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Opportunities and Challenges in Nutrigenomics and Health Promotion

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ABSTRACT

Not all individuals respond identically, or at times in the same direction, to dietary interventions. These inconsistencies likely arise because of diet and genomic interactions (nutrigenomics effects). A host of factors may influence the response to bioactive food components including specific polymorphisms (nutrigenetic effect), DNA methylation patterns and other epigenomic factors (nutritional epigenomic effects), capacity to induce and/or suppress specific mRNA expression and patterns (nutritional transcriptomics), the occurrence and activity of proteins (proteomic effects), and/or the dose and temporal changes in cellular small molecular weight compounds will not only provide clues about specificity in response to food components, but assist in the identification of surrogate tissues and biomarkers that can predict a response. While this "discovery" phase is critical for defining mechanisms and targets, and thus those who will benefit most from intervention, its true usefulness depends on moving this understanding into "development" (interventions for better prevention, detection, diagnosis, and treatment) and a "delivery" phase where information is provided to those most in need. It is incumbent on those involved with food and nutrition to embrace the "omics" that relate to nutrition when considering not only the nutritional value of foods and their food components, but also when addressing acceptability and safety. The future of "Nutrigenomics and Health Promotion" depends on the ability of the scientific community to identify appropriate biomarkers and susceptibility variants, effective communications about the merits of such undertakings with the health care community and with consumers, and doing all of this within a responsible bioethical framework.

Key words: functional foods, nutrigenetics, transcriptomics, proteomics, metabolomics, health

Dietary habits are implicated in the major causes of death among individuals throughout the world (1). Although the literature is replete with evidence that a western type diet is associated with a significant risk for heart disease and cancer, numerous inconsistencies are also evident. These inconsistencies are probably due to the multi-factorial and complex nature of health disparities, and the specificity and temporal effects that individual dietary constituents have in modifying genetic pathways leading to disease states.

While excess calories are generally linked to significant health risk, a large number of bioactive food components may modulate the response both positively and negatively (2). A multitude of compounds, both essential and non-essential dietary components, may influence disease risk. While historically essential nutrients such as calcium, zinc, selenium, and folate, vitamins C, D, and E have surfaced as disease risk modifiers, it is becoming increasingly clear that numerous non-essential food components including carotenoids, flavonoids, isothiocyanates, allyl sulfur constituents, conjugated linoleic acids, etc. can be as important (2). Bioactive food components arising from plant, animal, fungi and bacteria may modify simultaneously more than one cellular process including such

diverse events as carcinogen metabolism, hormonal homeostasis, cell signal regulation, cellular proliferation, apoptosis, and angiogenesis (3).

A variation in disease risk among, and within populations with similar dietary patterns suggests that an individual's response to foods or their components may reflect genomic interactions (Fig. 1). A greater understanding of "nutrigenomics", defined as the interaction between nutrition and an individual's genome, should provide important clues about those who will benefit most from dietary interventions, and those who might be placed at risk because of dietary change. While the techniques needed to unravel the links between dietary habits and genomics are identical to those used in modern molecular research, the understanding of the diet-gene connection will not be simple since evidence already exists that multiple genes and various dietary components can influence the phenotypic.

Examining only nutrigenetics (genetic variation including single nucleotide polymorphisms as a function of foods or their components) will be inadequate to identify what produces a phenotypic change. Additionally, DNA methylation and other epigenetic events (nutritional epigenomics) as well as variation in gene expression profiles (nutritional transcriptomics) can be modified by dietary habits. It is also increasingly apparent that several food components can influence the formation and/or bioactivation of a host of key cellular proteins (proteomics) and therefore influence phenotypes (Fig. 1). Shifts in small molecular weight constituents in fluids and tissues (metabolomics) as a function of the quantity and duration of exposure to food components may also be an important trigger in determining whether or not a response occurs. The wealth of information that will emerge from nutrigenomics, proteomics and metabolomics studies necessitate that integrative and interactive databases are created to incorporate modern techniques and approaches in bioinformatics.

