MINIREVIEW

Nutritional Interactions: Credentialing of Molecular Targets for Cancer Prevention

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Dietary behavior has been identified as one of the most important modifiable determinants of cancer risk. Which personalized modifications are needed remains an area of considerable controversy. Part of this uncertainty may arise from interactions among dietary bioactive compounds and/or food combinations. These interactions may either enhance or negate the response to specific foods. Evidence suggests that the cancer-protective effects of an individual's diet may reflect the combined effects of various vitamins, minerals, and other bioactive components such as flavonoids, isothiocyanates, and/or allium compounds rather than from the effect of a single ingredient. A better understanding of physiologically important interactions is needed to determine the merit of combining foods for maximum efficacy for cancer prevention. Furthermore, the response is complicated, since multiple cellular processes associated with carcinogenesis can be modified simultaneously, including sites such as drug metabolism, DNA repair, cell proliferation, apoptosis, inflammation, differentiation, and angiogenesis. Current evidence suggests that bioactive food components can typically influence more than one process. It is essential to have a better understanding of how the response relates to exposures and credentialing which process is most involved in bringing about a change in tumor incidence and/or tumor behavior. Credentialing is being defined as a determination of which cellular process(es) and which bioactive food components are most important for bringing about a phenotypic change. Additional attention is needed to determine the critical intake of dietary components, their duration, and when they should be provided to optimize the desired physiological response. Further research is also needed on the molecular targets for bioactive components and whether genetic and epigenetic events dictate the direction and magnitude of the response. Exp Biol Med 232:176–183, 2007

Key words: bioactive dietary components; cancer; prevention; molecular targets

Introduction

Dietary habits are recognized as an important modifiable environmental factor influencing cancer risk and tumor behavior. While some have estimated that about 30%–40% of all cancer cases relate to diet, the actual percentage is highly dependent on the dietary components and the specific type of cancer (1). Epidemiologic evidence suggests that regular consumption of fruits, vegetables, and whole grains may reduce cancer risk in some individuals. This association has been attributed to these foods being rich sources of numerous bioactive compounds. Plant foods contain a variety of components, including, but not limited to, essential nutrients, polyunsaturated fatty acids, and phytochemicals such as glucosinolates and flavonoids, many of which can inhibit cell proliferation and induce apoptosis, and which may act additively or synergistically when combined in the human diet. While optimizing the intake of specific foods and/or their bioactive components seems a prudent, noninvasive, and cost-effective strategy for reducing the cancer burden, this is far from a simple process (2). The magnitude of the problem of identifying which dietary components are most important in increasing or decreasing cancer risk is evident from the literally thousands of compounds consumed each day (2). Furthermore, the lack of information about some components limits the ability to unravel which bioactive components are the most important. It is estimated that >5000 individual phytochemicals have been identified in fruits, vegetables, and grains, but a large percentage still remain unknown and need to be identified before we can fully understand the health benefits of phytochemicals in whole foods (3). Furthermore, interactions between the different components within a food or

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through food combinations may explain why isolated dietary components may not be as efficacious for cancer prevention as whole foods.

A combination of wine, fish, dark chocolate, fruits, vegetables, garlic, and almonds has been suggested to offer additive benefits and to reduce cardiovascular disease by more than 75% (4). This hypothesis was recently tested when a combination of cholesterol-lowering foods (plant sterols, soy protein, viscous fibers, and almonds) was found to be as efficacious as certain statins in lowering LDL-cholesterol concentrations (5). Whether or not these, or possibly other, food combinations offer special protection against cancer risk and/or modify the behavior of tumors remains to be adequately evaluated. Some studies do suggest that combinations of foods may offer special advantages for cancer prevention. For example, combining soy phytochemicals and green tea extracts has been reported to be more effective in inhibiting tumor angiogenesis, reducing estrogen receptor (ER)-alpha, and lowering serum insulin-like growth factor (IGF)-1 in estrogen-dependent human breast tumors implanted into severe combined immunodeficient mice than when either is provided alone (6). Similarly, soy phytochemicals and green tea synergistically inhibited prostate tumorigenicity, final tumor weight, and metastasis in a mouse model of orthotopic androgen-sensitive human prostate cancer (7). The combination of orange, apple, grape, and blueberry displayed a combined beneficial effect in antioxidant activity. The median effective dose (EC_{50}) of the combination of fruits was five times lower than the EC_{50} of each fruit alone, suggesting at least additive effects after the combination of the four fruits (8). This may reflect different phytochemical profiles in the different fruits. These results also suggest that combinations of foods may be efficacious for cancer prevention. However, the merit of combining foods, as well as which foods should be combined for maximum cancer prevention, remains to be determined. A better understanding of the bioactive components present in food, as well as the mechanism(s) of action of these dietary components towards cancer prevention, is needed before this can be achieved.

