Nutritional “Omic” Technologies for Elucidating the Role(s) of Bioactive Food Components in Colon Cancer Prevention

Cindy D. Davis2 and Norman G. Hord*

Nutritional Sciences Research Group, NCI/National Institutes of Health, Rockville, MD 20892-7328 and
*Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824

ABSTRACT Evidence continues to implicate dietary components and genetic susceptibilities as important determinants of cancer risk and tumor behavior. Variation in cancer incidence among and within populations with similar dietary patterns suggests that an individual’s response may reflect interactions with genetic factors, which may modify gene, protein, and metabolite expression patterns. Nutrigenomics, defined as the interaction between nutrition and an individual’s genome, will likely provide important clues about responders and nonresponders. In this symposium, the role of bioactive food components in colon cancer susceptibility was used to exemplify the application of “omic” technologies for cancer prevention. Topics that were addressed included dietary changes and gene polymorphisms (nutrigenetics), DNA methylation (nutritional epigenomics), gene expression (nutritional transcriptomics), altered formation or bioactivation of proteins (proteomics), and characterizing how the quantity and timing of exposure influence small molecular weight cellular constituents (metabolomics). The final presentation focused on exfoliated cells as a surrogate sample for the evaluation of bioactive food components in cancer prevention. The goal of the symposium was to provide an example of each of the “omic” technologies as they relate to nutrition, cancer risk, and tumor behavior, and to help the participants understand that an integrated framework that simultaneously examines all of the “omic” technologies is needed. J. Nutr. 135: 2694–2697, 2005.

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Colon cancer is the third most common newly diagnosed cancer in the United States, accounting for ~11% of cancer deaths. Numerous epidemiologic, preclinical, and clinical studies (1) point to dietary bioactive components as one of the most important modifiable determinants of colon cancer incidence, as well as the biological behavior of tumors. However, despite consistent associations between colon cancer and certain dietary patterns, numerous inconsistencies are also evident (1). These inconsistencies may reflect the multifactorial and complex nature of cancer and the specificity that individual dietary constituents have in modifying genetic pathways. Epidemiologic studies of folate status and colon cancer risk highlight some of the inconsistencies in the epidemiologic literature (2). Among 15 published retrospectively conducted epidemiologic studies that investigated the relation between folate status (assessed by dietary folate or by the measurement of blood folate concentrations) and the risk of adenomas or colorectal cancer, the majority showed either a significant or equivocal inverse relation that was not statistically significant; however, the relation could not be distinguished from other factors in their relation to diet or became nonsignificant after adjustment for confounders (3–5). Nevertheless, most, but not all, of the studies have suggested that folate deficiency increases, whereas folate supplementation decreases the risk of colorectal cancer (2). This variability among studies probably relates to many factors, including differences among the cohorts and genetic variation.

The human genome project has documented that sequence variations are common; in fact, most genes have small sequence differences or polymorphisms that occur between individuals every 1000–2000 nucleotides (6). Some of these polymorphisms may affect how well the protein works and how the protein interacts with other proteins or substrates. There are 50–100 genes involved either directly or indirectly in folate metabolism, including receptors, binding proteins, enzymes, tissue specific gene products, and downstream factors that rely upon folate-derived metabolites (7). When one takes into account the variability that is known to occur within the
human genome, there are likely to be thousands of polymorphisms within such a number of genes. These polymorphisms and their interactions are likely contributors to the variation in biological response and outcomes in response to dietary folate among individuals.

A polymorphism in methylenetetrahydrofolate reductase (MTHFR), which causes the substitution of C to T at nucleotide 677, is the most important common variant known in folate metabolic pathways. This polymorphism, which occurs in 5–20% of the population worldwide (7), results in reduced conversion of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, the form of folate that circulates in plasma. Individuals with this polymorphism appear to have increased dietary folate and riboflavin requirements (8–10). This polymorphism also appears to alter the relation between folate status and colorectal cancer susceptibility (11,12). Compared with subjects with the CC or CT genotype having low folate levels, those with the TT genotype showed a decreased risk of colorectal adenomas when they had high levels of plasma folate [adjusted odds ratio (OR) = 0.58], and an increased risk when they had low folate levels (adjusted OR = 2.13) (11). Because there was no clear relation between plasma folate and colorectal adenomas among those with the CC or CT genotype, only a subset of the population may benefit from exaggerated folate intakes. Thus, to provide the best dietary recommendations for everyone for cancer prevention, it may be necessary to include the impact of genetic variation and to consider requirements for each individual, given their specific genomic profile.

