Molecular Targets for Bioactive Food Components

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ABSTRACT Mounting evidence points to dietary habits as an important determinant of cancer risk and tumor behavior. Although the linkages with diet are intriguing, the literature is also laden with inconsistencies. The reasons for these inconsistencies are likely multi-factorial, but probably reflect variations in the ability of bioactive constituents to reach or affect critical molecular targets. Fluctuations in the foods consumed not only influence the intake of particular bioactive components, but may alter metabolism and potentially influence the sites of action of both essential and nonessential nutrients. Genetic polymorphisms are increasingly recognized as another factor that can alter the response to dietary components (nutritional transcriptomic effect) by influencing the absorption, metabolism, or sites of action. Likewise, variation in DNA methylation patterns and other epigenetic events that influence overall gene expression can be influenced by dietary intakes. Furthermore, variation in the ability of food components to increase or depress gene expression (nutrigenomic effect) may account for some of the observed inconsistencies in the response to dietary change. Because a host of food components are recognized to influence phosphorylation and other posttranslational events, it is also likely that these and other proteomic modifications account for at least part of the response and variation that is reported in the literature. Collectively, it is clear that bioactive food components can influence a number of key molecular events that are involved in health and disease resistance. As the era of molecular nutrition unfolds, a greater understanding of how these foods and components influence cancer will surely arise. Such information will be critical in the development of effective tailored strategies for reducing cancer burden. Just as important, however, is that as this information unfolds it is utilized within a responsible bioethical framework.


KEY WORDS: bioactive • nutrigenomics • nutrigenetics • proteomics • metabolomics

During the past several decades, enormous progress has been made in developing national nutrition policies and research agendas that are fundamental to dealing with a host of problems faced by segments of the population. Despite the many successes, nutrition-related health issues remain commonplace and account for many of the leading causes of death of Americans. According to the USDA, about $71 billion could be saved per year if healthier diets were consumed because of reduced medical costs, increased productivity, and extended lives (1). Since this estimate only accounts for conditions such as coronary heart disease, stroke, cancer, and diabetes—and not other diet-related diseases—the savings could be far greater. Obesity alone has been estimated to cost $117 billion and osteoporosis another $14 billion per year in medical expenses. Thus, the appropriate use of diet can have profound benefits on both personal health and financial well-being.

The diet and cancer link

Some of the earliest descriptions of the symptoms of cancer appeared in ancient Chinese and Arabic medical writings (2). One of the first significant attempts to determine the geographical distribution of cancer and possible causes and linkages appeared in the early 19th century in Europe. Findings from their questionnaire that were published in 1806 by the Society for Investigating the Nature and Cure of Cancer noted “with regard to cancer, it is not only necessary to observe the effects of climate and local situation but to extend our views to different employments, as those in various metals and manufactures; in mines and collieries; in the army and navy; in those who lead sedentary or active lives; in the married or single; in the different sexes, and many other circumstances” (3). It is disheartening that some of the same issues continue to be debated almost 200 y after their synopsis was published. Regardless, evidence from a variety of sources points to dietary habits as a significant environmental factor in the overall cancer process. While optimizing the intake of specific foods...
and/or their bioactive components seems a prudent, noninvasive, and cost-effective strategy for reducing cancer burden, this is far from a simple process. The complexity of the diet coupled with the heterogeneity of the cancer process makes it a daunting task to establish true interrelationships. Nevertheless success is key to enormous societal and personal satisfaction.

**Bioactive food components**

While epidemiological and preclinical evidence continues to highlight dietary components ranging from macro- to microconstituents as likely modifiers of cancer processes, there are also alarming inconsistencies in the literature (4–6). Evidence already exists that not only can the magnitude of the response vary across studies, but even the direction of the response can shift. These inconsistencies may reflect the cumulative effects of numerous variables that influence the effective quantity of the bioactive component occurring at the target site or that influence its ability to bring about a metabolic or phenotypic change. **Figure 1** illustrates the multiple steps where bioactive food components can interact with genes and their expression in altering phenotypes. Undeniably genes can influence absorption, metabolism, or transport of a bioactive food component (nutritional transcriptomic effect) or its site of action and thus influence the overall response to the diet. Likewise, bioactive food components can alter the genetic expression of a host of cellular events (nutritional transcriptomic effect) and thus influence cancer outcomes. It has long been recognized that nutrients can modify proteins once formed through a variety of processes including phosphorylation. Thus, it is likely that nutritional proteomics is in some way key to the changes in tumor incidence or behavior that is observed in model systems following modification of the diet or as suggested by epidemiological and limited clinical studies. Finally, if the effective concentration of the bioactive component never reaches target tissue or is masked by other intermediates, then metabolomics becomes a limiting factor. Collectively, it is clear the response to bioactive food components is highly dependent on a number of cellular events and regulatory processes that attempt to maintain homeostasis and/or survival.

