

International Research Conference on Food, Nutrition, and Cancer

Diet, Nutrition, and Cancer Prevention: The Postgenomic Era^{1,2}

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ABSTRACT The genomic era of human nutrition is upon us: the human genome and several plant genomes have been characterized, and genetically modified foods are now abundantly available in the marketplace. The link between diet and cancer is well established, and new genomic technologies have made possible the investigation of nutritional modulation of the carcinogenesis pathway with nutrients, micronutrients, and phytochemicals. Current study of nutrient-modulated carcinogenesis involves exploring the effect of nutrients on DNA damage and repair mechanisms; DNA methylation, which influences gene expression and cellular phenotypes; antioxidant rearranging and oxidative stress; target receptors and signal transduction pathways; cell cycle controls and check points; apoptosis; and antiangiogenic processes. With nutritional genomics, proteomics, and metabolomics, scientists are able to simultaneously elucidate the biological effects of dietary constituents on cell function and global gene expression. This generation of new knowledge on nutrient-gene interactions provides the justification for a research framework for diet and cancer prevention that is focused on identifying and developing new biomarkers as well as a novel and contemporary paradigm for dietary intervention. *J. Nutr.* 133: 3830S–3836S, 2003.

KEY WORDS: • cancer • prevention • genomic • nutrition • diet

Half a century ago, Watson and Crick (1) revealed the structure of DNA. Within the 20 y after their discovery, science benefited from well-developed recombinant DNA technologies, and in 2001, the Human Genome Project and Celera Genomics presented the first complete draft of the human genome (2,3). This sequencing achievement subsequently heralded the beginning of the postgenomic era.

Parallel to the milestones in genomic development is the research in carcinogenesis, which has also advanced remarkably over the past several decades with astounding growth in technology and information. Cancer is now considered a genetic disease: tumor cells result from multiple genetic defects caused by exposure to environmental, dietary, and infectious agents as well as other lifestyle factors. Carcinogenesis is a multistep, multistage process and the progression from the premalignancy of intraepithelial neoplasia to a clinical, symptomatic, and invasive stage may span 20 y or more (4). Knowledge of the

genetic hallmarks of tumorigenesis provides us with the opportunity to use approaches such as dietary intervention to prevent cancer development. Genomic technologies are new weapons in the scientific arsenal that arm nutrition scientists with the ability to leave behind the reductionist method of investigating single nutrient effects on a biological system for a more holistic approach of exploring the molecular details of food nutrient effects on an entire biological organism. These technologies are integral to any new paradigm for nutrition research in diet and cancer prevention in the postgenomic era (5).

This year's American Institute for Cancer Research (AICR)⁴ and World Cancer Research Fund International (WCRF) conference centered on phytochemicals and gene regulation in cellular, animal and human models, the role of nutrients in cancer survivors, the intraepithelial neoplasia phase, and influence of nutrients on cancer prevention throughout the life cycle. These latter topics were discussed during the session on cancer prevention in the postgenomic era and are covered in depth in accompanying articles and abstracts. Thus, the focus here is on a key conceptual framework that outlines the application of nutritional genomics and proteomics to molecular epidemiology and diet in cancer prevention.

¹ Presented as part of a symposium, "International Research Conference on Food, Nutrition, and Cancer," given by the American Institute for Cancer Research and the World Cancer Research Fund International in Washington, D.C., July 17–18, 2003. This conference was supported by Balchem Corporation; BASF Aktiengesellschaft; California Dried Plum Board; The Campbell Soup Company; Danisco USA Inc.; Hill's Pet Nutrition, Inc.; IP-6 International, Inc.; Mead Johnson Nutritionals; Roche Vitamins, Inc.; Ross Products Division; Abbot Laboratories; and The Solae Company. Guest editors for this symposium were Helen A. Norman and Ritva R. Butrum.

² Supported in part by the National Cancer Institute/UCLA Clinical Nutrition Research Unit (grant CA42710) and the American Gastroenterological Association/Fiterman Foundation Award.

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⁴ Abbreviations used: AICR, American Institute for Cancer Research; DCP, Division of Cancer Prevention; EPIC, European Prospective Investigation into Cancer and Nutrition; NCI, National Cancer Institute; RAPID, Rapid Access to Preventive Intervention Development; RDA, Recommended Dietary Allowance; WCRF, World Cancer Research Fund International.

