling evidence that a variety of nutrients influence genetic and epigenetic processes and gene-regulated metabolic pathways through interactions with specific molecular targets. These molecular targets may be individual genes, molecules that result from gene expression or are otherwise affected by gene expression, or any other molecular events that are relevant to the process of carcinogenesis.

The focus of this review is limited to vitamin D, calcium, folate, selenium, genistein, and resveratrol, because they represent different classes of nutrients, and potential molecular targets related to cancer risk have been identified. Vitamin D, calcium, folate, genistein, and selenium are being investigated in chemoprevention trials (3). Although chemo-

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prevention, that is, the use of natural or laboratory-made substances to prevent cancer, is not the focus of this review, it seems logical that, in the future, the search for effective, population-based chemoprevention strategies will benefit from observations linking nutrients with specific cancer-related molecular targets. Table 1 lists some additional nutrients reported to modify cancer risk.

Undeniably, nutrition and cancer research will move from an observational to a molecular approach as knowledge about genomics and new technologies surfaces (4). Nutritional genomics offers opportunities to credential nutrients, that is, to determine which specific nutrient-related genetic and epigenetic changes bring about phenotypic changes that influence cancer risk, for purposes of establishing which interactions are the most important and under what circumstances. This knowledge should lead to the identification of molecular targets that can be manipulated for cancer prevention (5). In addition to describing interactions between selected nutrients and molecular targets, this review includes a discussion of certain nutrient-related genetic polymorphisms, that is, different allelic forms of genes, that may influence cancer risk, the implications of nutritional genomics for food production, and future research directions.

Specific Nutrients and Their Molecular Targets

This section provides clues to possible molecular targets for a few essential and nonessential nutrients. The diversity of molecular targets that are influenced demonstrates the complexity and breadth of nutrient interactions and supports the concept that nutrients act within numerous biochemical

Table 1. Partial List of Nutrients That May Modify Cancer Risk[a]

Group	Nutrient	Source
Vitamins	Vitamin D	Dairy products
	Folic acid	Vegetables
	Vitamin A	Vegetables
	Vitamin E (α-tocopherol)	Vegetable oils
	Ascorbic acid	Vegetables, fruits
Minerals	Calcium	Dairy products, vegetables
	Selenium	Vegetables, fruits, cereal grains, meat, fish
	Iron	Red meat
	Zinc	Vegetables
Carotenoids	Lycopene	Tomatoes
	Lutein	Dark green vegetables
	α-Carotene	Orange-yellow vegetables
	β-Carotene	Orange-yellow vegetables
Flavonoids	Genistein	Soybeans, soy products
	Resveratrol	Grapes, red wine
	Quercetin	Vegetables, fruits
	Rutin	Vegetables, fruits
	Tangeretin	Citrus fruits
	Nobiletin	Citrus fruits
	Catechins	Grapes
	(−)-Epigallocatechin-3-gallate	Green tea
	Anthocyanins	Vegetables, fruits, black tea
Organosulfur compounds	Diallyl sulfide	*Allium* vegetables (e.g., garlic, onion)
	Allyl mercaptan	*Allium* vegetables (e.g., garlic, onion)
	Allyl methyl trisulfide	*Allium* vegetables (e.g., garlic, onion)
	S-allylcysteine	*Allium* vegetables (e.g., garlic, onion)
Isothiocyanates	Allyl isothiocyanate	Cabbage
	2-Phenylethyl isothiocyanate	Cabbage
	Benzyl isothiocyanate	Cabbage, garden cress
	3-Methylsulfinylpropyl isothiocyanate	Broccoli
	Sulforaphane	Broccoli
Indoles	Indole-3-carbinol	Cruciferous vegetables
	Indole-3-acetonitrile	Cruciferous vegetables
Monoterpenes	D-Limonene	Citrus fruit oils
	D-Carvone	Caraway seed oil
Phenolic acids	Curcumin	Turmeric, curry, mustard
	Caffeic acid	Fruits, coffee beans, soybeans
	Ferulic acid	Fruits, soybeans
	Chlorogenic acid	Fruits, coffee beans, soybeans
Chlorophyll	Chlorophyll	Green vegetables
	Chlorophyllin	Green vegetables

a: Adapted from Huang et al. (165).

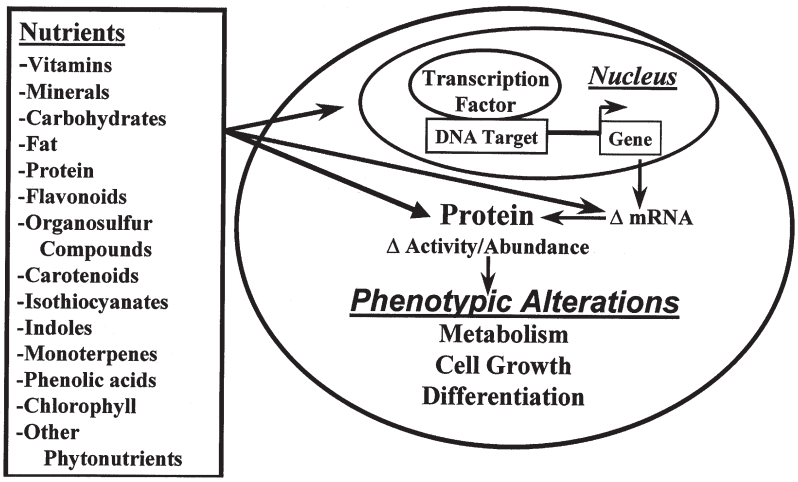

Figure 1. Selected nutrients as regulators of gene expression.

and molecular cascades, thereby serving as significant modulators of cancer risk. Figure 1 reveals that the pleiotropic effects of nutrients cannot be attributed to a single regulatory mechanism but are likely a manifestation of nuclear and cytoplasmic events that regulate the abundance and/or activity of specific proteins. Fluctuations in these proteins can lead to changes in overall cellular metabolism and can markedly influence the proportion of cells that are dividing, undergoing apoptosis, or differentiating.

Essential Nutrients and Phytonutrients

Vitamin D: Vitamin D is an essential nutrient produced in the skin as a photoproduct of 7-dehydrocholesterol or obtained through consumption of various foods, including dairy products (e.g., milk, cheese, and butter) and seafood (e.g., fish and oysters). Functionally, vitamin D is a prohormone that is integral to homeostatic regulation of serum calcium and phosphorus through its own homeostatic regulation at specific hydroxylation sites (6). The active form, 1,25-hydroxycholecalciferol [1,25$(OH)_2$D], binds intracellularly to cytoplasmic vitamin D receptor (VDR). This receptor is found widely dispersed throughout the body, especially in the intestines, bone, pancreas, breast, prostate, pituitary, gonads, mononuclear cells, activated T lymphocytes, and skin (7). The resulting 1,25$(OH)_2$D-VDR complex is recognized to interact with nuclear vitamin D-responsive DNA elements (VDRE) and, thereby, initiates or represses nuclear transcription of various target genes (7). Figure 2 summarizes some aspects of the vitamin D regulatory pathway.