Individual Foods Versus Their Isolated Constituents

There is evidence that individual foods may offer advantages over their isolated constituents, suggesting factors in the foods may influence the absorption, metabolism, or retention of the bioactive food components or that multiple bioactive compounds within the food are exerting additive or synergistic effects. For example, whole green tea is more efficacious than epigallocatechin gallate in inhibiting TNFα release and increasing the percentage of human lung cancer cells undergoing apoptosis (9). These effects appear to be mediated through enhanced incorporation of the tea polyphenols into the cells (9). Similarly, consumption of tomato powder, but not one of its principal components, lycopene, inhibited N-methyl-N-nitrosourea and testosterone-induced prostate cancer (10). A fat-soluble extract from vegetable powder was more efficacious than β-carotene in inhibiting cell proliferation and inducing morphologic changes consistent with apoptosis (cellular shrinkage, chromatin condensation, and nuclear fragmentation) in a lung cancer cell line (11). However, in other cases the food may not be as effective as its isolated components, suggesting the food may contain constituents that inhibit the response by again modifying the absorption, metabolism, or site of action of the bioactive food constituent. Such antagonistic components may explain the reduced ability of soy flour and full-fat soy flakes to inhibit aberrant crypt foci compared to isolated genistein (12). A better understanding of how the food matrix and combination of bioactive components present within food influence cancer prevention is needed.

Biology of Cancer

Malignant cells are characterized by the upregulation or constitutive activation of multiple signaling pathways that promote proliferation, inhibit apoptosis, and enable the cells to invade and migrate through tissues while evoking angiogenesis (13). Downregulation or loss of proteins and pathways that oppose these behaviors is also commonly seen. Hanahan and Weinberg (14) have summarized the derangements in signaling that are needed for the formation of a fully invasive tumor. These include:

1. Self-sufficiency in growth signals, such that the tumor cells produce their own, or the relevant pathways become constitutively active without the need for exogenous signals.
2. Insensitivity to signals which would normally inhibit proliferation.
3. Invoking survival pathways in order to avoid apoptosis; this would normally occur in irreversibly damaged cells.
4. Replicating indefinitely, thus avoiding terminal differentiation or senescence.
5. Initiating angiogenesis to ensure sufficient oxygen and nutrient supply to sustain tumor growth.
6. Undergoing invasion and metastasis.

During the carcinogenic process, cells often acquire multiple oncogenic mutations that are often functionally redundant; thus, inhibiting any single dysregulated pathway may have only a modest impact on tumor behavior (13). Therefore, the likelihood that any single agent or dietary component would have a "magic bullet"-like impact on cancer is lessened. Targeting multiple molecular targets that are prone to dysregulation in a given type of cancer seems to offer the best prospect for cancer prevention (13). While this strategy has been adopted for some time in the chemotherapeutic arena, it appears to be equally applicable in cancer prevention (15).

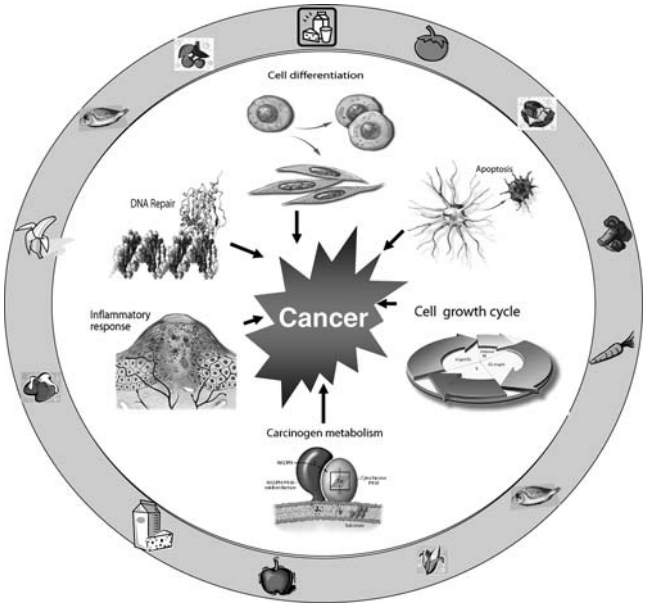

Figure 1. Bioactive components present in food can influence molecular targets associated with several biological processes. These processes, such as cell cycle control, apoptosis, carcinogen metabolism, cell differentiation, DNA repair, and the inflammatory response, have been associated with carcinogenesis.

Mechanisms of Dietary Cancer Prevention

All of the major signaling pathways, which are deregulated in cancer and which have been examined as targets for cancer prevention, have responded to one or more dietary components (Fig. 1; Ref. 16). These include, but are not limited to, carcinogen metabolism, DNA repair, cell proliferation/apoptosis, inflammation, differentiation, oxidant/antioxidant balance, and angiogenesis. So far, more than 1000 different phytochemicals have been credited with putative cancer preventive activities (17), and in recent years much effort has gone into elucidating the molecular mechanisms of a few of them (15). However, the response is complicated, since the effects of dietary components can be cell type- and dose-dependent, and any one agent may have multiple mechanisms of action. Furthermore, most of the detailed mechanistic data have been obtained in cell culture studies, often with physiologically unachievable concentrations of single agents, making extrapolation to humans difficult (15). Therefore, when interpreting the results from *in vitro* studies, care must be taken to consider dose, cell type, culture conditions, and treatment time, as each of these can affect the biological outcome. Credentialing which process(es) is/are most involved in bringing about a change in tumor incidence and/or tumor behavior is essential. In addition, since many of these processes are likely influenced by several food components, it is necessary to obtain a better understanding of physiologically relevant synergistic and antagonistic interactions.