Nutrigenomics, the study of how genes and dietary components interact to alter phenotype, will likely provide important clues about responders and nonresponders (13). Nutrigenomics encompasses an understanding about how the response to bioactive food components depend on an individual's genetic background or nutrigenetics, nutrient-induced changes in DNA methylation and chromatin alterations or nutritional epigenetics, and nutrient-induced changes in gene expression or nutritional transcriptomics (13). However, phenotype depends on a combination of genes and environmental factors, e.g., diet.

In addition to genomics, epigenetics is an important regulator of gene expression and can affect cancer susceptibility, and thus have an impact on dietary recommendations. The situation has been confusing, because virtually all types of cancer examined have both global hypomethylation and genespecific hypermethylation in the gene promoter regions (14). Hypermethylation of promoter regions, which is associated with transcriptional silencing, is at least as common as DNA mutations as a mechanism for inactivation of classical tumor suppressor genes in human cancers (15,16). Furthermore, a number of candidate tumor suppressor genes that are not commonly inactivated by mutation are transcriptionally silenced by this mechanism (17). Preclinical and clinical evidence suggest that part of the cancer-protective effects associated with several bioactive food components may be related to DNA methylation patterns. Dietary factors that are involved in one-carbon metabolism provide the most compelling data for the interactions of nutrients and DNA methylation, because they influence the supply of methyl groups and therefore the biochemical pathways of methylation processes (17). These nutrients include folate, vitamin B-12, vitamin B-6, methionine, and choline. In fact, dietary methyl (folate, choline, and methionine) deficiency in combination causes global hypomethylation, hepatic steatosis, cirrhosis, and ultimately hepatic tumorigenesis in rodents in the absence of carcinogen treatment. Because epigenetic events are reversible, they offer an important mechanism whereby environmental factors, e.g., diet, can influence biological processes and disease-related phenotypes.

Evidence suggests that dietary supplementation in vivo can lead to changes in DNA methylation, as well as profound effects on phenotypes. This has been well demonstrated using the agouti mouse model (18). Specifically, dietary supplementation of maternal diets led to a shift in DNA methylation patterns in offspring, from a hypomethylated to a hypermethylated long terminal repeat 5′ of the agouti gene. This causes a shift from a yellow to an agouti coat, which is traditionally associated with a lower risk of cancer, diabetes, obesity, and prolonged life. It should be noted that these nutrients were added to a diet considered to be nutritionally adequate. However, it is not known which component or combination is required to bring about this effect.

Another important site of regulation that will determine who will and who will not respond to various bioactive food components is the rate of transcription (nutritional transcriptomics) of various genes. The development of microarray technology is providing nutritionists with a powerful tool to examine potential sites of action of dietary components and their interactions with cellular processes. Genome-wide monitoring of gene expression using DNA microarrays allows the simultaneous assessment of the transcription of tens of thousands of genes and their relative expression between normal cells and tumor cells, as well as gene expression patterns before and after exposure to different dietary components. Gene expression analysis using microarray technologies should provide clues about the mechanisms that underlie the beneficial or adverse effects of dietary components, identify important genes that are altered in the predisease state and therefore possibly serve as “molecular biomarkers” and/or assist in identifying and characterizing the basic molecular pathways influenced by food components.

A limited number of studies have examined the effects of folate status on gene expression and colon cancer susceptibility (19,20). Folate depletion induced the upregulation of immune-related genes, urokinase, and inducible nitric oxide synthase, and the downregulation of adhesion molecules (protocadherin-4, midogen, and integrin αV) and vascular endothelial growth factor in normal rats (19). Mutant mice, particularly knockout mice, are becoming an invaluable tool to understand how dietary components regulate gene expression. Ma et al. (20) genetically engineered folate transporter knockout models to differentially manipulate folate status and to identify regulatory relation among folate-responsive genes considered to drive colonic tumor development. They investigated the effect of targeted ablation of 2 folate transport genes, folate binding protein 1 (Folbp1) and reduced folate carrier (RFC1), on gene expression and colon carcinogenesis. Microarray analysis of colonic mucosa showed distinct changes in gene expression specific to Folbp1 or RFC1 ablation. Only a limited number of genes were differentially expressed between the RFC1 and control mice. In contrast to the RFC1 knockout mice, a substantially greater number of genes were differentially up- or downregulated in Folbp1 knockout mice compared with controls. It is noteworthy that a large number of immune-related genes were differentially expressed. Because similar observations were reported in the colons of rats fed folate deficient diets (19), these results suggest altered immune
function as one mechanism for increased colon cancer susceptibility in folate deficient animals.

Dietary components can also modify the translation of RNA to proteins, as well as posttranslational modifications that can affect protein activity. The term proteome refers to all the proteins produced by a species, much as the genome is the entire set of genes. Unlike the genome, the proteome is dynamic and varies according to the cell type and the functional state of the cell. Thus, proteomic analysis only allows a point in time comparison after dietary interventions. However, 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. Proteomic analysis allows for the identification of protein modifications in response to dietary intervention.