Vitamin D and its homologs have been investigated as dietary anticarcinogens for decades, particularly as they relate to colon, rectum, prostate, and breast cancers (6). Observational and cohort studies generally have supported an inverse association between vitamin D and prostate cancer (6,8) and, possibly, colorectal and breast cancers (8). Incidence rates of colon and rectal cancer among men tend to increase with decreasing levels of solar radiation. Such observations raise intriguing questions about interrelationships between sunlight-derived and dietary sources of vitamin D in influencing the risk of colorectal cancer and the development of adenomatous polyps.

Vitamin D and its metabolites appear to act through a variety of molecular targets to inhibit carcinogenesis. The con-

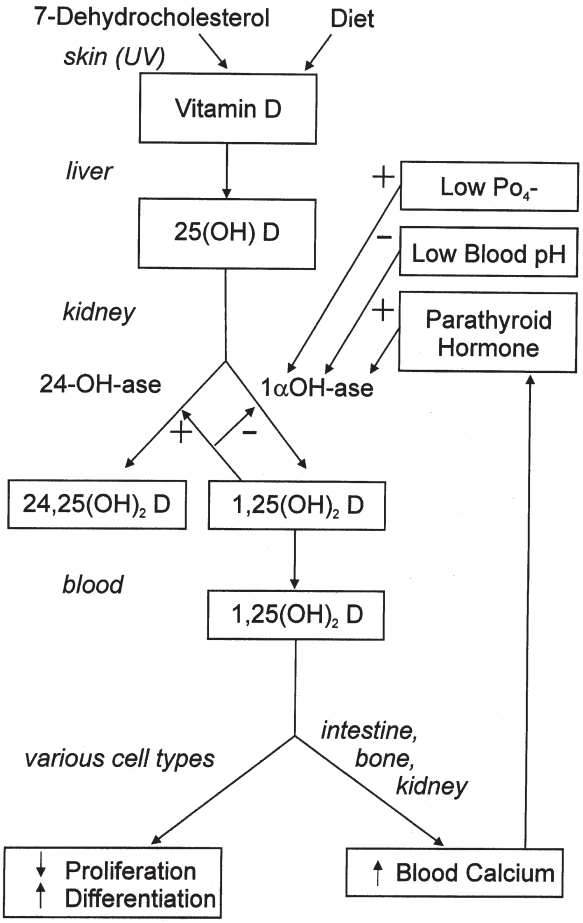

Figure 2. Vitamin D regulatory pathway. [From Platz and Giovannucci (6), reprinted with permission from Academic Press.]

sequence of vitamin D binding to epithelial growth factor receptor is a reduction in the availability of epithelial growth factor, with subsequent inhibition of growth and increased differentiation in normal and malignant cells (9). Studies with breast cancer cell lines (i.e., murine CS-2 and human MCF-7) also indicated that depressed proliferation and enhanced apoptosis contribute to the preventive activities associated with vitamin D (10,11). Vitamin D has been shown to directly regulate *BRCA-1* expression via VDR-induced transcriptional activation of the *BRCA-1* promoter in MCF-7 cells, a pathway that is disrupted during development of prostate and breast cancers (12). In CS-2 cells, vitamin D induces apoptosis by upregulating endonuclease and downregulating genes (e.g., *GRP-78*, α-*prothymosin*, and *calmodulin*) needed for proliferation (11).

Because vitamin D acts as a hormone, interest in its ability to bind with estrogen receptors (ERs) in breast and prostate tumors has surfaced. Nolan and colleagues (10) reported that apoptotic events caused by vitamin D occur in ER (+) or ER (–) breast cancer cells, suggesting that the effect is independent of estrogen status. Vitamin D also interacts with androgens via androgen receptors (AR) through the action of the VDRs. In human prostate cancer cells (LNCaP), vitamin D acts to suppress growth through an AR-dependent mechanism after VDR-mediated expression of AR (13). Insulin and insulin-like growth factor (IGF) I are recognized to be involved in promoting growth and development of breast tumors. Vitamin D reverses the mitogenic effects of insulin and IGF-I by interruption of late-growth signaling pathways, rather than by a direct effect or by binding to insulin or IGF-I receptors (14). Vitamin D, through the action of VDR, also has been shown to be immunosuppressive by repression of interleukin-2 cytokine gene transcription (15). VDR binds to the DNA adjacent to the *jun-fos* gene complex, inactivating *jun*, which is an activating gene for transcription (15). This results in the repression of normal cell transcription and may be a mechanism for vitamin D inhibition of cell proliferation.

The ability of vitamin D to inhibit growth and induce apoptosis in tumor cell lines independent of *p53* tumor suppressor gene signaling pathways is of particular interest. In an investigation of apoptotic pathways in MCF-7 and T47D cells, it was determined that vitamin D activates apoptosis through a *Bcl-2*-regulated pathway, independent of known caspase cascades or *p53* status (16). Another molecular target for vitamin D is the c-*myc* protooncogene, which induces proliferation and tumor growth. In human Caco-2 colon and HL-60 leukemic cancer cells treated with vitamin D, VDR binding is increased and c-*myc* expression is downregulated, which suppresses cell division and induces differentiation (17).

Overall, considerable evidence reveals a direct functional consequence of vitamin D and its metabolites on a number of physiological processes. Receptor polymorphism is clearly an important determinant in the cellular response to $1,25(OH)_2D$ (18). Such evidence provides insights into how receptor genotype-phenotype association can account for a plethora of cellular responses. Variances in responsiveness among individuals may occur on the basis of naturally occurring variants of a single gene. For a full understanding of the response to $1,25(OH)_2D$, a more detailed understanding of its interactions with cofactors is essential. It is certainly conceivable that these cofactors will vary among responses and cell types.

Calcium: Dietary calcium is provided primarily by dairy products, although lesser amounts can be found in dark green vegetables, nuts, grains, and beans. Several studies have documented a weak inverse association between calcium intake and various cancers, particularly colorectal cancer (8,19). However, human observational and intervention studies have yielded conflicting evidence (6). In contrast, some evidence suggests that calcium intake may be positively associated with prostate cancer, particularly for the advanced state (6,20).

Calcium and vitamin D are recognized to operate in a complex relationship to facilitate various functions. In human keratinocyte cells, $1,25(OH)_2D$ transcriptionally activates the *CYP24* gene through two VDREs, which act as a negative-feedback system to regulate its concentration and the prevention of hypercalcemia (21). A functional Ras-dependent Ets-binding site was located downstream from the proximal VDRE and was critical to $1,25(OH)_2D$-mediated induction (21).

Some have proposed that if circulating calcium levels are increased, there might be a concomitant reduction of vitamin D available to inhibit cellular proliferation or increase differentiation in premalignant or malignant neoplastic cells (i.e., colon and rectum), as well as an increase in the saponification of fatty acids and bile acids by calcium (6). Because calcium concentrations are rather tightly regulated, it is unclear whether this process truly occurs under normal circumstances. Nevertheless, evidence does exist that suggests that consumption of calcium-rich foods such as dairy products may increase the risk of prostate cancer (7).