The evolving definition of nutrients

We have made tremendous scientific progress since President Nixon's 1971 declaration of the war on cancer and the publication of *Diet, Nutrition, and Cancer* two decades ago by the National Academy of Sciences (6,7). With the great gains in knowledge about nutrient-gene interactions, the definition of nutrients has continued to evolve. A *nutrient* is classically defined as a constituent of food necessary for normal physiological function and *essential nutrients* are those required for optimal health (8). Nutrients are thus traditionally known as chemical substances obtained from food and needed by the body for growth, maintenance, and repair of tissue. Essential nutrients are not formed metabolically within the cell and must be present in food that is ingested, whereas nonessential nutrients can be synthesized by the cell (9). For the past century, our nutrition research archetype has been focused on the identification of a single nutrient in its deficiency state and the role of particular single nutrients in intermediary metabolism and cell growth, development, and maintenance, which has led to the formulation of a Recommended Dietary Allowance (RDA) of each nutrient. Since the publication of the first list of RDAs by the Food and Nutrition Board of the U.S. National Research Council 60 y ago, other nations and international agencies such as the World Health Organization have continued to provide health professionals and the public with a wide array of dietary guidelines and nutrient intake recommendations (10,11). These public policy actions have helped successfully eradicate and prevent recurrence of acute nutrient-deficiency diseases such as beriberi, scurvy, rickets, and pellagra.

With our expanding working knowledge of the role of nutrients in gene expression and cellular response to changes in nutrient availability, various academic societies and editorializing experts have led the ongoing pursuit of a definitive meaning of the term nutrient (12): what exactly is a nutrient in this day and age? Young (13) defined a nutrient in the postgenomic era as a "fully characterized (physical, chemical, physiological) constituent of a diet, natural or designed, that

serves as a significant energy yielding substrate or a precursor for the synthesis of macromolecules or of other components needed for normal cell differentiation, growth, renewal, repair, defense and/or maintenance or a required signaling molecule, cofactor or determinant of normal molecular structure/function and/or a promoter of cell and organ integrity." In addition, nutrients can catalyze reactions and promote the assembly of mechanistic structures. A comprehensive definition along these lines is timely in the postgenomic age because nutrients can influence or regulate the transcription, translation, and post-translational metabolic processes. Nutrient-genome interaction may differ according to the life cycle of the organism and have a profound influence on health maintenance and disease prevention. Within this mechanistic definition of nutrients, it must be taken into consideration that the requirement range of a particular nutrient is contingent upon the functionality of the cell and organism, that the required amount may vary depending on whether the nutrient is needed for normal cell growth or cancer prevention, and that certain nutrients may also be harmful in supernormal doses. These corollaries of the nutrient definition are clearly illustrated in the case of folate deficiency and dietary supplementation with folate.

This new definition of nutrients can provide the appropriate mode of gene-nutrient analysis needed at the genome, transcriptome, proteome, metabolome, physiome/phenome, and populome level to generate appropriate biomarkers (Fig. 1). With the development of novel technologies and the advent of nutritional genomics, proteomics and other so-called "-omics" sciences, there is renewed interest in dietary components that affect global gene expression and the integrative physiological and metabolic functions of an organism. Nutrition science has thus evolved into a multidisciplinary field that applies molecular biochemistry and integration of individual health to the epidemiologic investigation of population health. Therefore, there exists ample justification for creating an innovative research model to further explore the role of diet in health promotion and disease prevention, including cancer and other chronic illnesses.