The complexity of the diet is illustrated by the number of essential and nonessential food components that have been proposed to modify one or more steps in the cancer process (7). Table 1 provides an incomplete list of some of the numerous food components that may be important in modifying cancer risk and tumor behavior. Collectively, evidence exists that both essential and nonessential dietary components can alter the cancer process. These dietary components are not limited to plants, since zoochemicals arising from the consumption of animal products can provide compounds like conjugated linoleic acid and n-3 fatty acids that may also influence cancer. Likewise, compounds arising from mushrooms (fungochemicals) have been proposed to have anticancer properties (8). Finally, it is conceivable that microorganisms residing in the gastrointestinal tract may play a key role by forming compounds (bacteriochemicals) that may increase or decrease cancer (9,10).

The magnitude of the problem of identifying which dietary component is most important in increasing or decreasing risk is evident by the literally thousands of compounds consumed through the diet each day. Likewise, the dearth of information about some components limits the ability to unravel which bioactive components are most important. For example, whereas it is estimated that humans may be exposed to >5000 flavonoids, only a few have antiproliferation properties for their anticarcinogenic effects. Examination of classes of food components as suggested in Table 1 may assist in expediting this process. Nevertheless, this will be a complicated process because it is likely that multiple steps in the cancer process are being modified simultaneously, including sites such as drug detoxification, DNA repair, cell proliferation, apoptosis, differentiation, and angiogenesis (Fig. 2). Overgeneralizations may also lead to false impressions about what are key facts influencing the cancer process. Clearly, absolute intake, duration of exposure, and speciation are fundamental to the ability of any of these bioactive food components to influence the cancer process, at least in model systems (11,12). Although there is good reason to believe that similar responses will occur in humans, the literature is less plentiful with data. To move this area forward additional attention is needed to determine the critical intake and duration required to bring about a desired physiological change.

It is already evident that considerable variation exists in the amount of bioactive components that occur in a particular food. For example, the selenium content of foods is known to be quite variable, likely because of the variation in the content of the soil. This variation in soil content may also manifest itself in other food items (such as animal products) that are consumed. Recent evidence demonstrates the content of selenium in beef, which is a significant source of selenium in the North America, can range more than 2-fold depending on where the cattle were raised (13). The content of organic components can also vary significantly within and among plants. α-tocopherol in broccoli has been reported to vary from 0.46 to 4.25 mg/100 g fresh weight, with a mean of 1.62 (14). Assessing the intake of some constituents is particularly daunting because of significant limitations in available compositional databases. Even a single database may be inadequate because the content of food component in plants can depend not only on preparation techniques, but also on various environmental factors, including soil conditions, climate, season, horticultural practices, and the type and age of the plant. Such variations in food content can lead to wide fluxes in exposures at potential target sites for cancer prevention. The variability in intake of polyphenols arising from tea consumption illustrates the complexity of trying to examine a dietary factor as a modifier of cancer risk. Although the daily mean intake of polyphenols from tea for 116 individuals was estimated to be 80.8 mg, the range varied from 3 to 588 (15). Relatively few studies have examined reproducibility of intake for specific food items using the food frequency questionnaires. Using 2 different questionnaires for tea the correlation for black tea

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**Molecular Targets for Food Components**

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**Figure 1** Influence of bioactive food components on genetic, epigenetic, and proteomic events. A host of bioactive food components are known to influence one or more stages of this process.
consumption was 0.68. Nevertheless, complicating this issue is the uncertainty about the health benefits that might be attributed to intermittent vs. sustained intake of a given amount of a particular food or its component(s).

Some of the most compelling evidence that diet can influence the cancer process comes from a rather recent nutritional intervention study with selenium-yeast (16). Although speculation about the propensity of selenium to serve as an anticancer agent has been expressed for >30 y (17), this intervention study provided some of the most intriguing evidence for a biological response, although admittedly the protective effects only surfaced in secondary analysis. Nevertheless, these findings are largely supportive by population studies and randomized clinical trials, suggesting an inverse association exists between increased selenium intake and a reduction in esophageal, gastric, lung, prostate, and colorectal cancers. Preclinical studies with animal models supply additional evidence that an inverse association exists between the dietary intake of selenium and carcinogenesis (18). Although the majority of selenium’s role in health promotion has focused on its antioxidant activity, it has several diverse biologic effects including an ability to suppress cell proliferation, enhance immune response, alter the metabolism of carcinogens, and induce apoptosis. New genomic and proteomic technologies offer exciting opportunities to examine each of these events simultaneously and ultimately to determine which is most instrumental in bringing about a change in the incidence or behavior of a tumor.