Calcium may also influence the cancer process independent of its relationship with vitamin D. Calcium has been associated with factors recognized to inhibit proliferation and stimulate differentiation. The calcium sensitivity of cells is linked to calcium-sensing receptors of the G protein-coupled cell surface receptor family, which are found on the plasma membrane of numerous cell types (17). In colon cells, the calcium signaling pathway regulates c-*myc* protooncogene expression: high levels of calcium inhibit c-*myc* expression, and low levels of calcium allow increased expression of c-*myc*, which leads to hyperproliferation of colon cells and a possible increased risk of colon cancer (17). Experimental studies have suggested that calcium inhibits colon carcinogenesis by suppressing induction of the tumor-promotion enzyme ornithine decarboxylase, which is involved in DNA synthesis (22,23).

Another possible mechanism for the antiproliferative action of calcium, in conjunction with vitamin D, is direct interference with the formation of k-*ras* mutations, such as reported for experimentally induced colorectal tumors (24).

A population-based study of colorectal cancer patients indicated that high calcium intake is associated with decreased risk of k-*ras* mutations in colorectal tumors (25). One recent study reported that high calcium intake is positively associated with colon tumors with k-*ras* G-to-A mutations in codon 12 but inversely associated with k-*ras* G-to-A mutations in codon 13 (26). Although the significance of these findings remains to be established, the implications are that some individuals may benefit from calcium, whereas others may be placed at risk. As indicated earlier, it is conceivable that the identification of critical molecular targets for nutrients will be useful in establishing who will and will not benefit from intervention strategies.

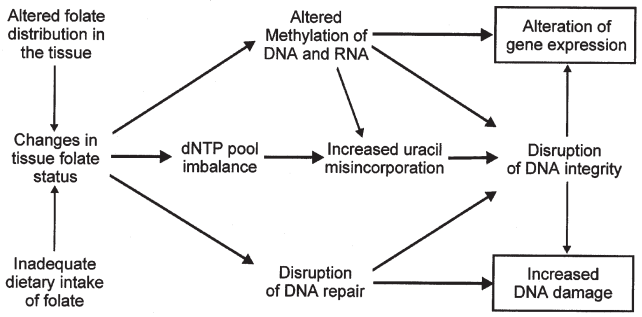

Figure 3. Molecular effects of folate depletion. [From Choi and Mason (29), reprinted with permission from American Society for Nutritional Sciences.]

Folate: Folate is a generic term that reflects the endogenous form of the vitamin occurring naturally in food and the synthetic form, folic acid, found in supplements and fortified foods. Folate functions as a coenzyme in one-carbon transfer reactions in the metabolism of nucleic and amino acids (27). Through this function, folate is essential for the de novo biosynthesis of purines and thymidylate, which affects DNA replication and cell division, and for the synthesis of *S*-adenosylmethionine (SAM), which is a methyl donor for >100 biochemical reactions, including methylation of DNA (28–30). These biosynthetic pathways, each of which is important to DNA metabolism, appear to compete when the dietary methyl supply is inadequate, as in folate deficiency, possibly resulting in altered DNA methylation, disruption of DNA integrity, and disruption of DNA repair and, consequently, increased risk for carcinogenesis (29,30). DNA methylation is recognized as an essential step in normal cellular homeostasis and occurs by the addition of a methyl group to a cytosine base that is part of a CpG dinucleotide (31,32). Instead of normal DNA methylation patterns, cancer cells often show global hypomethylation, which promotes genomic instability, coexisting with region-specific hypermethylation (31,32). DNA methylation has been linked inversely to gene expression; thus DNA hypomethylation may lead to overexpression of oncogenes, and hypermethylation can suppress gene transcription and silence tumor suppressor genes (29–32). The possible molecular effects of folate deficiency and their likely interrelationships are illustrated in Fig. 3 (29). A recent comprehensive review of the epidemiological, preclinical, and clinical evidence linking folate deficiency with cancer risk concluded that the most compelling evidence exists for a direct association with colorectal cancer risk; that the evidence regarding risk for cancers of the lung, uterine cervix, esophagus, stomach, pancreas, liver, breast, and colon/rectum is somewhat weaker; and that folate deficiency likely acts as a potentiator or cocarcinogen with other risk factors (30). For instance, it has been suggested that hypomethylation and DNA strand breaks resulting from folate deficiency might enhance the incorporation of tumorigenic viruses such as human papilloma virus into human DNA (29,33). In humans, lower serum folate or localized folate deficiency has been associated with increased global DNA hypomethylation in leukocytes in healthy postmenopausal women (34), squamous cell lung cancer (35), cervical intraepithelial neoplasia (36), gastric cancer (37), and colorectal cancer (38); folate administration significantly reversed hypomethylation in patients with chronic atrophic gastritis (37) and colorectal cancer (38). Notably, only moderate folate depletion (intake of 118 μg/day for 7 wk), with no clinical signs of folate deficiency, was required to increase global DNA hypomethylation in leukocytes in postmenopausal women. Furthermore, increasing dietary folate (200 or 415 μg/day for 7 wk) did not significantly decrease hypomethylation, suggesting a delayed DNA methylation response to folate repletion in this population (Fig. 4) (34). In animal studies, folate deficiency induced exon-specific hypomethylation in the *p53* tumor suppressor gene (28,39), but not global hypomethylation in liver (28,40) or colonic mucosa (40). Hypomethylation in *p53* was reversed in some exons by increasing dietary folate (39).

Evidence exists that hypomethylation increases the susceptibility of DNA to nuclease attack, which can result in DNA strand breaks, thus disrupting DNA integrity (30,33). In addition, folate deficiency, which results in a dinucleotide imbalance (reduction of thymidylate synthesis from deoxyuridylate), may thereby increase the likelihood that uracil (rather than thymine) will be mistakenly incorporated into DNA. The increased requirement for excision of uracil resi-

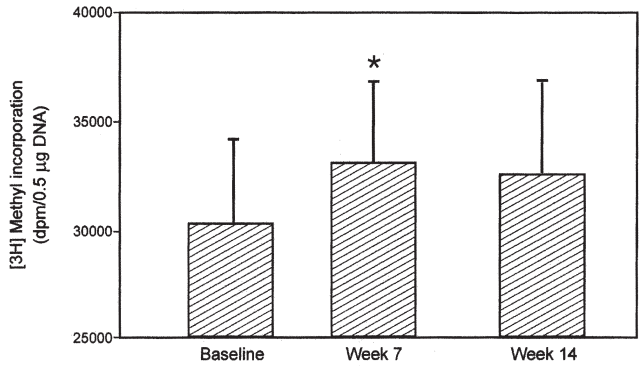

Figure 4. [^{3}H]methyl incorporation into leukocyte DNA of all subjects at baseline, Week 7 (postdepletion), and Week 14 (postrepletion). *, Significantly greater ($P = 0.0025$) than baseline. [From Rampersaud et al. (34), reprinted with permission from the American Society for Clinical Nutrition.]

dues and repair of DNA may hinder the repair process and result in single- or double-strand DNA breaks (29,41). A recent in vitro study reported that folate deficiency substantially decreased DNA stability in normal human colon epithelial cells by inducing DNA hypomethylation and uracil misincorporation and by inhibiting DNA excision repair (42). In one clinical study, there were nine times as many uracil molecules per cell in bone marrow DNA from folate-deficient individuals as from normal-folate individuals, and micronuclei frequency (a measure of chromosome breaks) was elevated. Both defects were reversed by folate administration (41). In animals, sustained folate deficiency (6 wk) was associated with parallel increases in hypomethylation and DNA strand breaks in the *p53* gene (28). Strand breaks in the *p53* gene might contribute to loss of its tumor suppressor function (30).