Tissue and Cellular Assessment

A fundamental issue at the frontier of nutrition research is which biological specimens are most predictive of the response to bioactive food components. Since some tissues are inaccessible there is a need for the identi-

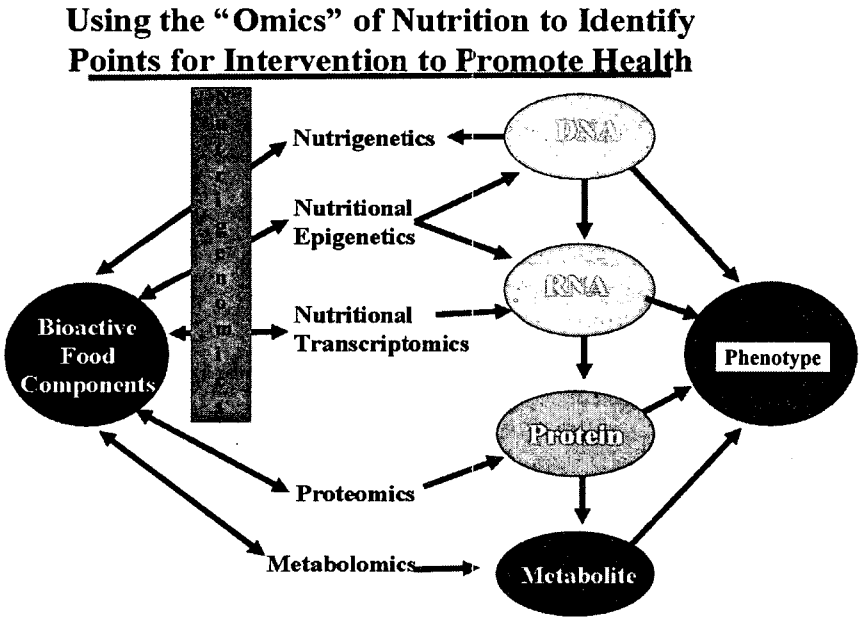

Fig. 1. Using the "Omics: of Nutrition to Identify Points for Intervention to Promote Health.

fication of suitable surrogate samples that provide predictive information about the response to diet. Although blood or blood constituents have often been employed to assess the response to food components, they may not always adequately reflect the concentration and molecular/biochemical effects that are brought about by bioactive food components in target tissue (4). It remains unclear if exfoliated, sloughed cells or surrogate tissue biopsies offer advantages in predicting the merits of dietary intervention strategies. While limitation in the collection of these exfoliated and surrogate samples are evident, there is intriguing evidence that some of these preparations may predict tissue uptake and changes in gene, protein and metabolome expression profiles caused by dietary exposures.

Nutrigenetics

Preclinical studies provide some of the most compelling evidence that genetic backgrounds can influence the response to food components (5). For example, fat resistant A/J mice, but not fat sensitive C57BL/6J mice, increase thermogenic capacity in response to high fat diets (6). Studies with transgenic and knockout animals are beginning to reveal the physiologic consequences of gene expression patterns. For example the $p21^{WAF1/cip1}$ gene product, downstream effectors of p53 is an inhibitor of cyclin-dependent kinase activity and is therefore key in cell cycle regulation, apoptosis and cell differentiation. Targeted inactivation of the $p21^{WAF1/cip1}$ gene has been found to enhance tumor formation and decrease survival in the multiple intestinal neoplasia (Min) mice, a genetic model for human colon cancer susceptibility (7). This effect was magnified when mice consumed a Western-style high risk diet which contains high fat and phosphate and low calcium and vitamin D (7). Transgenic studies are also shedding light about the tissue specificity and sensitivity in gene-nutrient interactions. For example zinc deficiency in p53 knockout mice accelerated the induction and progression of *N*-nitrosomethylbenzylamine (NMBA)-induced tumors in the forestomach more than in the esophagus (8). While data from this study indicate that all tissues are not equality sensitive to nutrient imbalances they also suggest diet can ameliorate some genetic predisposition to cancer and presumably other disease conditions.

The occurrence of polymorphisms in humans are beginning to provide clues about why variation in response to diet might occur. Common polymorphisms involved in the absorption, metabolism or excretion of nutrient are logical modifiers of the response to a food or its component. In women with a polymorphism that causes a valine to alanine change in the 9 position in the signal sequence for the enzyme manganese dependent superoxide dismutase who consume fruits and vegetables below the medium intake have been reported to be at greatest risk of breast cancer (9). Higher intakes of fruits and vegetables in these women appeared to mask the effects of this polymorphism. Likewise, interactions between glutathione-s-transferase (GST) genotypes and dietary isothiocyanates, occurring in cruciferous vegetables, are linked with a lower risk for colorectal cancer among individuals who had both GSTM1 and T1-null phenotypes, presumably because of a slower rate metabolism and excretion of this food constituent (10).