New technologies and nutritional genomics

DNA microarrays (19,20) and serial analysis of gene expression (SAGE) (21) are being increasingly employed to
identify multi-site gene-food component interactions that were merely dreams just a few years ago. Today, the expression of literally thousands of genes can now be monitored simultaneously between normal and abnormal tissue and used to identify and characterize the response to prevention and therapeutic interventions. As the understanding about how bioactive food components modify genetic events becomes clearer, the current conundrum surrounding appropriate dietary recommendations should evolve into rational and personalized intervention strategies to improve health and minimize the risk of disease.

The term nutritional genomics has been used to describe work at the interface of plant biochemistry, genomics, and human nutrition. The term was coined by DellaPenna (22) to enhance the understanding of reactions and interactions at the molecular or genomic level. As this area continues to unfold, it is becoming increasingly apparent that a host of genomic interrelationships with the diet exist which encompass the broad topic of nutrigenomics. Genetic changes arising as a result of single point mutations, rearrangements, or copy number involving either deletions or additions can likely influence the loss of bone mass resulting from dietary variables including caffeine consumption (24).

Molecular or genomic level. As this area continues to unfold, it is becoming increasingly apparent that a host of genomic interrelationships with the diet exist which encompass the broad topic of nutrigenomics. Genetic changes arising as a result of single point mutations, rearrangements, or copy number involving either deletions or additions can likely influence the response to various dietary components. Unfortunately, relatively few causal diet and chronic disease associations can be articulated with any degree of certainty. It is noteworthy that important exceptions may include the relationship between dietary fat and coronary heart disease and the interrelationship between calcium consumption and fracture risk. In both cases a biomarker, i.e., plasma lipoproteins and bone mineral density, respectively, was identified that was responsive to dietary intervention. Unfortunately, few if any surrogate or intermediate biomarkers have been validated for cancer. The absolute response to diseased conditions is becoming increasingly recognized to depend on a number of gene polymorphisms, and thus may contribute to variation in response and possibly account for the dearth of validated cancer biomarkers. For example, those that are linked to dietary habits appear to have a pivotal role in lipoprotein metabolism and cardiovascular disease including apoproteins (apo) E, B, A-IV, and C-III, LDL receptor, microsomal transfer protein (MTP), fatty acid-binding protein (FABP), cholesteryl ester transfer protein (CETP), lipoprotein lipase, and hepatic lipase (23). Likewise, evidence exists that vitamin D receptor polymorphisms may influence the loss of bone mass resulting from other dietary variables including caffeine consumption (24).

The health benefits attributed to soluble fiber also appear to be linked with genetic backgrounds because data from Hegele et al. (25) suggest that polymorphism occurring with the angiotensinogen gene can influence the response in blood pressure brought about by these carbohydrates.

Vineis et al. (26) reviewed how metabolic polymorphisms likely influence the interrelationship between diet and cancer. The prevalence of these polymorphisms is increasingly being recognized as a significant variable that may influence the interpretation of results of otherwise solidly-designed studies. Linkages between polymorphisms in genes associated with drug metabolism and dietary habits are becoming more apparent (27,28) and are not only important for explaining alterations in the initiation phase of carcinogenesis, but also interactions with chemotherapeutic agents. A low prevalence of polymorphisms in genes coding for activation (phase I) enzymes CYP1A1 (0.07) and CYP2E1 (0.02) was observed in a sample of subjects involved with the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC Study), while there was also a high prevalence in genes coding for detoxification (phase II) enzymes GSTM1 (0.40) and NQO1 (0.20) (29). It is not clear what impact these gene profiles had on the overall outcome of the study. Interestingly, 7 of 10 members of this sample carried the VDR-TaqI polymorphism (t) associated with lower risk for prostate cancer, which may account in part for lower cancer rates in Finland when compared with the United States (29). Further, in a nested case-control study within the ATBC 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 for lung cancer risk of 1.8 for heterozygotes and 2.3 for homozygous variants compared with homozygote wild types (30). More recently, polymorphism in glutathione peroxidase has been linked to an increased risk of breast cancer (31).