Allelic variants of folate genes may play a significant role in several disease conditions. Particular emphasis has been given to the role of two common polymorphisms [methylenetetrahydrofolate reductase (MTHFR) 677C → T, 1298A → C] in cardiovascular disease, cerebrovascular disease, venous thrombosis, longevity, neural tube defects, pregnancy/preeclampsia, diabetes, cancer, psychiatric disease, renal failure, and renal replacement therapy (43). Uncovering how these and other polymorphisms influence folate metabolism will lead to a greater understanding of this essential nutrient and the many genes that support its enzymatic utilization in a plethora of critical biosynthetic reactions and that, under some conditions, may promote disease.

Selenium: The essential trace mineral selenium is of fundamental importance to human health. The amount in vegetables and fruits is highly dependent on the soil content. As a constituent of selenoproteins, selenium has several structural and enzymatic roles, in the latter context being best known as an antioxidant. Although investigation of selenium's role in health promotion has focused on its antioxidant activity (44), it has diverse biological functions, including the ability to suppress cell proliferation (45), enhance immune response (46), alter the metabolism of carcinogens (47), and induce apoptosis (45). Providing selenium in its inorganic (e.g., selenite and selenate) or organic [e.g., selenocysteine and selenomethionine (Se Met)] forms has been found to meet nutritional needs. The biological activity of selenium actually reflects its expression in various compound forms, rather than as tissue content per se (47). Kuchan and Milner (48) provided rather compelling evidence that intracellular concentrations of glutathione were instrumental in determining the ability of selenite to alter cellular proliferation. Thus it is possible that the various forms of selenium will not be equal in their efficacy but will be highly dependent on not only the quantity provided, but how it is metabolized.

Results from population studies and randomized clinical trials have indicated inverse associations between selenium and esophageal and gastric cancers (49), lung cancer (50, 51), prostate cancer (51,52), and colorectal and total cancers (51). Animal studies have consistently supported an inverse association between selenium and carcinogenesis (reviewed in Ref. 47). Many selenoproteins (>11 have been identified, each containing selenocysteine) bring about numerous physiological actions that may relate to the cancer process. Nevertheless, most evidence suggests that selenium beyond that needed to optimize the activity of selenium-containing proteins is required to bring about a reduction in experimentally induced cancers.

Research on the gamut of molecular targets for selenium has supported its ability to function as an antioxidant and alter several events that lead to changes in cell proliferation, differentiation, and apoptosis (53). Considerable attention has been devoted to its role in the thioredoxin system, a major antioxidant system (44,45). The activity of thioredoxin reductase (TR), a selenoenzyme, has also been linked to nuclear transcription factor nuclear factor-κB (NF-κB) activation through its ability to regulate thioredoxin concentrations. NF-κB is an inducible oncogenic nuclear transcription factor that responds to the redox state of the cells and has a pivotal role in inducing genes involved in a number of physiological processes, including those associated with cytokines, growth factors, cell adhesion molecules, and immunoreceptors. TR specifically reduces oxidized thioredoxin to its reduced form using NADPH. The reduced thioredoxin reduces disulfide binding of several proteins, including NF-κB. Selenium availability is a key factor that determines overall TR activity in cells in culture and in vivo (45,54). Providing supplemental selenium to HT-29 human colon cancer cells grown in serum-free medium markedly increased TR activity (54). Recent studies by Ganther and Ip (55) provide evidence that supraphysiological exposure to selenium does not affect the activity of this enzyme. Thus, although it may be important to aberration in neoplastic cells, it may not be a target for exaggerated selenium intakes per se.

Various forms of selenium markedly retard the growth of neoplasms (56). This may relate to a direct genetic effect of selenium, such as inhibition of DNA synthesis and induced DNA strand breakage (47). For instance, selenite has been shown to increase DNA strand breakage, increase cdc2/cdk2 kinase activities without changing cyclins bound to cdk2, and arrest cell growth in the $S/G_2/M$ phase (57). The selenocompound methylselenocysteine also has been reported to increase the number of double-strand DNA breaks, thus inducing apoptosis and arresting cell growth in the G_1 phase (57).

In addition, recent studies reveal that selenium availability can alter DNA methylation. Davis and colleagues (58) found that DNA isolated from Caco-2 cells, a human colon cancer cell line, not treated with selenite was significantly hypomethylated compared with that from cells treated with 1 or 2 μM selenite. In addition, methylation of the p53 promoter region of Caco-2 cells decreased when cells were cultured in the absence of selenite. In an in vivo study, rats fed selenium-deficient diets had significantly hypomethylated liver and colon DNA compared with rats fed diets containing

selenium at 0.1 or 2.0 μg as selenite or selenomethionine. Thus alterations in DNA methylation may be a potential mechanism by which selenium may alter tumorigenesis. Although these findings are exciting, additional studies are needed to determine whether DNA from other tissues is also influenced and how other dietary components might affect this response.

The blocking of intraductal proliferation caused by methylselenocysteine in mammary tissue, a precursor lesion for tumor formation, has been correlated with increased expression of $p27^{Kip-1}$, which is involved in differentiation (59). Also, experimental data indicated that methylselenocysteine decreased cdk2 kinase activities and cyclin E-cdk2 availability, which may represent another pathway by which it inhibits tumor growth and proliferation (57).

The p53 protein is a factor that enhances transcription of several genes, including *gadd45*, *p21* (*WAF1* and *Cip-1*), *mdm2*, *cyclin G*, *bax*, and IGF binding protein-3. Generally, the p53 protein is maintained at a low concentration, although it can be induced by physical or chemical DNA damage. Interestingly, apoptosis can be triggered by selenium supplementation independent of DNA damage and in cells that have a null p53 phenotype (60). Both p73 and p63 have homology to p53 in their respective transactivation, DNA binding, and oligomerization domains. Both p73 and p63 transactivate p53-regulated promoters and induce apoptosis. Evidence suggests that p73 and p63 mediate apoptosis by mechanisms different from p53. It remains to be determined whether selenium alters these or other factors associated with non-p53-mediated apoptosis.

Se-Met is the predominant form of selenium in dietary supplements and is the form most widely used in prevention trials (61). Because methionine-tRNA cannot distinguish methionine from Se-Met, incorporation of Se-Met into tissue proteins may account for its lower toxicity than other forms of selenium. The efficacy of Se-Met has also been found to depend on the intake of methionine, again pointing to the need to understand the dynamic interactions that can occur among the various dietary components (47). Nevertheless, even when methionine intake is adequate, Se-Met can influence pathways related to carcinogenesis, such as interruption of polyamine biosynthesis, which is required for normal cellular proliferation and development (61–63).