Virtually all dietary or environmental carcinogens to which humans are exposed require enzymatic biotransformation, known as metabolic activation, to exert their carcinogenic effects. Biotransformation enzymes, also referred to as xenobiotic- or drug-metabolizing enzymes, play a major role in regulating the mutagenic and neoplastic effects of chemical carcinogens, as well as metabolizing other drugs and endogenous compounds, such as steroid hormones. The drug-metabolizing enzyme system comprises phase I (oxidation, reduction, and hydrolysis) usually catalyzed by cytochrome P-450 enzymes and phase II (glucoronidation, sulfation, acetylation, methylation, and conjugation with glutathione) enzymes. The induction of Phase II enzymes is largely mediated by the antioxidant response element (ARE), which is located in the promoter region of specific genes (18). Generally, the transcription factor nuclear factor E2-related factor 2 (Nrf2) binds to the ARE sequence to initiate gene expression. Many enzyme inducers, including dietary components, also lead to the activation of several signal transduction pathways, such as the mitogen-activated protein kinases (MAPK), protein kinase C (PKC), and phosphatidylinositol 3-kinase (PI3K) pathways. The consequences of the activation of these signaling cascades are dissociation of Nrf2 from another cytosolic protein, Keap1; nuclear translocation; and accumulation of Nrf2 protein, which leads to increased expression of detoxifying enzymes through activation of the ARE. Bioactive components present in fruits and vegetables can prevent carcinogenesis by blocking metabolic activation, by increasing detoxification, or by providing alternative targets for electrophilic metabolites. Numerous constituents of plant foods, including flavonoids (such as quercetin, rutin, and genistein), phenols (such as curcumin, epigallocatin-3-gallate and resveratrol), isothiocyanates, allyl sulfur compounds, indoles, and selenium have been found to be potent modulators of detoxification enzymes *in vitro* and in preclinical models (19, 20).

Activated carcinogens exert their biological effects by forming covalent adducts with the individual nucleic acids of DNA or RNA. Similarly, reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl radicals, have been found to attack both DNA bases and the deoxyribosyl backbone of DNA (21). DNA adducts distort the shape of the DNA molecule, potentially causing mistranslation of the DNA sequence. Second, when the DNA replicates, an adducted base that persists unrepaired can be misread, producing mutations in critical genes, such as oncogenes and tumor suppressor genes. Third, repair of bulky adducts can result in breakages of the DNA strand, which can, in turn, result in mutations or deletions of genetic material (22). Numerous DNA repair pathways exist to prevent the persistence of damage and are integral to the maintenance of genome stability and prevention of cancer (23). DNA repair mechanisms include direct repair, base excision repair, nucleotide excision repair, double-strand break repair, and repair of interstrand cross-links (24). DNA repair capacity can be assessed from mRNA or protein levels or enzyme activity. Folate deficiency has been found to disrupt DNA repair pathways

(25). Dietary components that scavenge activated oxygen species, such as flavonoids, vitamins E and C, and isothiocyanates, have been shown to stimulate repair of oxidative DNA damage. Moreover, it has been shown that dietary supplementation with cooked carrots increased the repair of 8-oxodG (an indicator of oxidative DNA damage) in white blood cells, whereas a similar amount of α-carotene and β-carotene provided as capsules had no effect (26). This strengthens the notion that whole food products rather than single bioactive components have beneficial effects against cancer. It can be further speculated that this effect is a result of other constituents in carrots, or, alternatively, it may be interactions between antioxidants and other components that render antioxidants more bioactive in a food matrix (27).

DNA damage can also arrest cell cycle progression to allow for repair and prevention of the alteration to become permanent or activate apoptosis to eliminate cells with potentially catastrophic mutations (24). Alterations in DNA repair, cell cycle progression, and apoptosis are all important molecular targets for dietary components in cancer prevention.

Generally, the growth rate of preneoplastic or neoplastic cells outpaces that of normal cells because of malfunctioning or dysregulation of their cell-growth and cell-death machineries (28). Therefore, induction of cell cycle arrest or apoptosis by dietary bioactive compounds can be an excellent approach to inhibit the promotion and progression of carcinogenesis (29). Cell-cycle progression is a sequential process that directs dividing mammalian cells through G1, S, G2, and M phases. Transitions between G1-S or G2-M phases function as checkpoints to halt cell division if necessary. Because the balance between the interactions among cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors (CDIs) governs the progression of the cell cycle (30), perturbation of any of the cell cycle–specific proteins by dietary components can potentially affect and block the continuous proliferation of neoplastic cells. Examples of dietary components that modulate cell proliferation include phenolic compounds, such as genistein and epigallocatechin-3-gallate, that elicit cell-cycle arrest through the induction of CDIs (p21 and p27) and the inhibition of CDK4, CDK2, cyclin D1, and cyclin E (31). Isothiocyanates can also induce p21 expression and inhibit cell proliferation at the G2-M checkpoint (32).