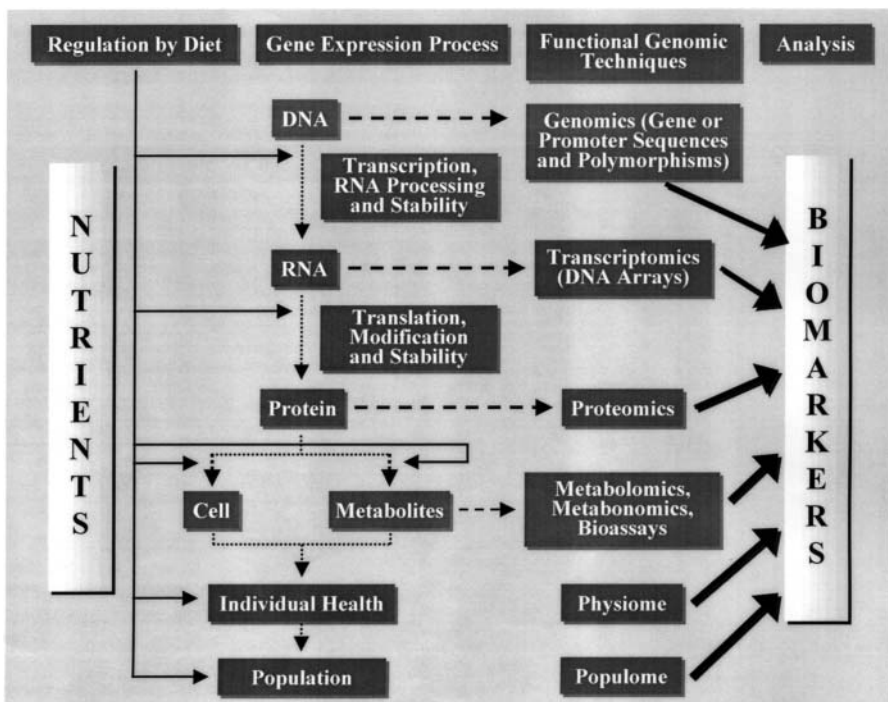


FIGURE 1 Nutritional genomics and biomarker discovery. The steps involved in gene expression (*center*), the stages at which diet, represented by nutrients, can modulate these processes from cell to population (*left*), and the functional genomics techniques used to analyze each stage, with appropriate biomarkers (*right*). Modified from Elliott and Ong (17).

Genomics, proteomics, and metabolomics

A wealth of recently developed novel genomic, proteomic, and metabolomic techniques with high throughput capacities in nutrition research (14–17) promises to facilitate the study of food nutrients and other diet constituents so that researchers may define the important factors in nutrient-gene interaction at the cell, individual, and population level. Nutritional genomics has the potential to assist scientists in interpreting the complex nutrient-gene interaction and the link between genetic abnormalities (i.e., epigenetic polymorphisms) and predisposition to cancer, analyze and integrate the vast data sets that these techniques and studies produce, and then identify new biomarkers (17). Nutritional genomics technologies can be integrated with data bases of genomic sequences (18), interindividual genetic variability (19), and disease susceptibility, the results of which, along with biomarkers to identify individuals at risk and predisposed to cancer, will be conducive to the development of nutrition and cancer prevention strategies. Levels of nutrient-gene analysis using the various technologies are listed in Table 1 (13,20). Propelled by the recent mapping of the human genome and accompanying technological developments, nutrition science has introduced an encompassing new term into our vocabulary: nutrigenomics. Nutrigenomics provides researchers with the tools for the exploitation of systems biology in the nutrition and health arena (21). The melding of functional genomics, or systems biology, into nutrition research has resulted in the integrated discipline of nutrigenomics. The principles of some of the key players in nutrigenomics—genomics, proteomics, and metabolomics—are briefly discussed below.

Genomics uses either classical DNA-sequencer technology or more advanced technologies such as DNA arrays (14). Microarrays can profile gene expression patterns containing tens of thousands of genes in a single experiment, thus allowing systemic

analysis of DNA and RNA variations and providing basic genetic information and insight into any heterogeneity in the coding regions (i.e., single nucleotide polymorphisms) or control elements (i.e., promoters) of genes. With transcriptomics, or expression profiling, scientists use a fluorescence-based detection system to determine RNA expression levels in biological samples (14). This entails using polymerase chain reaction techniques and Northern blot analysis, or annealing an immobilized capture oligonucleotide to its corresponding fragment from tissue onto a DNA microchip in a sequence specific-fashion (14). This expression profiling enables simultaneous analysis of the mRNA of a few genes up to several thousand genes. Genetic polymorphisms related to cancer are now widely investigated (19), and it is likely that many chronic diseases in addition to cancer also result from the connection between genetic susceptibility and environmental and lifestyle factors, including diet.