Essential and nonessential nutrients cannot be considered to operate in isolation; rather, they work in a dynamic, constantly changing milieu (64). Although interactions among nutrients have been inadequately examined, a few examples of negative and positive interactions with the response to selenium are available. Vitamin C has been reported to reduce selenium's effectiveness against chemically induced colon cancer (65). The significance of such interactions may be even more pronounced, because selenium has been shown to enhance the ability of garlic to inhibit chemically induced mammary cancer in experimental animals (66). Greater attention to all components of the diet and elaboration of their interactions should make possible specific and appropriate recommendations for the general population and allow for recommendations tailored to specific subgroups or individuals. The importance of such understanding is exemplified in the recent review by Alaejos and colleagues (67) in which they presented data indicating that several factors, including α-tocopherol, β-carotene, retinol, and vitamin C, might account for variability in the response to dietary selenium.

Nonessential Phytonutrients

Worldwide evidence from epidemiological studies supports an inverse association between cancer risk and consumption of vegetables and fruits. Even though considerable variations in methodologies exist among international studies, convincing data link vegetable and fruit intake with reduced risk for cancers of the mouth and pharynx, esophagus, lung, stomach, colon, and rectum (8,68). Vegetables, which are derived from various parts of plants, including roots (e.g., carrots and parsnips), leaves (e.g., spinach and lettuce), flowers (e.g., globe artichoke and broccoli crowns), stalks (e.g., celery and rhubarb), and seeds (e.g., corn and peas), and fruits contain thousands of chemically diverse phytonutrients. Certain classes of phytonutrients, such as flavonoids, carotenoids, organosulfur compounds, terpenes, and isothiocyanates, have been the focus of experimental research designed to determine their effects on cancer risk and the specific mechanisms by which they exert their effects (66,69–73). Because of the chemical and biological diversity of phytonutrients, the range of their molecular targets, and their possible interactions, cancer-related phytonutrient research is proving to be not only a tremendously exciting field, but also an immense challenge. Even individual phytonutrients can have multiple molecular targets. To illustrate the complexity and variety of possible interactions of individual phytonutrients with molecular targets, the discussion here highlights two of the many available excellent examples: genistein, an isoflavone found in high concentrations in the soybean, and resveratrol, a polyphenol found in grapes and other plants.

Genistein: Genistein, which has a chemical structure very similar to that of mammalian estrogen, is a phytoestrogen, that is, a plant-derived compound that can bind to the ER and exhibits estrogen-like biological activity (74,75). Epidemiological data suggest that consumption of soy products is associated with decreased risk for certain hormone-related cancers, including breast (76), endometrial (77), and prostate (78,79) cancers. A few studies have suggested that consumption of soy foods during the neonatal or prepubertal period of life may reduce subsequent risk of cancer, especially in breast tissue (80). Still others suggest that genistein may create a high estrogenic environment in utero and, thereby, increase subsequent breast cancer risk (81).

Genistein exists in the soybean as an inactive glycoside (genistin) that is hydrolyzed after ingestion to release the aglycone (genistein), which can be absorbed, excreted, or further metabolized by intestinal microflora to *p*-ethylphe-

nol before excretion (75,82). Although specific values vary among studies, data suggest that high-soy diets result in ~0.5–5 μM genistein in plasma (82–85). Large variations exist among individuals in the uptake, metabolism, and excretion of genistein and other isoflavones, possibly because of differences in intestinal microflora (82,84).

Experimental studies on the cancer-related effects of genistein demonstrate that this phytonutrient may influence the process of carcinogenesis through various mechanisms, including some that are ER related. On a molar basis relative to physiological estrogens, genistein is quite weak, with ~1×10^{-4} and 1×10^{-3} the activity of 17β-estradiol. Nevertheless, although it has a relatively low potency, it may exert physiological effects, because concentrations in blood can reach several orders of magnitude greater than physiological estrogens (86). Genistein may also function through antioxidant effects (87,88), effects on cell cycle kinetics and apoptosis (89–94), and effects on angiogenesis and metastasis (95–98). Consequently, genistein likely has various molecular targets potentially important to cancer risk. New genomic and other high-throughput technologies offer exciting opportunities to identify more targets and help understand the underlying complexity of the metabolic pathways that may contribute to tumor initiation and progression and how these pathways can be modified by genistein and other dietary components.

Genistein, like other flavonoids, has been reported to scavenge free radicals that can cause DNA damage and lipid peroxidation (88,99) and to enhance the activities of antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase (99). In contrast, one recent study in colon cancer cells reported that genistein enhanced the expression of the antioxidant enzyme metallothionein, but not catalase or superoxide dismutase (87).

In vitro studies indicate that genistein concentration is a critical determinant of the observed response. At low physiologically relevant concentrations (<10 μM), studies indicate that genistein acts as an estrogen agonist, on the basis of stimulation of ER (+) cell proliferation (100,101), induction of the estrogen-responsive *pS2* gene (93,100,102), and enhanced estradiol-induced DNA synthesis (103). In contrast, genistein inhibits breast cancer cell growth at higher concentrations (>10 μM) (93,100,103). The mechanisms responsible for the dual effects of genistein are not clear. It has been postulated that, even while acting as an estrogen agonist, at higher concentrations genistein also acts through other ER-independent mechanisms to inhibit cell proliferation induced by genistein through ER pathways (100). Several possible ER-independent mechanisms are discussed below.

Genistein has been shown to induce cell cycle arrest and apoptosis in numerous cancer cell lines, including ER (+) (93,94,104) and ER (–) (94,105) human breast carcinoma, prostate cancer (92), colon cancer (89), lung cancer (106), head and neck squamous cell carcinoma (107), leukemia (90), and neuroblastoma (108). Although the mechanisms by which genistein exerts these effects and the relevant molecular targets remain unclear, data support various possibilities. For example, the growth-inhibitory protein $p21^{WAF1}$ inhibits activity of cyclin-dependent kinases (cdk), which, in turn, can inhibit progression through the cell cycle and can lead to growth arrest and apoptosis (92,94). Genistein has been reported to upregulate $p21^{WAF1}$, accompanied by cell cycle arrest in the G_2/M phase and apoptosis, in ER (+) and ER (–) breast cancer cells (94) and in prostate cancer cells (92). In another study that reported cell cycle arrest in the G_2/M phase and apoptosis in ER (+) breast cancer cells, genistein inactivated *bcl-2*, an antiapoptotic gene, through phosphorylation (104). The authors proposed that the increase in phosphorylation of *bcl-2* in response to genistein is probably the result of increased serine phosphorylation, made possible through the inactivation by genistein of a serine phosphatase that normally dephosphorylates *bcl-2* (104). Studies by Darbon et al. (109) provide evidence that genistein induced G_2 arrest in melanoma cells related to an impairment of Cdc25C-dependent dephosphorylation of Tyr^{15} of Cdk1. This cell cycle arrest was also found to likely be due in part to the activation of a check-point kinase (Chk2, a serine/threonine kinase). Chk2 is known to directly phosphorylate and inhibit Cdc25C, a mitotic activator. The ability of caffeine to totally override the arrest in the G_2 phase of the cell cycle caused by genistein again points to the need to understand the dynamic interactions that are possible among dietary components.

Cell cycle arrest at the G_2/M phase can also result from inhibition of topoisomerase II (Topo II) (89,104), a nuclear enzyme important to DNA structural integrity that is critical for DNA replication and transcription and is a potential target for genistein. In one recent study, genistein inhibited Topo II in colon cancer cells, as measured by Topo II-mediated DNA breakage, and cell cycle arrest at the G_2/M phase was observed. Furthermore, data indicated that Topo II-mediated DNA breakage was not required for apoptosis (89).