Apoptosis is one of the most potent defenses against cancer, since this process eliminates potentially deleterious, mutated cells. Many dietary cancer-preventive compounds, including selenium, epigallocatechin-3-gallate, phenylethyl isothiocyanate, retinoic acid, sulforaphane, curcumin, apigenin, quercetin, and resveratrol, inhibit apoptosis (33, 34). Distinct from the apoptotic events in the normal physiological process, which are mediated mainly by the interaction between death receptors and their relevant ligands (35), many bioactive dietary components appear to induce apoptosis through the mitochondria-mediated pathway. Dietary compounds generally induce oxidative stress, which downregulates antiapoptotic molecules such as Bcl-2 or Bcl-x and upregulates proapoptotic molecules such as Bax or Bak (29). The imbalance between antiapoptotic and proapoptotic proteins elicits the release of cytochrome c from the mitochondrial membrane, which forms a complex with caspase-9 with the subsequent activation of caspases-3, –6, and –7 (36). The activated caspases degrade important intracellular proteins, leading to the morphological changes and the phenotype of apoptotic cells (37). To enhance this mitochondria-mediated apoptosis, dietary components also activate proapoptotic c-Jun N-terminal kinase (JNK) and inhibit antiapoptotic NF-κB signaling pathways (29). Thus, the cytotoxic effects of dietary components on cells can be monitored by measuring their effects on mitochondria, caspases, and other apoptosis-related proteins.

Inflammation represents a physiological response to invading microorganisms, trauma, chemical irritation, or foreign tissues. Although acute inflammation is usually beneficial, chronic inflammation is often detrimental to the host. Epidemiologic data show an association between chronic inflammatory conditions and subsequent malignant transformation in the inflamed tissue (38). Evidence indicates that there are multiple mechanisms linking inflammation to cancer and that there are multiple targets for cancer prevention by bioactive dietary components. At the molecular level, free radicals and aldehydes produced during chronic inflammation can induce gene mutations and post-translational modifications of key cancer-related proteins (39). In response to an inflammatory insult, pro-inflammatory cytokines such as tumor necrosis factor-a (TNF-a), interleukin-1 (IL-1), IL-6, IL-12, and γ-interferon are synthesized and secreted resulting in an elevation in reactive oxygen and nitrogen species. This process is followed by the secretion of anti-inflammatory cytokines (e.g., IL-4, IL-10, and TGF-β) to reduce the accumulation of reactive species. The binding of pro-inflammatory cytokines to their receptors triggers many signaling pathways including the mitogen-activated protein kinase (MAPK) pathway, which can activate two redox-sensitive transcription factors, namely, nuclear factor κB (NFκB) and the c-Jun part of activating protein-1 (AP-1). These transcription factors activate the expression of a wide variety of genes including inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). These enzymes, in turn, directly influence ROS and eicosanoid levels. ROS profoundly affects numerous critical cellular functions, and the absence of efficient cellular detoxification mechanisms which remove these radicals can increase cancer risk (40). ROS can also specifically activate certain intracellular signaling cascades and thus contribute to tumor development and metastasis through the regulation of cellular phenotypes such as proliferation, death, and motility (40).

Chronic inflammation results in increased DNA damage, cellular proliferation, the disruption of DNA repair pathways, inhibition of apoptosis, and the promotion of

angiogenesis and invasion (38), all of which are important during the cancer process. Several of these mechanisms are amenable to influence by dietary constituents. Evidence exists that selected dietary components, including conjugated linoleic acid, long chain omega-3 fattty acids such as those in fish oil, butyrate, epigallocatechin-3-gallate, curcumin, resveratrol, genistein, luteolin, quercetin, and vitamins A and D, may influence the inflammatory process at various sites (41–46).

Angiogenesis, the development of new blood vessels from endothelial cells, is a crucial process in tumor pathogenesis as it sustains malignant cells with nutrients and oxygen (47). During angiogenesis, endothelial cells are stimulated by various growth factors, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), and are attracted to the site where the new blood supply is needed by inflammatory cytokines and chemoattractants (48, 49). Chemotactic migration along this gradient is, however, possible only through the degradation of extracellular matrix components (50). This is accomplished *via* matrix metalloproteinases (MMPs) (51, 52). Preventing the expansion of new blood vessel networks results in reduced tumor size and metastasis and is another mechanism whereby dietary components inhibit tumor growth. Dietary components that inhibit angiogenesis include polyunsaturated fatty acids (53) and polyphenols such as epigallocatechin-3-gallate, resveratrol, curcumin, and genistein (54–56).

Combinations of Individual Cancer Protective Components

Strategies that use a combination of agents (dietary bioactive food components or drugs) with distinct molecular mechanisms, rather than individual agents, offer an exciting opportunity for maximizing cancer prevention while minimizing toxicity. The combination can be designed to target multiple pathways or to reinforce the effects on a particular pathway. This approach has increased the understanding of the merits of combining drugs. For example, a combination of the nonsteroidal anti-inflammatory drug sulindac, which inhibits COX-2, and the EGFR inhibitor EKB-569 almost completely protected APC^{min} mice from adenoma formation (57). Furthermore, the effective dose of sulindac could be reduced by 75% (57).