Proteomics enables researchers to identify all proteins expressed in a cell or organ and detect any posttranslational modification or change in the protein expression pattern. Proteome analysis requires first isolating proteins from a sample, separating them by two-dimensional polyacrylamide-gel electrophoresis, and staining the proteins in the gel (14). The pattern of protein expression can be determined by computer-based comparison of gels (i.e., before and after the treatment of cells or organisms). To identify the protein of interest (which displays increased or decreased levels of expression), the protein is isolated from the gel and digested with trypsin or other specific protease, and then the resulting peptide fragments are analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, yielding the fingerprint of peptide masses characteristic of a given protein (14). Data base comparison of this information with known amino acid or DNA sequences identifies the protein. Any deviations of the measured peptide fragment mass from the corresponding mass of the expected amino acid sequence may indicate posttranslational modifications such as phosphorylation, glycosylation, or myristylation (14). New proteomic technologies are now being applied clinically for use in early detection; therapeutic targeting; and, at long last, patient-tailored therapy (22).

Functional genomics and proteomics can also be applied to enzymes involved in the metabolism of nutrients (23). The multistep pathway from genome to phenotype, along with the involved process of identifying gene function, spurs continual technological development and investigation of metabolic pathways and metabolic flux analysis, or the biochemical profiling that is now known as metabolomics (13,24). Tumor cells possess the potential for proliferation, differentiation, cell cycle arrest, and apoptosis. There is a specific metabolic phenotype associated with each of these processes that is characterized by the production of energy and special substrates necessary for the cells to function in that particular state (25). The stable isotopes approach, in combination with biological mass spectrometry, is composed of the new technologies that are used for metabolic profiling, which measures the expression, transcription, and activation of metabolic enzymes (23). These technologies equip scientists with the ability to determine the metabolic phenotype characteristics of tumor cells.

The development of genomics, proteomics, and metabolomics has transformed the biomarker concept of nutrient-gene interaction from a reductionist pursuit of one ideal marker into a holistic one, in which a significant fraction of all regulated genes and metabolites can be quantified concurrently. Validation of these biomarkers requires that nutrition scientists understand the methodological, demographical, environmental, and dietary characteristics of populations in relation to genetic damage and the molecular epidemiology of

TABLE 1

Levels of gene-nutrient analysis for assessment of nutrient requirements^{1,2,3}

Level	Definition	Example of analysis
1 Genome	Genomic imprint	Nucleotide sequencing
2 Methylome	DNA methylation modifications	Microarray analyses
3 Transcriptome	mRNA expression	Hybridization assays; temporal
4 Proteome	Set(s) of cellular proteins	Mass spectrometry; two hybrid; 2D gel, posttranslational modifications
5 Metabolome	Low molecular weight metabolites in cells/organs	μ TAS: IR, NMR
6 Physiome/phenome	Quantitative integration of cell and organ processes	Viable cell, organ, and whole-body systems, with focus on flux and mass balance models
7 Populome	Complete nutritional characterization of a population group, from data sets 1–6	The above, as relevant, plus dietary and sociocultural data

¹ Levels 1 and 2 are gene-centric in foci and are largely context independent. Other levels include a supra-genome strategy and are context dependent.

² Abbreviations: 2D, two-dimensional; μ TA, micrototal analytic systems; IR, infrared; NMR, nuclear magnetic resonance.

³ From Young (13) and modified from Oliver (20).

cancer. Separation, detection, and computing technologies are simultaneously merging in response to the quest for new tools with which to study the intricate interaction that occurs in biological systems (15). However, the challenge of interpretive bioinformatics persists and remains to be aggressively pursued. Ideally, new nutrigenomics tools will allow nutrition researchers to effectively address the interface of diet and metabolism as well as examine the pathways and mechanisms by which diet and nutrition may prevent cancer.

During a recent National Cancer Institute (NCI) conference on nutritional genomics and proteomics, Milner et al. suggested that a genomic approach to biomarker discovery can proceed along two pathways: 1) It can focus on the disease state, whereby investigators identify the earliest genes involved in disease and use them as targets, aiming to pinpoint nutritional agents capable of modifying the gene expression; or 2) it can focus on the healthy condition, where researchers examine the effects of dietary components on global gene expression, seeking links between gene expression patterns and the processes of disease development (26). Milner et al. proposed that a major future research effort should be to identify and validate cancer-related biomarkers that are modulated by nutrients, and they further affirmed that panels of biomarkers rather than single biomarkers may provide the best approach (26).