Several genetic commonplace polymorphisms can influence folate homeostasis and thus may assist in explaining the linkage between this B-vitamin and cancer (32,33). Polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) gene code for the protein that converts 5,10-methylenetetrahydrofolate (methyleneTHF), the major form of intracellular folate, to 5-methyltetrahydrofolate (methylTHF), the major form of circulating folate in plasma. MethyleneTHF, a cofactor for methylating deoxyuridylate to produce thymidylate, is the rate-limiting nucleotide in DNA synthesis. Depressed MTHFR activity increases the availability of methyleneTHF, thereby reducing the chances of inadequate thymidylate and misincorporation of uracil into DNA. The reduction in uracil incorporation into DNA reduces chromosome instability and potentially reduced risk of cancer (32). Epidemiologic studies revealed that when folate intake is adequate, colorectal cancer risk is reduced by >50% in individuals with the MTHFR 677TT genotype compared with the MTHFR 677CC genotype, and risk of adult acute lymphocytic leukemia (ALL) is reduced by 77% (34). When folate intake was inadequate, however, the genetic profile became less important. Recent clinical data demonstrated that genomic DNA hypomethylation was greater in leukocytes of individuals with the MTHFR 677TT genotype compared to those with the MTHFR 677CC genotype and was inversely correlated with folate status (35).

Several studies suggested that reducing MTHFR activity may limit the availability of methyl groups for synthesis of S-adenosylmethionine (SAM) and increase DNA hypomethylation, and that when folate status is low this mechanism may negate the beneficial effect of the MTHFR 677TT polymorphism (35). MTHFR 1298 A-C polymorphism may also have an influence on adult ALL (34).

The diversity of molecular targets that can be influenced by food components highlights the complexity and breadth of interactions. Figure 2 and 3 reveal that the effects of a bioactive food component may be a manifestation of nuclear and cytoplasmic events that regulate the abundance and/or activity of specific proteins involving several cancer processes. Fluctuations in these proteins can lead not only to changes in overall cellular metabolism, but also can markedly influence the proportion of cells that are dividing, undergoing apoptosis, or differentiating. Characterizing which of these molecular events is most important in bringing about a phenotypic effect is the primary thrust of an RFA that has been released by NCI during the last year. This RFA (CA 03–001), entitled “Collaborations on Nutritional Modulation of Genetic Pathways Leading to Cancer,” will provide financial support to assist in building teams to address fundamental molecular issues about the mechanism(s) by which bioactive food components influence the cancer process. It is anticipated that this RFA will be re-released soon.
Nutrigenomics

Rather compelling evidence that a variety of nutrients interact with specific molecular targets (36–39). Invariably, studies reveal that a host of messages are modified by the presence or absence of a food component. Our understanding of the cellular events that allow for these collective changes remains obscure. However, as presented in this symposium common events such as mRNA stability and polymorphisms may help explain the multitude of effects that are observed (40). While understanding the basis for the multiple nutrigenomic effects is important, it is also fundamental to understand that several processes are influenced simultaneously. Thus, it is likely that the clustering of gene expression changes and then expressing these in terms of a ratio with some other process may be most informative. Such an approach was used with some success in examining shifts in cell proliferation and apoptosis (41–43). Additional characterization of these message profiles will be needed to examine for commonality and disparities in response among various nutrients so that potential synergies and antagonisms can be identified. Figure 3 provides an example for the way that multiple bioactive food components may influence one or more steps in a process. Such interrelationships will need to be examined to understand the influence of the entire diet or possibly the food matrix on biological responses. Knowledge about genes and food components influencing key cellular processes will surely be fundamental for determining who will and will not respond to intervention strategies.

Transgenic and knockout models provide evidence that genes can markedly influence the response to dietary components (43–45). Likewise, the use of transgenic and knockout models can provide valuable clues about the site(s) of action of specific bioactive food components (36,43,46,47). Without question, more information is needed to examine how genetic profiles influence the ability of specific dietary components to bring about phenotypic changes. Understanding the dynamic interactions between food components and molecular targets is the basis of a recent RFA that focuses on prostate cancer (RFA 03–003). It is believed that enhanced emphasis on molecular targets for prostate cancer will energize the nutrition community to explore molecular sites of actions in other tissues.