Recently, dietary components have been found to exert additive or synergistic effects with pharmaceutical agents by modifying different molecular targets. Synergy is used to describe an outcome in which the response to the combination is statistically greater than the sum of the response to the two single-agent treatments. For example, diets containing high levels of olive oil exert a significant protective effect against colon tumor development that is additive with the inhibitory effects of sulindac, possibly related to the regulation of the expression and activity of key proteins involved in prostaglandin-biosynthesis (COX-2) and apoptosis-induction (Bcl-2 and caspase-3) pathways (58). The soy isoflavone daidzein has been reported to improve the capacity of tamoxifen to reduce mammary tumor burden, incidence, and multiplicity, as well as increasing tumor latency (59). These effects appear to be mediated through protection against oxidative DNA damage (59). Recent cell culture and animal studies also suggest that dietary compounds, including genistein, curcumin, epigallocatechin-3-gallate, resveratrol, indole-3-carbinol, proanthocyanadin, and vitamin D3, enhance the efficacy of cancer chemotherapeutic drugs and radiotherapy by modifying the activity of key cell proliferation and survival pathways, such as those controlled by Akt, nuclear factor-6B, and cyclooxygenase-2 (reviewed in Ref. 60). Additive or synergistic relationships may relate to different molecular targets and thus a combined effect or a maximum response to one cancer process.

Dietary components that modify the same molecular targets as drugs may allow lower amounts of the drug to be used for cancer prevention and thus minimize potential adverse effects of the drugs. For example, a diet enriched in omega-3 fatty acids can inhibit COX-2 activity and have synergistic effects with low doses of celocoxib for colon cancer prevention (61). Indole-3-carbinol, the hydrolysis product of glucosinolates occurring in cruciferous vegetables, is protective against the hepatoxicity of the antitumor drug ET743 or Trabectidin without compromising its efficacy (62). Similarly, docosahexaenoic acid (DHA) and genistein attenuated the induction of HMG-CoA reductase activity in mevastatin-treated MCF-7 cells (63). This suggests that dietary genistein or DHA may lower the dose of statin required to achieve the same level of reductase inhibition, thereby decreasing the potential for adverse effects of these drugs for breast cancer prevention.

Combinations of dietary components may also modify the dose of nutrients that are required to bring about a physiological effect. This is exemplified by the fact that low doses of 9-cis-retinoic acid and vitamin D3 that were not effective in preventing mammary cancer when given alone were effective when given in combination and bypassed any potential adverse effects of higher intakes (64). The mechanism responsible for this interaction is not known but may involve binding of the RARs, RXRs, and VDR, which could regulate the genes involved with cell proliferation, differentiation, and/or apoptosis. Similarly, low doses of *S*-allylcysteine and lycopene in combination were able to suppress the development of MNNG-induced gastric cancer *via* modulation of apoptosis-associated proteins (decreased Bcl-2/Bax ratio and upregulation of Bim and caspases 8 and 3) at much lower intakes than when given in isolation (65). Finally, the combination of vitamin D3 and genistein caused growth inhibition of DU145 prostate cancer cells at lower and biologically achievable concentrations of both compounds (66). Genistein appears to potentiate the action of vitamin D3 by directly inhibiting CYP24 enzyme activity and therefore increasing the half-

life of vitamin D3, which results in homologous upregulation of cellular VDR levels (66). This dual action of genistein leads to enhanced vitamin D–mediated responses and target gene activation, rendering the cells more sensitive to the growth inhibitory and proapoptotic signals of vitamin D3 (66). However, while there is increasing understanding of the additive and synergistic combinations of drugs, knowledge regarding such combinations of bioactive food components remains limited.

Dietary components that alter multiple molecular targets within a specific biological process or pathway have been shown to exert additive or synergistic effects. For example, dietary components that inhibit different phases of the cell cycle cooperate to inhibit tumor growth. Quercetin and genistein synergistically inhibit proliferation of ovarian carcinoma cells by modifying different stages in the cell cycle and different signal transduction pathways (67). Quercetin arrests the cell cycle at the G_1 and S phase boundary, whereas genistein affects the G_2 and/or early M phase. Similarly, quercetin interacted synergistically with resveratrol in causing transient cell cycle arrest in human leukemia cells (68), and epigallocatechin-3-gallate and curcumin synergistically inhibit growth of normal, premalignant, and malignant human oral epithelial cells (69). Whereas EGCG blocked cells in G_1, curcumin blocks cells in S/G_2M. Thus synergistic interactions between/among dietary phytochemicals likely contribute to inhibition of cell proliferation.

Apoptosis is another cellular process whereby inhibiting multiple molecular targets by different dietary components increases the cancer-protective effect. Selenium and vitamin E have been shown to have synergistic effects on apoptosis induction in human prostate cancer cells (70). This synergy was accounted for primarily by selenium and vitamin E modifying distinct signaling pathways of caspase activation. Selenium activated caspases-1 and -12, whereas vitamin E activated caspase-9. Thus selenium and vitamin E in combination may activate multiple molecular targets for apoptosis induction, the endoplasmic reticulum stress/cytokine signaling pathway and mitochondrial pathway, respectively. By targeting the entire battery of initiator caspases, selenium and vitamin E in combination may act in a cooperative fashion to "switch on the full force of the apoptotic machinery" (70). These studies suggest that combinations of nutrients with different mechanisms of action can likely have synergistic effects in cancer prevention. However, a much better understanding of how and which dietary components work in combination is sorely needed for better preventative strategies.