Diet and cancer prevention

Current dietary recommendations for cancer prevention largely stem from epidemiologic studies that compare dietary patterns (i.e., intake of particular food items) between countries of low and high incidence for a particular cancer. Most of these studies have been conducted over the past 25 y; the National Research Council completed the first comprehensive review in 1982, and AICR brought the issue to a global perspective in 1997 (6,27). AICR and WCRF are currently planning to publish an updated report in 2006. In general, most of the recommendations from the federal government, preventive health organizations, and world bodies are for increased intake of fiber and a variety of fruits and vegetables, consumption of alcohol and salt only in moderation, reduced fat intake, and increased physical activity (10,11,27).

Largely based on these recommendations, NCI and other funding agencies have initiated and supported various prospective, large-scale dietary and cancer prevention clinical trials (28). Many of these studies, however, have yielded negative or unexpected results (28–32). One such study is a recently completed polyp prevention trial in which men and women who had undergone polypectomy were randomly divided into two groups. The first group was assigned to an intervention arm: a diet low in fat (20%), high in fiber (18 g/1000 kcal consumed), and containing at least 3.5 servings of fruits and vegetables per day. The second group was provided with a brochure on dietary recommendations and asked to continue with their usual diets. The recurrence rates in both groups at the 4-y follow-up were similar, suggesting that dietary changes had no effect on polyp prevention (30). Two other large-scale β -carotene intervention trials within populations of smokers and asbestos-exposed individuals revealed that the risk of lung cancer increased rather than decreased, as expected, in the groups supplemented with β -carotene, which suggests that this supposedly promising chemopreventive agent instead has prooxidant activity (31,32). Although these studies were bolstered with strong epidemiologic evidence linking consumption of carotenoid-rich fruits and vegetables with a reduced risk of cancer, the trial outcomes failed to support the hypothesis that carotenoids (namely β -carotene) are responsible for the beneficial effects.

It may therefore be necessary to design different clinical experiments using whole-plant food extracts and high throughput genomic assays to determine the mechanistic health benefits derived from fruits and vegetables. Broad, multicenter, projective cohort studies similar to the European Prospective Investigation into Cancer and Nutrition (EPIC), which was constructed specifically to explore the relationship of nutrition and cancer, are ideal (33). First results of some of the studies nested within the EPIC cohort were published earlier this year, one of which revealed that dietary fiber in whole foods was inversely related to incidence of large bowel cancer, suggesting that increase in dietary fiber may reduce the risk of colorectal cancer (34). Additional results from the EPIC are highly anticipated. New approaches and strategies in diet, nutrition, and cancer are rigorous and ongoing at the NCI Division of Cancer Prevention (DCP), with detailed descriptions available at the NCI internet site (35). Furthermore, more recent and rapid accumulation of experimental evidence indicates that dietary constituents, particularly phytochemicals and some minerals and vitamins, can modulate the complex multistep, multistage carcinogenesis process at the initiation, promotion, and progression phases of neoplasia (1,36).

Our broadening biomolecular-based knowledge of cancer has opened new avenues and targets for prevention trials. Similarly, the focus on a molecular target for chemoprevention has now shifted to the intraepithelial neoplasm stage of epithelial malignancy (Fig. 2). Genotypic and phenotypic biomarkers have been used as surrogate endpoints because they correlate with histological modulation at intraepithelial neoplasia. The goals for cancer prevention may also have to be repositioned to the in utero and early childhood stages of the human life cycle, if nutrition programming in relation to cancer risk does actually occur during these stages (13). Evidence of the health benefits of folate, vitamin B-12, and vitamin B-6 cyclooxygenase-2 inhibitors and other plant nonsteroidal antiinflammatory drugs, tea catechins, and polyphenols were presented at the AICR/WCRF conference. Using genomic technologies coupled with molecular analysis, investigators have observed and subsequently documented that dietary constituents can indeed modulate carcinogenesis via one of several pathways, with different tissue specificities and potencies. The nutrient-modulated pathways presented in the conference include altering carcinogen activation by inhibiting Phase I drug metabolizing enzymes through the cytochrome p450 superfamily; modifying carcinogen detoxification through Phase 2 drug metabolizing enzymes; scavenging reactive DNA agents and enhancing DNA repair mechanisms; interacting with signal transduction; inhibiting angiogenesis; and suppressing abnormal proliferative characteristics, either by influencing apoptosis or cell cycle checkpoint activities.