Genistein also has been demonstrated to be a protein tyrosine kinase (PTK) inhibitor, resulting in inhibition of cell growth (90,99,108). In a study in which genistein inhibited the growth of neuroblastoma cells and induced cell differentiation, it inhibited PTK activity by 33% and IGF-stimulated PTK activity by 75% (108). Also, in this study, genistein reduced expression of the n-*myc* oncogene. An investigation into the mechanism by which genistein induced apoptosis in prostate cells determined that genistein inhibited the antiapoptotic transcription factor, NF-κB, a heterodimer (110). NF-κB exists in the cytoplasm as a trimer composed of the IκBα inhibitory protein and p50 and p65 subunits. The IκBα inhibitory protein must be phosphorylated and ultimately degraded to allow the subunits to translocate to the nucleus. Davis et al. (110) provided evidence that genistein reduces the amount of phosphorylated IκBα, thereby maintaining the NF-κB complex and blocking the translocation of the p50 and p65 subunits to the nucleus and the subsequent activation of the transcription factor.

Genistein recently has been reported to suppress in vitro and in vivo invasion and angiogenesis of breast cancer cells (95) and in vitro invasion of melanoma cells (96), as well as lung metastasis in mice induced by melanoma cells (97) and peritoneal metastasis of intestinal adenocarcinomas in rats (98). Inhibition of invasion of ER (+) and ER (−) breast cancer cells was accompanied by transcriptional downregulation of matrix metalloproteinase-9, most likely at activation protein-1 sites, and upregulation of tissue inhibitor of metalloproteinase-1, two common effector molecules implicated in regulating tumor cell invasion (95). In this study, genistein significantly suppressed angiogenesis only in ER (−) cell xenografts (in mice), as measured by decreased vessel density and decreased expression of vascular endothelial growth factor and transforming growth factor-β_1. In melanoma cells, genistein suppressed adhesion-induced tyrosine phosphorylation of proteins, one of the early events in the cancer cell-extracellular matrix interaction, at the cell periphery, thus interfering with the cell-extracellular matrix interaction and decreasing invasive potential (96).

Significant physiological effects are attributed to dietary genistein. The actual response relates to the quantity and timing of its exposure. Although many molecular targets have been identified, it remains unclear which is most critical in explaining the overwhelming evidence for its anticancer properties. Finally, it should be emphasized that genistein under some circumstances may increase cancer risk (81,111). The identification of those who benefit from or are vulnerable to supplemented soy or genistein is a matter of immediate concern, because many individuals are self-medicating.

Resveratrol: Resveratrol (3,4′,5-trihydroxystilbene), a natural phytoalexin formed in a variety of plant species, such as grapes, mulberries, and peanuts, in response to environmental stress and fungal infections, is present in high concentrations in fresh grape skins and red wine (112). As with genistein, recent findings indicate that resveratrol has an impressively wide range of molecular targets that could influence carcinogenesis at various stages. Specifically, resveratrol has been reported to inhibit *CYP1A1* gene expression by preventing the binding of the aryl hydrocarbon acceptor to promotor sequences that regulate transcription (113); inhibit activity of ornithine decarboxylase, a key enzyme in polyamine biosynthesis, which is enhanced in cancer cell proliferation (114); inhibit cyclooxygenase-2-(COX-2) activity, directly and by suppressing the activation of *COX-2* gene expression through inhibition of the protein kinase C signal transduction pathway (115); influence the expression of *bax* and *p21*, genes involved in the regulation of cell proliferation and apoptosis, in aberrant crypt foci (*bax* and *p21*) and surrounding mucosa (*bax* and *p21*) (116); induce apoptosis through activation of *p53*-dependent transcription (117); induce caspase-mediated apoptosis and *CD95* signaling-dependent apoptosis in tumor cells (118); act as an ER antagonist in the presence of estrogen, leading to growth inhibition of breast cancer cells (119); reduce expression of the AR, thus inhibiting androgen-stimulated cell growth and androgen-upregulated genes in prostate cancer cells (120); inhibit the transcription factor NF-κB, linked to inflammation and oncogenesis (121,122); and inhibit ribonucleotide reductase, the enzyme that provides proliferating cells with deoxyribonucleotides required for DNA synthesis (123). It is postulated that the inhibition of ribonucleotide reductase may result from the ability of resveratrol to scavenge a tyrosyl radical, important to the enzyme's activity, on the small R2 protein of ribonucleotide reductase (123). Data suggest that inhibition of ribonucleotide reductase and, thus, of deoxyribonucleotide/DNA synthesis by resveratrol may influence the cell division cycle (124,125). Della Ragione and colleagues (124) demonstrated that resveratrol blocked proliferation of the promyelocytic cell line HL-60 at the S-G_2 boundary, causing elongation of the S phase of the cell division cycle and rapid induction of cell differentiation.

Influence of Genetic Polymorphisms

As cancer prevention researchers try to identify nutrients that have specific molecular targets related to cancer risk, it is becoming clear that genetic differences play some role in the ability of individuals to withstand exposure to exogenous carcinogens or to inhibit initiation, promotion, or proliferation in carcinogenesis. Polymorphisms related to diet have not been as extensively examined as have those generally related to cancer or to cancer risk associated with tobacco use (126). A comprehensive review of metabolic polymorphisms, including a proposed nomenclature system and review of classes of polymorphisms relevant to the nutrition-cancer relationship, has been published recently (126). The impact of different polymorphic forms of the same gene or gene product likely has contributed to contradictory results from intervention studies (127,128). It is becoming apparent that the prevalence of polymorphisms is variable among studied populations, and these differences could influence the interpretation of results of otherwise solidly designed trials. For example, in a random sample of participants in the α-Tocopherol, β-Carotene Cancer Prevention Study, there was a low prevalence of polymorphisms in genes coding for activation (phase I) enzymes cytochrome *P*-450 1A1 (0.07) and 2E1 (0.02) and a high prevalence in genes coding for detoxification (phase II) enzymes glutathione *S*-transferase M1 (0.40) and NQO1 (0.20) (129). Interestingly, 7 of 10 members of this sample carried the VDR-*Taq*I polymorphism (t) associated with lower risk for prostate cancer, which may account in part for lower cancer rates in Finland than in the United States (129). Furthermore, in a nested case-control study within the α-Tocopherol, β-Carotene Cancer Prevention Study, glutathione peroxidase-1 (hGPX1), a selenium-dependent enzyme involved in detoxification of hydrogen peroxide, was found to have a polymorphism exhibiting a proline-to-leucine replacement at codon 198. This polymorphism conferred a relative risk (RR) for lung cancer risk of

1.8 for heterozygotes and 2.3 for homozygous variants compared with homozygote wild types (130). Numerous genetic and metabolic polymorphisms are relevant to cancer prevention (131); this review will focus on selected polymorphisms related to nutrients discussed in the previous sections.