A critical unanswered question is what additive or antagonistic responses occur among dietary components that have the same molecular target. For example, both garlic organosulfur compounds and sulforaphane, which is present in broccoli, induce expression of detoxifying enzymes *via* the binding of the transcription factor Nrf2 to the ARE which is located in the promoter region of related genes. Can these findings be interpreted to mean that if an individual consumes sufficient organosulfur compounds, than sulforaphane will no longer have any anticancer effects? Or might other molecular targets be important? Thus, additional information is needed to determine the biological significance of nutrient-nutrient interactions.

Summary

Combinations of foods and/or bioactive dietary constituents may be efficacious for cancer prevention. However, which foods and/or components to combine for maximum cancer prevention remain to be determined. Credentialing, or prioritizing, bioactive components present in food, as well as the mechanism(s) of action of these dietary constituents towards cancer prevention, is needed before this can be achieved. The response is complicated, since the effects of dietary components can be cell- and dose-dependent, and any one agent may have multiple mechanisms of action. Furthermore, most of the detailed mechanistic data have been obtained in cell culture studies, often with concentrations of single dietary components that cannot be achieved *in vivo*. These limitations make extrapolations of results to humans difficult. New genetic technologies should be employed to study the impact of isolated and interactive dietary components on complex cellular and molecular networks to better understand the basis for complex interactions of food components on cancer prevention. Credentialing which process(es) is/are most involved in bringing about a change in tumor incidence and/or tumor behavior is also essential for optimizing cancer prevention.

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1. World Cancer Research Fund, American Institute for Cancer Research. Food, Nutrition and the Prevention of Cancer: A global perspective. Washington, D.C.: American Institute for Cancer Research, 1997.
2. Milner, JA. Molecular targets for bioactive food components. J Nutr 134:2492s–2498s, 2004.
3. Liu, RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 78:517S–520S, 2003.
4. Franco OH, Bonneux L, de Laet C, Peters A, Steyerberg EW, Mackenbach JP. The Polymeal: a more natural, safer, and probably tastier (than the Polypill) strategy to reduce cardiovascular disease by more than 75%. BMJ 329:1447–1450, 2004.
5. Jenkins DJA, Kendall CWC, Falkner DA, Nguyen T, Kemp T, Marchie A, Wong JMW, de Souza R, Eman A, Vidgen E, Trautwein EA, Lapsley KG, Holmes C, Josse RG, Leiter LA, Connelly PW, Singer W. Assessment of the longer-term effects of a dietary portfolio of cholesterol-lowering foods in hypercholesterolemia. Am J Clin Nutr 83:582–591, 2006.
6. Zhou JR, Yu L, Mai Z, Blackburn GL. Combined inhibition of estrogen-dependent human breast carcinoma by soy and tea bioactive components in mice. Int J Cancer 108:8–14, 2004.
7. Zhou JR, Yu L, Zhong Y, Blackburn GL. Soy phytochemicals and tea