It is logical to assume that multiple, rather than single polymorphisms, within, and across genes may contribute to phenotypic changes induced by food components. For example, polymorphisms in the 5' (the *FokI* restriction site) and in the 3' (*Bsm*I, *Taq*I restriction sites and polyA tail) end of the gene for vitamin D nuclear receptor (VDR) may influence the response to 1, 25 D_3 (11). The *Fok1* polymorphism has been associated with a decrease in the intracellular activity of 1, 25 D_3 and the 3' polymorphisms are associated with decreased transcription of the gene (11). How polymorphisms interact to bring about phenotypic change and influence disease risk remains an area of intense investigation.

Epigenomics

Epigenetic events represent an important mechanism for selective activation or inactivation of genes. Since epigenetic events are susceptible to change they offer another potential explanation for how dietary habits may influence health. Abnormal methylation patterns are nearly a universal finding in cancer, as changes in DNA methylation have been observed in many cancer tissues (e.g., colon, stomach, uterine cervix, prostate, thyroid, and breast). Site-specific alterations in DNA methylation may also have a significant role in gene regulation. Dietary factors may be involved with DNA methylation through at least 4 mechanisms (12). First, they may influence the supply of methyl groups available for the formation of S-adenosylmethionine (SAM) and thus the precursor for methylation reactions. Second, dietary factors may modify the utilization of methyl groups by processes including shifts in DNA methyltransferase activity. A third plausible mechanism may relate to DNA demethylation activity. Finally, the DNA methylation patterns may influence the response to a bioactive food component.

A polymorphism (677C-->T) in a key folate enzyme, methylenetetrahydrofolate reductase (MTHFR), may impair DNA methylation when folate intake is inadequate and may increase the risk of reproductive abnormalities (13). Women with the TT polymorphism were particularly susceptible to a hypomethylation caused by folate inadequacy. Likewise, recent information about the effect of diet on gene methylation and the release of hidden genetic variation by impairment of heat shock protein 90-mediated buffering systems offer eloquent examples of how epigenetic mechanisms might affect gene-environment interactions (14). Evidence that phenotypic and epigenetic changes can occur following the addition of selected nutrients to a diet considered adequate for reproduction raises intriguing questions about when the role of in utero exposures on subsequent disease risk (15).

Nutritional Transcriptomics

It is also clear that dietary habits can markedly influence gene expression profiles. High-throughput genomics technologies are increasingly being used to identify multiple molecular pathways that are influenced by food components. These investigations provide fundamental information about plausible mechanism(s) that underlie the beneficial or adverse effects of a specific dietary component, and assist in identifying important genes that are altered in the pre-disease state that may be used as potential molecular biomarkers. A relevant example about the ability of food components to alter gene expression profiles comes from studies with selenium supplementation. These studies with rodent models or cells in culture have revealed major increases in the expression of genes involved in DNA damage repair, oxidative stress and cell-cycle control yet decreased the expression of genes involved with drug detoxification (16). Because of the large number of genes whose expression can be modified by selenium cluster analysis have been used to help identify key sites of action. It is likely that similar clustering approaches will be needed to evaluate the effects of other food components especially since multiple targets are likely to be influenced simultaneously. Likewise, these approaches may be particularly useful in the identification of interactions among various dietary components that potentially modify common cellular events including drug metabolism, cellular proliferation, apoptosis, etc. A greater understanding about why multiple genes are modified simultaneously is needed to adequately interpret nutritional transcriptomics. It remains unclear if these shifts in gene expression represent some cellular signal such as intracellular calcium, radical formation, mRNA stability or some other overarching mechanism that would bring about multiple positive and negative changes in transcriptomic expression. A cautionary note must be including that single point determination are clearly inadequate to evaluate the short and long term response and acclimations that occur as a result of exposures to specific food components.

Proteomics

The term proteome refers to all the proteins produced by a species, much as the genome is the entire set of genes. However, unlike the genome, the proteome is very dynamic and thus varies widely according to the cell type and its physiological state. It is well recognized that protein expression does not always correlate with mRNA expression. Several factors may account for this inconsistency including alternative splicing resulting in multiple proteins from a single gene, post-translational modifications (i.e. glycosylation, phosphorylation, acetylation, oxidation, and reduction) or simply shifts in rates of synthesis and degradation (17).