Functional proteomic studies have been useful for elucidating the molecular targets of dietary components in human colon cancer cells. Both quercetin and butyrate-treated HT-29 cells exhibited growth inhibition accompanied by proteome alterations in the cells (21,22). Using 2-dimensional electrophoresis and identification of proteins by mass spectrometry of peptide fragments generated by tryptic digestion, quercetin was found to alter the expression of 20 proteins at least 2-fold, including heat shock proteins and annexins, both of which play a crucial role in apoptosis, as well as proteins involved in metabolism, detoxification, and gene regulation (21). In contrast, 35 differentially expressed protein spots were observed as a result of butyrate treatment (22). Butyrate treatment altered various components of the ubiquitin-proteasome system, suggesting that proteolysis could be a means by which butyrate may regulate key players in the control of cell cycle, apoptosis, and differentiation (22). Also, the authors demonstrated that butyrate simultaneously upregulated both proapoptotic (caspase-4 and cathepsin D) and antiapoptotic proteins (hsp27, antioxidant protein-2, and pyruvate dehydrogenase E1), which may account for the relative resistance of HT-29 cells to butyrate-induced apoptosis (22). Therefore, proteomic analysis identified numerous molecular targets that can be linked to the cancer protective effects of dietary components in colonic tissue.

One of the newest “omics” in nutrition is metabolomics, which refers to the dose and the temporal changes in cellular small-molecular-weight compounds in response to dietary treatments. For example, one study used a metabolomic approach to evaluate all of the biochemical changes after dietary intervention in humans. In this study, plasma profiles of healthy premenopausal women were analyzed before and after consumption of 60 g of soy (23). Despite the presence of substantial intersubject variability, the metabolic analysis enabled the identification of biomarkers related to the dietary intervention. Soy intervention changed the plasma lipoprotein, amino acid, and carbohydrate profiles, suggesting soy-induced alterations in fat, protein, and carbohydrate metabolism. It is possible that these types of studies will allow for the identification of individuals, based on their metabolic propensities, who would benefit from a variety of foods and/or food patterns for cancer prevention.

A fundamental issue in the utilization of “omic” technologies for elucidating the role of bioactive food components in cancer prevention research is which biological samples are most predictive of the response to a bioactive food component in a target tissue. We acknowledge the challenges in answering 3 important questions regarding the utility of biomarkers, such as these, as indicators of cancer risk: 1) What is the relation of the surrogate end point to cancer? 2) What is the relation of the intervention (or exposure) to the surrogate? 3) To what extent does the surrogate end point mediate the relation between intervention (exposure) and cancer? (24). As with other putative biomarkers of cancer risk, we recommend caution in extrapolating from surrogate effects or associations between these markers and cancer risk.

One limitation to the validation of biomarkers in target tissue is their relative inaccessibility. Although blood and blood constituents have frequently been used to evaluate the response to bioactive food components, the concentration and the molecular/biochemical effects of these agents in the blood and in the target tissue of interest may not be related. Exfoliated or sloughed cells hold the potential for monitoring human exposure to bioactive food components in target tissues but have not been adequately evaluated (25). Examples include colonic epithelial cells obtained from stool samples, bladder urothelial cells present in urine samples, airway epithelial cells in sputum or bronchoalveolar lavage, buccal mucosal cells obtained by rinsing the mouth, and mammary epithelial cells obtained from ductal lavage or nipple aspirate fluid. DNA, RNA, and protein isolated from exfoliated cells have been analyzed for various types of genetic and epigenetic changes (25). Although there are collection limitations, the limited access to some cancer sites raises the intriguing possibility that some exfoliated cells may serve as surrogate indicators of the response to diet both in terms of cellular uptake and shifts in gene, protein, and metabolite expression profiles.

In conclusion, significant advances have been made in understanding the relation between dietary factors and cancer prevention; however, the identification of those who will or will not benefit from dietary intervention strategies remains a major obstacle. Adequate knowledge about how the responses depend on an individual’s genetic background (nutrigenetic effects), the cumulative effects of food components on genetic expression profiles (nutritional transcriptomics and nutritional epigenomics effects), the occurrence and activity of proteins (proteomic effects), or the dose and the temporal changes in cellular small molecular weight compounds (metabolomics effects) may assist in identifying responders and nonresponders. Deciphering the importance of each of these potential sites of regulation will be particularly challenging but does hold promise in explaining many of the inconsistencies in the literature. As the era of molecular nutrition unfolds, a greater understanding of how foods and dietary bioactive components influence cancer will surely arise. Such information will be critical in the development of effective delivery of tailored approaches to reduce the cancer burden. As this information unfolds, it is critical that it is used within a responsible bioethical framework.

LITERATURE CITED

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