bioactive components synergistically inhibit androgen-sensitive human prostate tumors in mice. J Nutr 133:516–521, 2003.
8. Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. J Nutr 134:3479S–3485S, 2004.
9. Suganuma M, Okabe S, Kai Y, Sueoka N, Sueoka E, Fujiki H. Synergistic effects of (–)-epigallocatechin gallate with (–)-epicatechin, sulindac, or tamoxifen on cancer-preventive activity in the human lung cancer cell line PC-9. Cancer Res 59:44–47, 1999.
10. Boileau TW, Liao Z, Kim S, Lemeshow S, Erdman JW Jr, Clinton SK. Prostate carcinogenesis in N-methyl-N-nitrosourea (NMU)-testosterone-treated rats fed tomato powder, lycopene, or energy-restricted diets. J Natl Cancer Inst 95:1578–1586, 2003.
11. Lu QJ, Huang CY, Yao SX, Wang RS, Wu XN. Effects of fat-soluble extracts from vegetable powder and beta-carotene on proliferation and apoptosis of lung cancer cell YTMLC-90. Biomed Environ Sci 16:237–245, 2003.
12. Thiagarajan DG, Bennink MR, Bourquin LD, Kavas FA. Prevention of precancerous colonic lesions in rats by soy flakes, soy flour, genistein, and calcium. Am J Clin Nutr 68:1394S–1399S, 1998.
13. McCarty MF. Targeting multiple signaling pathways as a strategy for managing prostate cancer: multifocal signal modulation therapy. Integr Cancer Therapies 3:349–380, 2004.
14. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 100:57–70, 2000.
15. Manson, MM. Inhibition of survival signaling by dietary polyphenols and indole-3-carbinol. Eur J Cancer 41:1842–1853, 2005.
16. Davis CD, Milner JA. Diet and cancer prevention. In: Temple NJ, Wilson T, Jacobs DV, Eds. Nutritional Health: Strategies for disease prevention. Totowa: Humana Press, pp151–171, 2006.
17. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 3:768–780, 2003.
18. Lee JS, Surh YJ. Nrf2 as a novel molecular target for chemoprevention. Cancer Lett 224:171–184, 2005.
19. Keum YS, Jeong WS, Kong AN. Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms. Mutat Res 555: 191–202, 2004.
20. Milner JA. A historical perspective on garlic and cancer. J Nutr 131: 1027S–1031S, 2001.
21. Bartsch H. DNA adducts in human carcinogenesis: etiological relevance and structure-activity relationship. Mutat Res 340:67–79, 1996.
22. Chao EC, Lipkin SM. Molecular models for the tissue specificity of DNA mismatch repair-deficient carcinogenesis. Nucleic Acids Res 34: 840–852, 2006.
23. Cooke MS, Evans MD, Dove R, Rozalski R, Gackowski D, Siomek A, Lunec J, Olinski R. DNA repair is responsible for the presence of oxidatively damaged DNA lesions in urine. Mutat Res 574:58–66, 2005.
24. Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem 73:39–85, 2004.
25. Frisco S, Choi SW. Gene-nutrient interactions in one-carbon metabolism. Curr Drug Metab 6:37–46, 2005.
26. Astley SB, Elliott RM, Archer DB, Southon S. Evidence that dietary supplementation with carotenoids and carotenoid-rick food modulate the DNA damage: repair balance in human lymphocytes. Br J Nutr 91: 63–72, 2004.
27. Moller P, Loft S. Interventions with antioxidants and nutrients in relation to oxidative DNA damage and repair. Mutat Res 551:79–89, 2004.
28. Jacks T, Weinberg RA. Taking the study of cancer cell survival to a new dimension. Cell 111:923–925, 2002.
29. Chen C, Kong AN. Dietary cancer-chemopreventive compounds: from signaling and gene expression to pharmacological effects. Trends Pharmacol Sci 26:318–326, 2005.
30. Weinstein IB. Disorders in cell circuitry during multistage carcinogenesis: the role of homeostasis. Carcinogenesis 21:857–864, 2000.
31. Agarwal R. Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents. Biochem Pharmacol 60:1051–1059, 2000.
32. Visanji JM, Duthie SJ, Pirie L, Thompson DG, Padfield PJ. Dietary isothiocyanates inhibit Caco-2 cell proliferation and induce G2/M phase cell cycle arrest, DNA damage, and G2/M checkpoint activation. J Nutr 134:3121–3126, 2004.
33. Martin KR. Targeting apoptosis with dietary bioactive agents. Exp Biol Med 231:117–129, 2006.
34. Hu R, Kong AN. Activation of MAP kinases, apoptosis and nutrigenomics of gene expression elicited by dietary cancer-prevention compounds. Nutrition 20:83–88, 2004.
35. Krammer PH. CD95′s deadly mission in the immune system. Nature 407:789–795, 2000.
36. Watson RW, Fitzpatrick JM. Targeting apoptosis in prostate cancer: focus on caspases and inhibitors of apoptosis proteins. BJU Int 96:30–34, 2005.
37. Thornberry NA, Lazebnik Y. Caspases: enemies within. Science 281: 1312–1316, 1998.
38. Hofseth LJ, Ying L. Identifying and defusing weapons of mass inflammation in carcinogenesis. Biochim Biophys Acta 1765:74–84, 2006.
39. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer 3:276–285, 2003.
40. Storz P. Reactive oxygen species in tumor progression. Front Biosci 10: 1881–1896, 2005.
41. Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James MJ. The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Am J Clin Nutr 63:116–122, 1996.
42. Shany S, Levy Y, Lahav-Cohen M. The effects of 1alpha,24(S)-dihydroxyvitamin D(2) analog on cancer cell proliferation and cytokine expression, Steroids 66:319–325, 2001.
43. Chan MM. Inhibition of tumor necrosis factor by curcumin, a phytochemical. Biochemical Pharmacology 49:1551–1556, 1995.
44. Xagorari A, Papapetropoulos A, Mauromatis A, Economou M, Fotsis T, Roussos C. Luteolin inhibits an endotoxin-stimulated phosphorylation cascade and proinflammatory cytokine production in macrophages. J Pharmacol Exp Ther 296:181–187, 2001.
45. Davis JN, Kucuk O, Djuric Z, Sarkar FH. Soy isoflavone supplementation in healthy men prevents NF-kappaB activation by TNF-alpha in blood lymphocytes. Free Radic Biol Med 30:1293–1302, 2001.
46. Manna SK, Aggarwal BB. All-trans-retinoic acid upregulates TNF receptors and potentiates TNF-induced activation of nuclear factors-kappaB, activated protein-1 and apoptosis in human lung cancer cells. Oncogene 19:2110–2119, 2000.
47. Fayette J, Soria JC, Armand JP. Use of angiogenesis inhibitors in tumour treatment. Eur J Cancer 41:1109–1116, 2005.
48. Presta M, Dell'Era P, Mitola S, Moroni E, Ronca R, Rusnati M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev 16:159–178, 2005.
49. Albini A, Tosetti F, Benelli R, Noonan DM. Tumor inflammatory angiogenesis and its chemoprevention. Cancer Res 65:10637–10641, 2005.
50. Pfeffer U, Ferrari N, Morini M, Benelli R, Noonan DM, Albini A. Antiangiogenic activity of chemopreventive drugs. Int J Biol Markers 18:70–74, 2003.
51. Cockett MI, Murphy G, Birch ML, O'Connell JP, Crabbe T, Millican AT, Hart IR, Docherty AJ. Matrix metalloproteinases and metastatic cancer. Biochem Soc Symp 63:295–313, 1998.