Several dietary components may modify proteins post-translationally and thereby regulate their activity. For example, shifts in phoshorylation in selected proteins occur after exposure to diallyl disulfide (DADS), a compound found in processed garlic (18) and sulforaphane, a compound found in broccoli (19). What cellular events that accounts for these shifts in phosphorylation remains to be resolved.

Calorie restriction is also recognized to extend the lifespan and reduce cancer risk in a number of species (20). Part of this protection may arise from an alteration in the expression of the regulatory gene, SIR (21). SIR2 (in yeast) and the analogous mammalian gene, SIRT1 code for a deacetylase enzyme known as sirtuin. Resveratrol, a potent activator of the sirtuin deacetylase activity results in deacetylation of proteins, including p53 and histones. Deacetylation results in the cell's ability to recover from insults rather than committing suicide; thereby extending life (21).

The future of nutritional proteomics will surely depend on the development of new and sensitive technologies that allow the rapid analysis and identification of protein products or groups of related proteins. Data is already surfacing that demonstrates the usefulness of proteomic technologies for elucidating the mechanisms of action and of food components. For example, the growth inhibition of HT-29 colon cancer cells resulting from sodium butyrate treatment provided evidence for a change in the ubiquitin-proteasome system, suggesting that proteolysis could be a means by which butyrate may regulate the cell cycle, apoptosis and differentiation (22). These authors also demonstrated that butyrate upregulated both proapoptotic (caspase-4 and cathepsin D) and antiapoptotic proteins (hsp27, antioxidant protein-2 and pyruvate dehydrogenase E1) which may account for some variation in response to butyrate-induced apoptosis (22).

Metabolomics

Metabolomics is the study of the metabolome, which is the entire metabolic content of a cell or organism at a given moment (23). While the focus of metabolomic research has largely been with biofluids, including serum and urine more attention is needed for what occurs within cells as a function of the quantity and duration of exposure to bioactive food components. Quantitative lipid metabolome data has already begun to identify the differential effects of dietary fats on cardiac and liver phospholipid metabolism (24). Likewise, evidence about changes in metabolome has been suggested to predict the effects of excess amino acids (25).

To date few studies have quantitated changes in small molecular weight compounds caused by specific bioactive food components. A study that has used a metabolomic approach to evaluate the effects of soy in five healthy premenopausal women found differences in the plasma lipoprotein, amino acid, and carbohydrate profiles suggesting this dietary component could influence energy metabolism (26). Regardless, the large individual variability seen in this study is particularly noteworthy and raises significant concerns about how best to characterize changes in metabolomic profiles. It is prudent that more attention is given to what specimens are examined and when measurements are made relative to dietary intervention if any sense is to be made from these analyses.

Summary

Dietary components continue to captivate consumer, scientists and legislators as potentially significant determinants of health. While the linkages are fascinating, the literature is less than ideal and thus makes it difficult to make firm conclusions about who will benefit most from specific dietary interventions. It is becoming increasingly clear that not all individuals respond identically to dietary interventions. Genetic polymorphisms (nutrigenetic effect) likely account for this inconsistency in response. However, DNA methylation and other epigenomic events can influence gene expression and therefore modify the response to food components and visa versa. Furthermore, variation in the ability of food components to enhance or suppress gene expression patterns (nutritional transcriptomic response) may account for inconsistencies in the response to food components among individuals. Since a host of dietary constituents are recognized to influence postranslational events, these also likely account for at least part of the response variation. While a bioactive food component may influence a number of key molecular events that are involved with health, to do so it must achieve an effective concentration within the target site, be in the correct metabolic form and lead to changes in small molecular weight signals in the cellular milieu (metabolomic effects). Elemental to assessing and evaluating the significance of the interrelationships among a dietary component with nutrigenetics, nutritional epigenomics, nutritional transcriptomics, proteomics and metabolomics is knowledge about the appropriate tissue/cell or surrogate to monitor, the time of exposure to bring about an effect and the effective quantity need to bring about a phenotypic change. As the era of molecular nutrition unfolds, a greater understanding of how foods and components influence health will surely arise. This information is undeniably fundamental to the development of effective delivery of tailored approaches to achieve one's genetic potential, increase both physical and cognitive performance and reduce the risk of diseases.

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