A number of polymorphisms in the *VDR* gene have been identified; common polymorphisms include *Bsm*I, *Taq*I in intron 8 and exon 9, and a poly(A) site in the 3′ end of the gene (7). A recent population study of *VDR* polymorphisms and risk of breast cancer among Latinas in the United States noted that the *Bsm*I B and short poly(A) morphisms in the 3′ end of the *VDR* gene were associated with increased risk (132). *Bsm*I B polymorphisms (BB and Bb) also have been associated with decreased risk of prostate cancer and benign prostatic hyperplasia compared with *Bsm*I bb genotypes among Japanese men (133). The *Taq*I homozygous genotype (TT) does not influence the risk of breast cancer but may reduce the risk of lymph node metastasis and inhibit tumor progression and increase survival among ER (+), tamoxifen-treated women (134). Furthermore, *Taq*I polymorphisms have been associated with as much as a 60% reduction in prostate cancer risk for individuals with homozygous variants at codon 352 (tt) compared with individuals who are homozygote wild types or heterozygotes (TT or Tt) (135).

Several common genetic polymorphisms appear to modulate cancer risk through their influence on folate metabolism, including two polymorphisms of the *MTHFR* gene, 677 C → T (alanine → valine) and 1298 A → C (glutamate → alanine), and a polymorphism of *MTR*, the gene that codes for methionine synthase, 2756 A → G (aspartate → glycine); all these polymorphisms reduce enzyme activity (136–141). MTHFR irreversibly converts 5,10-methylenetetrahydrofolate (methylene THF), the major form of intracellular folate, to 5-methyl tetrahydrofolate (methyl THF), the major form of circulating folate in plasma. Methylene THF is the cofactor for methylating deoxyuridylate to produce thymidylate, the rate-limiting nucleotide in DNA synthesis. Decreased activity of MTHFR increases the availability of methylene THF, thus reducing the chances of insufficient thymidylate and misincorporation of uracil into DNA. Reduced incorporation of uracil into DNA leads to fewer chromosome breaks and possibly less cancer risk (136,137,141). Epidemiological studies have reported that when folate intake was supposedly adequate, colorectal cancer risk was reduced (>50%) in individuals with the MTHFR 677TT genotype compared with the MTHFR 677CC genotype (140,142,143), and risk of adult acute lymphocytic leukemia (ALL) was reduced by 77% (141). When folate intake was low, however, the genetic profile became unimportant. Recent clinical data demonstrated that genomic DNA hypomethylation was greater in leukocytes of individuals with the MTHFR 677TT than with the MTHFR 677CC genotype and was inversely correlated with folate status (144).

Several authors suggested that lower MTHFR activity limits availability of methyl groups for synthesis of SAM, reducing the cellular capacity to methylate DNA (increasing hypomethylation) and that when folate status is low, this mechanism negates the beneficial effect of the MTHFR 677TT polymorphism that results from increased availability of thymidylate (142–144). Recent data suggest that the MTHFR 1298AC polymorphism may have an important role in adult ALL (141). Compared with the MTHFR l298AA genotype, risk for this disease was reduced by 67% and 93% in individuals with the MTHFR 1298AC and MTHFR 1298CC genotypes, respectively, suggesting that enhanced availability of methylene THF may contribute to inhibition of adult ALL development (141).

Methionine synthase, which catalyzes the transfer of a methyl group from 5-methyl THF to homocysteine, producing methionine and tetrahydrofolate, is essential for maintaining adequate intracellular methionine, the precursor of the methyl donor SAM (138). One epidemiological study reported that when folate status was adequate, individuals with the MTR 2756GG genotype had a 41% lower risk of colorectal cancer than individuals with the MTR 2756AA genotype, a finding that was contrary to the authors' hypothesis. As with the MTHFR 677TT genotype, reduced risks were comparable among genotypes when folate intake was low (138). Excessive intake of alcohol (a methyl group antagonist) can interfere with folate metabolism by decreasing SAM levels, thereby inducing hypomethylation of DNA, and thus might be expected to influence the effects of *MTHFR* and *MTR* polymorphisms on cancer risk (138,142, 143). Data support a slightly greater direct association of alcohol with colorectal cancer risk among folate-deficient men with the MTHFR 677TT genotype than in those with MTHFR 677CT and MTHFR 677CC genotypes (142,143). Interestingly, men with the MTR 2756GG genotype who consumed less than one drink per day were at lower risk (RR = 0.27) for colorectal cancer than those who consumed more than one drink per day (RR = 2.64), independent of folate status. Not all polymorphisms are equally susceptible to the impact of alcohol, considering that its intake did not affect risk for MTR 2756AG or MTR 2756AA genotypes (138).

Implications for Food Production

Given the strikingly large number of nutrients that can potentially influence cancer risk through their interactions with cancer-related molecular targets, it is essential to consider what implications such insights might have for food production. For example, natural garlic sold in grocery stores contains <0.05 ppm (dry wt) selenium (47). Ip and Lisk (145) increased the selenium concentration in garlic (110–150 ppm dry wt) and onion (28 ppm dry wt) through selenium fertilization and demonstrated that the high-selenium vegetables inhibited mammary cancer in rats. Interestingly, garlic was more effective than onion at the same level of selenium supplementation (145), suggesting that the food matrix can influence the response. The complexity of

such interactions is illustrated by the ability of dietary selenium supplements to increase the efficacy of garlic to retard carcinogen bioactivation and tumor formation (146). Thus, to assess the overall response, one must consider not only variation in the nutrient content within a food source but across the entire diet.

Standard agronomic approaches can now be complemented or, in some cases, replaced by advances in biotechnology, defined here as any technique that enhances the genetic information of living organisms, including plants, to create new genetic combinations designed to emphasize or express certain traits (147), that have made it possible to develop genetically modified (GM) foods. In plants, genetic modification can result in improved agronomic traits, such as herbicide/pesticide tolerance, or improved quality traits, including nutrient content. Approaches using genomic technologies can identify the genes important for plant metabolic pathways that have human nutritional importance (2). Foods in which physiologically active ingredients such as essential nutrients or phytonutrients have been manipulated (increased, decreased, or modified), whether as a result of genetic modification (GM foods) or food processing (e.g., fortified and low-fat foods), can be categorized as "functional foods," foods that provide potential health benefits, including cancer risk reduction, beyond basic nutrition (148,149).

There is evidence that the overexpression of genes can markedly influence nutrient content of foods. For example, enhancing the gene involved in encoding the final enzyme in vitamin E synthesis, γ-tocopherol methyltransferase, using a genomics-based approach increased α-tocopherol concentration by nearly ninefold in *Arabdopsis* seed oil (150). As another example of genetic modification of plant metabolism, transgenic soybeans have been developed in which the relative level of oleic acid (a monounsaturated fatty acid) has been raised from 25% to 85%, thus increasing the shelf life of soy-related products by reducing the potential for oxidative damage (151).