52. Ii M, Yamamoto H, Adachi Y, Maruyama Y, Shinomura Y. Role of matrix metalloproteinase-7 (matrilysin) in human cancer invasion, apoptosis, growth, and angiogenesis. Exp Biol Med (Maywood) 231: 20–27, 2006.
53. Rose DP, Connolly JM. Regulation of tumor angiogenesis by dietary fatty acids and eicosanoids. Nutr Cancer 37:119–127, 2000.
54. Cao Y, Cao R, Brakenhielm E. Angioangiogenic mechanisms of diet-derived polyphenols. J Nutr Biochem 13:380–390, 2002.
55. Dulak J. Nutraceuticals as anti-angiogenic agents: hope and reality. J Physiol Pharmacol 56:51–69, 2005.
56. Oak MH, El Bedoui J, Schini-Kerth VB. Antioangiogenic properties of natural polyphenols from red wine and green tea. J Nutr Biochem 16:1–8, 2005.
57. Torrance CJ, Jackson PE, Montgomery E, Kinzler KW, Vogelstein B, Wissner A, Nunes M, Frost P, Discafani CM. Combinatorial chemoprevention of intestinal neoplasia. Nat Med 6:1024–1028, 2000.
58. Schwartz B, Birk Y, Raz A, Madar Z. Nutritional-pharmacological combinations–a novel approach to reducing colon cancer incidence. Eur J Nutr 43:221–229, 2004.
59. Constantinou AI, White BE, Tonetti D, Yang Y, Liang W, Li W, van Breemen RB. The soy isoflavone daidzein improves the capacity of tamoxifen to prevent mammary tumours. Eur J Cancer 41:647–654, 2005.
60. Sarkar FH, Li Y. Using chemopreventive agents to enhance the efficacy of cancer therapy. Cancer Res 66:3347–3350, 2006.
61. Reddy BS, Patlolla JM, Simi B, Wang SH, Rao CV. Prevention of colon cancer by low doses of celecoxib, a cyclooxygenase inhibitor, administered in diet rich in omega-3 polyunsaturated fatty acids. Cancer Res 65:8022–8027, 2005.
62. Donald S, Verschoyle RD, Greaves P, Colombo T, Zucchetti M, Falcioni C, Zaffaroni M, D'Incalci M, Manson MM, Jimeno J, Steward WP, Gescher AJ. Dietary agent indole-3-carbinol protects female rats against the hepatotoxicity of the antitumor drug ET-743 (trabectidin) without compromising efficacy in a rat mammary carcinoma. Int J Cancer 111:961–967, 2004.
63. Duncan RE, El-Sohemy A, Archer MC. Regulation of HMG-CoA reductase in MCF-7 cells by genistein, EPA, and DHA, alone and in combination with mevastatin. Cancer Lett 224:221–228, 2005.
64. Cope MB, Steele VE, Eto I, Juliana MM, Hill DL, Grubbs CJ. Prevention of methylnitrosourea-induced mammary cancers by 9-cis-retinoic acid and/or vitamin D3. Oncol Rep 9:533–537, 2002.
65. Velmurugan B, Mani A, Nagini S. Combination of S-allylcysteine and lycopene induces apoptosis by modulating Bcl-2, Bax, Bim and caspases during experimental gastric carcinogenesis. Eur J Cancer Prev 14:387–393, 2005.
66. Swami S, Krishnan AV, Peehl DM, Feldman D. Genistein potentiates the growth inhibitory effects of 1,25-dihydroxyvitamin D_3 in DU145 human prostate cancer cells: Role of the direct inhibition of CYP24 enzyme activity. Molec Cell Endocrinol 241:49–61, 2005.
67. Shen F, Weber G. Synergistic action of quercetin and genistein in human ovarian carcinoma cells. Oncol Res 9:597–602, 1997.
68. Mertens-Talcott SU, Percival SS. Ellagic acid and quercetin interact synergistically with resveratrol in the induction of apoptosis and cause transient cell cycle arrest in human leukemia cells. Cancer Lett 21:141–151, 2005.
69. Khafif A, Schantz SP, Chou TC, Edelstein D, Sacks PG. Quantitation of chemopreventive synergism between (-)-epigallocatechin-3-gallate and curcumin in normal, premalignant and malignant human oral epithelial cells. Carcinogenesis 19:419–424, 1998.
70. Zu K, Ip C. Synergy between selenium and vitamin E in apoptosis induction is associated with activation of distinctive initiator caspases in human prostate cancer cells. Cancer Res 63:6988–6995, 2003.