This experimental molecular evidence forms the rationale for ongoing clinical chemoprevention trials and has become the key molecular target for nutrients involved in cancer prevention (36,37). Potential cancer preventive agents from dietary constituents are available to the research community through the Rapid Access to Preventive Intervention Development (RAPID) Program of the NCI DCP. The RAPID program provides investigators with the resources and infrastructure needed to evaluate possible chemopreventive bioactive compounds using genomic and proteomic approaches to assess the potential targets and effects of these agents (38). It is hoped that relevant biomarkers will be the target for future chemoprevention trials. Current projects under the assistance of the NCI RAPID program strive to reveal novel molecular biomarkers modulated by agents that can then be used on animal or preclinical models in cancer prevention experiments

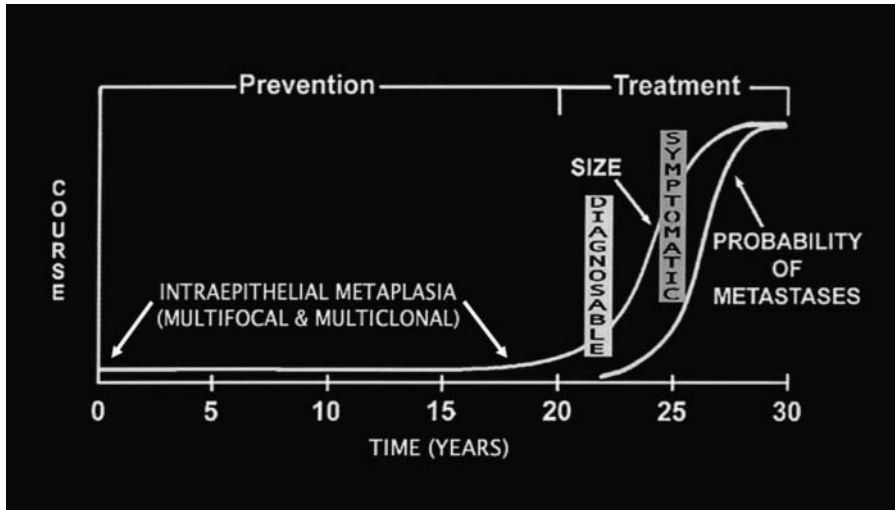


FIGURE 2 Clinical course of epithelial tumors over time, from the intraepithelial neoplasia stage to metastases. Modified from Go et al. (5).

and to facilitate the movement from experimental studies to Phase I and Phase II clinical trials. The NCI DCP and Chemopreventive Agent Development Research Group have undertaken a vigorous program to identify and test potential cancer chemoprevention agents through collaborations and partnerships with academic research centers, the pharmaceutical industry, and private businesses involved in the development of emerging technologies (39). Milner (40) presents NCI's current strategy and future direction in this issue.

In any diet and cancer prevention strategy, the constantly changing food supply must be studied, monitored, and considered, because the era of functional foods has also arrived. Several recent studies serve as example projects that integrate genomics and nutrition to determine the effect of functional food components on health (41–45). Genomics for food biotechnology and genome-level DNA sequencing of whole plants, in conjunction with improved methods of profiling natural products, have made possible combined genetic and biochemical approaches to deciphering biosynthetic pathways and engineering new pathways in transgenic plants (41). Investigators must embrace the genetically modified foods that result and actively pursue the effects of these foods on animal and human health and disease prevention. Combined efforts from industry, government, and academia are essential in

developing a comprehensive and integrated strategy for research on nutraceuticals and functional foods in relation to cancer prevention (Fig. 3).

In September 2002, NCI convened, under the leadership of Kim and Milner, a national conference on nutritional genomics and proteomics in cancer prevention, which was designed to highlight molecular mechanisms by which nutrients may influence cancer prevention and provide genomic and proteomic models that may be useful in future nutrition investigations (37). Milner et al. (26) summarized the opportunities and challenges that investigators currently face, which include identifying and validating cancer biomarkers; investigating the relationship between nutrients (bioactive food components) and cancer prevention; examining possible tissue specificity in response to certain nutrients; defining the interactions among food components as determinants of response; and elucidating the mechanisms of action of bioactive food components. Milner et al. noted, however, that simply acknowledging this research agenda has little effect unless investigators are willing to undertake the focus on nutritional genomics and proteomics.