Microarray technology that allows parallel analysis of expression patterns of a large number of genes in a single experiment has created an exciting new frontier for the nutrition and health care community. Unfortunately, too frequently conclusions are being drawn on the basis of limited observations. It is becoming increasingly apparent that cellular variation in gene expression profiles requires that multiple chips be examined for each experimental condition. Studies by Wang et al. (48) suggest a minimum of 5 cDNA chips may be needed to detect a doubling or reduction in half of the expression of a gene. As the desire to detect even small changes in gene expression grows, it will likely be necessary to examine even greater numbers of samples. The vast amount of gene expression data that will be generated from these multiple samples requires a robust database system that will facilitate efficient data storage, retrieval, secure access, data dissemination, and integrated data analyses. It will also be necessary to examine if commonality in response across tissues is occurring. A recent NIH Announcement (CA 03–027) is aimed at supporting research to determine if bioactive food components alter gene expression across various tissues and if there is consistency in response among animal models and what occurs in humans.

Proteomics

Techniques are being developed and refined to assist in the identification of the proteome, defined as all of the proteins present in a particular cell at a particular moment (49). The importance of these types of investigations stems from the fact that gene expression does not always correlate with protein expression and the influence of food components may be either translational or posttranslational, rather than at the transcriptional level (50,51). There are a host of examples about how specific dietary components can alter protein phosphorylation and glycosylation (52–54). Some of these changes may occur because of several processes including oxidative stress (55,56). In some cases this may be through shifts in the quantity or expres-
Molecular targets for food components

The response to a bioactive food component cannot be considered to occur in isolation but must be considered in the context of the entire diet (57). Although interactions occurring among nutrients remain an area poorly examined, there are a few examples that illustrate potential negative and positive interactions. For example, ascorbic acid can reduce the ability of selenium to retard a chemically induced tumor, presumably by reducing it to elemental selenium (59). However, other constituents of the diet such as allyl sulfur compounds found in processed garlic can enhance the anticancer potential of selenium (59). Studies aimed at examining interactions may simultaneously provide important insights into sites of action of various food components. Verification of the importance of such changes in terms of phenotypic outcome will be key to the usefulness of these or other types of predictive models. One approach to examining interactions among nutrients may be through metabolomic research. Metabolomics involves the systematic estimation of metabolomes, i.e., the characterization of all metabolites and small molecular weight compounds occurring in an organism. While methods for metabolomics are in the early stages of development, they nonetheless offer exciting opportunities for the determination of relationships between exposures and phenotypic outcomes and possible interactions among nutrients. Various approaches have been suggested and many analyses and visualizations are currently being explored to obtain an operational view of metabolomics (60–62).

As with other techniques, how to deal with the reams of data are of paramount importance (63). Several methods, including principal component analysis and clustering, are being examined to analyze metabolomic data. Metabolomic subsets, such as the lipid and amino acid metabolome, are already suggesting that some very useful information can be obtained (64,65).

Future research directions

Nutritional sciences remain an exciting and relevant research area. The success of this discipline depends on dedicated scientists who use the rapidly developing technologies to identify molecular sites of action of bioactive food components and how these sites relate to disease prevention. Without a doubt, identifying the mechanisms will be challenging, especially considering the multiple sites of interactions among individual food components, and the influence of genetic susceptibilities. Nevertheless, innovative strategies should identify components of this mechanistic puzzle so that a greater insight into the relationship between diet and health can be established. Resolving conflicting and inconsistent data among studies will surely be one of the most important aspects of basic research on the mechanisms of action of bioactive food components.

Many research questions relevant to nutrition and cancer remain to be addressed. For instance, how do interactions among individual food components influence genetic expressions occurring within a cell? Can the enhanced antiproliferative effects of combining bioactive food components found in vitro (66) be verified in vivo? Furthermore, is there commonality in response to bioactive components across tissues? How do age and gender influence the response to food components on molecular targets? How can interindividual variance in nutrient metabolism best be taken into account? Can biomarkers that indicate nutrient status be identified and validated? How might exfoliated cells be used as predictors of the response to dietary intervention strategies? 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 endpoint biomarkers for clinical disease be identified and validated? Will currently used study designs and analytical techniques be adequate in molecular-based nutrition and cancer research?

The answers to these and other questions may depend on the development of effective collaborations between nutrition scientists with others because it is unlikely that single individuals and/or institutions will have sufficient capabilities to adequately address some of the overarching issues. Perhaps it is important to recognize that developing and implementing this new research paradigm in nutrition and cancer prevention will take considerable time and patience. While the challenges will be enormous the potential rewards in terms of both cancer morbidity and mortality will be of equally great magnitude.

LITERATURE CITED