The deliberate manipulation of phytonutrients in plant-derived foods is associated with a number of relevant issues that can be most readily examined by considering a specific example, such as genistein in soybeans. A great deal of research effort has already been directed to gene discovery for purposes of improving soybean quality traits, in terms of oil and protein composition (151). Similar genomic approaches could be used to increase the concentrations of genistein and possibly other constituents in soybeans. If it is assumed that the genistein-related genes can be identified and their regulation implemented, a decision would have to be made regarding what concentration and chemical form (glycoside vs. aglycone) of genistein in soybeans are desired. Clearly, any decision must consider the intended use and health consequences for consumers. Nevertheless, the answer to this question is not straightforward. It will depend on numerous factors, including interindividual differences in genistein metabolism, effects of processing and food storage on the genistein in soy products, and the concentrations required in humans for effective (but not harmful) biological activity. Genistein excretion has been reported to vary more than eightfold (8.5–69.5%) among individuals (84). Evidence already indicates a curvilinear relationship between absorption of genistein from food (as the glycoside) and plasma concentrations of genistein (as the aglycone), indicating that it might be difficult to achieve supraphysiological levels from foods (152). Likewise, the literature contains evidence that the glycoside form of genistein is absorbed in lower amounts than the aglycone (153). Fermentation of soy foods (e.g., tempeh) is recognized as a method to improve bioavailability, because it leads to increased hydrolysis of the glycoside (154). Basically, it remains to be demonstrated that increasing the genistein content of such soy products and integrating them into dietary patterns could increase plasma concentrations of genistein to the relatively high levels (10–100 μM) used to bring about cancer-inhibitory effects in cell cultures (74,89,92,104,109,110). Although the development of GM and functional foods has tremendous potential for enhancing the benefits of the food supply for reducing disease risk, including cancer risk, the evidence base required to support and implement this area of research needs to be greatly expanded.

Evidence exists that citizens of the United States are receptive to GM foods. A 1997 survey indicated that a majority of US consumers would be "very likely" (43%) or "somewhat likely" (34%) to purchase foods produced from GM insect-protected plants and "very likely" (22%) or "somewhat likely" (40%) to purchase foods from GM plants developed to give "better-tasting or fresher" produce (155). These findings are supported by a 1999 nationwide survey that reported that ~70% of consumers support foods produced through biotechnology and 80% have confidence in the safety of the food supply (156). Not all parts of the world appear to be equally receptive to this new technology (157,158). The perception of safety, however, is only one of several factors important for consumer acceptance of foods. Data from a study in a national sample of US adults indicated that taste is the most important influence on food choices, followed by cost (159). Thus it is important to recognize that many phytonutrients, including flavonoids, isoflavones (e.g., genistein), terpenes, isothiocyanates, and glucosinolates, are usually bitter, acrid, or astringent, even in very small amounts (160), and that sensitivity to bitter taste is a heritable trait that can influence food preferences (160,161). Debittering processes, which remove bitter peptides, oxidized soy lipids, and isoflavones, are commonly used when producing soy products (160). It is evident that a significant challenge of any strategy designed to manipulate phytonutrients in plant-derived foods to potentially reduce disease risk will be to ensure the sensory acceptability of such foods.

Future Research Directions

Research in nutrition and cancer prevention in this new millennium must give top priority to studies that seek to un-

derstand the basic molecular and genetic mechanisms by which nutrients influence the various steps in carcinogenesis. A well-coordinated, multidisciplinary effort among scientists, including nutritional scientists, molecular biologists, geneticists, statisticians, and clinical cancer researchers, will be required to advance a molecular approach to nutrition-related cancer research, a complex research area that presents enormous challenges. Some challenges will be readily met and overcome through current and future technological advances; others may be harder or even not possible to overcome in the near future. However, in all these circumstances, rigorous and concentrated research efforts will help build our knowledge of the underlying molecular events that govern nutrient-cancer relationships, knowledge that will be essential for developing evidence-based strategies for cancer prevention through dietary modification.

Many important research questions and issues relevant to nutrition and cancer must be addressed. For instance, how do interactions among individual nutrients influence cancer risk? Such interactions might have beneficial or adverse cancer-related effects. The independent effects of individual nutrients on molecular targets with consequences for carcinogenesis can be determined in experimental studies that focus solely on these nutrients, but such studies do not provide information about the possible interactions that might occur among nutrients of interest, when consumed as part of a whole food, and numerous other nutrients, in the same food or in other foods consumed at the same time. To illustrate, black tea and soybean products are commonly consumed together in a typical Japanese diet. In one study, thearubigen (a polyphenol in black tea) did not alter the in vitro growth of human prostate cancer cells when administered alone. However, a small amount (0.5 μg/ml) of thearubigen administered with genistein (20 μg/ml) synergistically inhibited cell growth and increased the DNA distribution at the G_2M phase of the cell division cycle by 34.2% compared with genistein alone (162). Such interactive effects may help explain why epidemiological studies investigating cancer risk that focus exclusively on consumption of black tea or soy products might not readily uncover an existing association. Given the huge number of nutrients in foods, particularly plant-based foods, that can potentially interact to influence cancer risk, in addition to interindividual differences in susceptibility (e.g., genetic polymorphisms), it is not surprising that findings from animal and in vitro studies often are not consistent with epidemiological findings. In fact, resolving conflicting data and inconsistencies among study results may be one of the more important aspects of basic research on the mechanisms of nutrient interactions with molecular targets and the distribution of specific genetic polymorphisms that might affect such nutrient interactions.

Many other questions readily come to mind. For example, are the effects of some nutrients on molecular targets influenced by age and gender? Would these determinants be easily recognized? How can the large interindividual differences in nutrient metabolism best be taken into account? Can biomarkers that indicate nutrient status be identified and validated? Does inhibition of a cancer-related pathway by the interaction of a nutrient with a specific molecular target cause compensatory activity to be initiated through other pathways, possibly negating any beneficial effect of the nutrient? If nutrients interact with cancer-related molecular targets through several mechanisms, how can the targets/mechanisms most important for reducing cancer risk be identified? Can nutrient-modulated biomarkers that can serve as surrogate end-point biomarkers for clinical disease be identified and validated? Will currently used study designs and analytic techniques be adequate in molecular-based nutrition and cancer research? Innovative approaches using sophisticated methodologies such as differential polymerase chain reaction and DNA microarray technology may need to be developed, particularly to delineate interactions and specific nutrient effects on gene expression (163). For instance, one study on rat mammary carcinomas treated with limonene (a monoterpene found particularly in citrus fruits) identified 42 limonene-induced genes (9 identified) and 58 limonene-suppressed genes (1 identified) by using subtractive display, a gene expression screening method based on polymerase chain reaction amplification that was developed by combining aspects of subtractive hybridization and differential display. Several of the identified genes are involved in the mitoinhibitory transforming growth factor-β signal transduction pathway, supporting the authors' hypothesis that monoterpenes may initiate mitoinhibitory and apoptotic signaling through a signal transduction-related mechanism (164). Such analytic techniques capable of determining nutrient effects on gene expression in vivo can make significant contributions to the nutrition and cancer knowledge base and should be one focus of future research.

The development and implementation of a successful multidisciplinary effort that emphasizes a molecular approach to nutrition-related cancer research will require collaboration, motivation, dedication, and training and education across all relevant disciplines. Perhaps it is just as important to recognize that developing and implementing this new research paradigm will require considerable time and patience. As noted above, the challenges to researchers will be enormous. The potential rewards, however, in terms of reducing cancer morbidity and mortality, will be of equally great magnitude.

Acknowledgments and Notes

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