For the past century, our nutrition research exemplar has been based on studying a single nutrient in a clinical deficiency state, considering the role of that particular single nutrient in

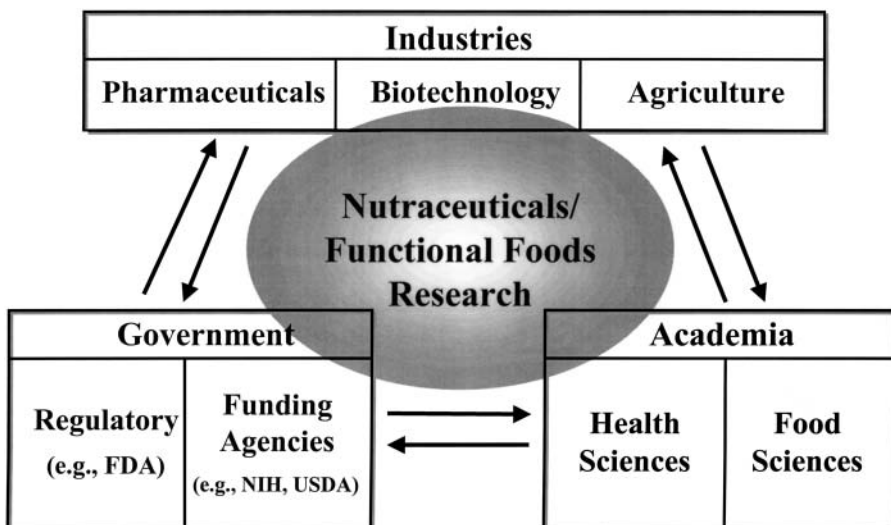


FIGURE 3 Interrelated strategies for research on nutraceuticals and functional foods. The interrelationship among members of industry, government, and academia forms a strong basis for research on the role of functional foods and nutraceuticals in cancer prevention strategy. Figure designed with assistance from Yu Wang.

cell growth and development of an organism, and then extrapolating from the results the RDA of the nutrient. The aggregate RDA of various nutrients forms the basis of our current dietary guidelines. Using the molecular epidemiology of genomics, proteomics, and metabolomics in this postgenomic era will enable complex nutrient-gene interactions to be investigated in clinical and dietary intervention studies at different stages of the life cycle (e.g., in utero, adolescence, adulthood, or advanced age). Furthermore, researchers will be able to determine the effects of timing in the continuum of various cancer phases, from nutrition programming in utero to the intraepithelial neoplasia phase and to the invasive stage and metastasis. The resulting robust data bases of information should be sufficient to yield information on whether a particular nutrient, food, or diet intervention is appropriate for health at a particular point in the life cycle or at a specific stage of carcinogenesis of a given cancer.

In this postgenomic age, the nutrition sciences are undergoing a renaissance that serves as a catalyst for the study and understanding of the integrative biology of living organisms. Consequently, the complexities of the interactions among genotype, diet, and environment will unravel, and personalized nutrition recommendations for individuals will become a feasible and long-term challenge (13). In the near future, diet, nutrition, and cancer prevention will have a dual focus on public health programs that target cancer risk management in the population at large and on individual programs that will focus on particular cancer risk profiles.

Nutritional genomics, proteomics, and metabolic profiling use high throughput technologies that enable researchers to analyze thousands of genes and their complex expression simultaneously. The resulting data facilitate molecular analysis of bioactive food components and identification of appropriate biomarkers that target individuals who are at risk and predisposed to cancer. Increasing stores of evidence substantiating the beneficial effects of certain nutrients in the carcinogenesis pathway pave the way for eventual modification of nutritional requirements as a cancer prevention strategy. Concurrent with the rapid progression in the field of human genomics, agricultural industries have developed genomic-assisted plant improvement and now produce flora enriched with certain nutrients. As the nutrition sciences unfold at the levels of molecular genetics and nutrient-gene interactions, new knowledge will emerge on how nutrients may modify cancer risk and how food (functional or nutraceutical) that is altered with agronomic approaches and biotechnology may be used in cancer prevention. An effective multidisciplinary approach to these developments and the ever-widening knowledge base is paramount because our efforts will ultimately affect clinical applications in health promotion and disease prevention. Plainly stated 50 years ago, it is more true today than ever that nutrition remains the cornerstone of preventive medicine, the handmaiden of curative medicine, and the responsibility of us all (46).

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