Perspective

Cruciferous Vegetable Intake and Cancer Prevention: Role of Nutrigenetics

Perspective on Navarro et al., p. 345

Christine B. Ambrosone and Li Tang

The field of nutritional epidemiology has used an observational study approach in identifying food groups, foods, and specific nutrients that seem to be associated with reduced cancer risk. These findings led to a number of chemoprevention trials, particularly among people at a high risk for specific cancers. The results of many of these trials, however, have been disappointing. For example, strong epidemiologic relationships between dietary sources of carotenoids and lung cancer risk led to the large-scale Beta-Carotene and Retinol Efficacy Trial (1) and Alpha-Tocopherol and Beta-Carotene Study (2) in individuals at a high risk for lung cancer primarily due to smoking. Both trials were terminated early because of a starting increase in lung cancer incidence among participants randomized to the β-carotene arms. More recently, the large-scale Selenium and Vitamin E [prostate] Cancer Prevention Trial stopped supplements early because of a lack of efficacy of either agent alone or in combination (3).

A major lesson learned from these trials is that favorable associations between cancer risk and dietary and serum levels of nutrients from complex foods ingested for as long as a lifetime do not necessarily extrapolate to limited-duration chemoprevention trials of specific micronutrients isolated from their complex food sources. Furthermore, the effects of specific nutrients and/or active food components may vary depending on the metabolic constitution of the individual at risk. A dietary component or nutrient may only reduce cancer risk in people with specific genetic makeups or exposures to other factors that can induce or inhibit enzymes in the mechanistic pathway of a nutrient’s preventive effect.

These concepts are well illustrated in the continuing research on how differences in genes that encode enzymes that are involved in the metabolism of cruciferous vegetable components or are induced or inhibited by consuming cruciferous vegetables potentially modify relationships between cruciferous vegetable intake and cancer risk. Epidemiologic studies indicate inverse associations between consumption of cruciferous vegetables and the risk of lung, colorectal, stomach, breast, prostate, and other cancers (4), but these studies are not all clearly consistent. In 1998, Lin and colleagues hypothesized that the effects of cruciferase may vary depending on genetic makeup. This group conducted a case-control study showing that higher intake of cruciferous vegetables was associated with reduced risk of recurrent colorectal adenomas mainly among patients with glutathione S-transferase (GST)M1-null genotypes (5). In an editorial accompanying this article, Ketterer proposed that sulforaphane, an anticarcinogenic component of cruciferae, is metabolized by GSTM1, leading via the glutathione pump to greater excretion of sulforaphane. Therefore, adenoma patients with genotypes null for the M1 allele would have less excretion and higher levels of sulforaphane and, thus, a reduced cancer risk (6). This early research in nutrigenetics, the study of the role of genetic variability in modifying relationships between diet and health outcomes, was followed by a number of epidemiologic studies that considered GST genotypes in evaluating relationships between cancer risk and consumption of cruciferous vegetables, again with inconsistent results (7).

A possible reason for these study inconsistencies is that the anticarcinogenic properties of cruciferous vegetables derive from their effects on enzymes that metabolize carcinogens, which may act through complex pathways. Phase I enzymes, such as the cytochrome P450 family, catalyze multiple reactions and play a primary role in the activation of a number of drugs, carcinogens, and metabolic products such as bilirubin (metabolized from hemoglobin). In turn, phase II enzymes, such as the GSTs, sulfotransferases, and UDP-glucuronosyltransferases (UGT), catalyze conjugation of reactive intermediates, resulting in their excretion. Figure 1 illustrates this process, with the metabolism of benz[a]pyrene, a prototype of carcinogenic polycyclic aromatic hydrocarbons (PAH), as an example. Mechanistically, higher activation of carcinogens by phase I enzymes or lower detoxification by phase II enzymes can result in higher levels of reactive carcinogen metabolites, which can bind to DNA, form DNA adducts, and, if not removed by DNA repair enzymes, cause mutations and/or replication errors that can initiate the carcinogenic cascade.

Overexpression of phase II enzymes protects cells against carcinogen-induced DNA damage, and knockout of phase II enzymes in rodent models significantly increases susceptibility to carcinogenic challenges. The GST superfamily member GSTP1-1 is particularly important in the metabolism of PAHs, carcinogens present in cigarette smoke and automobile exhaust (8). Hu et al. (9) found that allelic variants of human GSTP1-1 with disparate enzyme activity were differentially protective against PAH-induced DNA damage and that the formation of DNA adducts was significantly reduced in cells transfected with GSTP1-1 compared with cells transfected with an empty vector. Ryberg et al. (10) reported that the level of PAH-DNA adducts was higher in lung tissues of smokers carrying low-activity allele for GSTP1 and GSTM1 versus those encoding higher activity, and Henderson et al. (11) found a 3.4-fold increase in the number of skin papillomas in GSTP1-1 knockout mice. These studies provide direct...
ages 20 to 40 years found that these relationships were controlled feeding study in healthy nonsmoking participants. Their report dose-dependent relationships between levels of the journal, Navarro and colleagues in the Lampe group. Cruciferous vegetable intake has been on the GSTs. However, efficiency.

Fig. 1. Simplified schema of interactions between phase I enzymes (P450s) and phase II enzymes (EH, GST, UGT, SULT, and NQO1) in the metabolism of B[a]P, a prototype of carcinogenic PAHs. EH, epoxide hydrolase; SULT, sulfotransferase; NQO1, NAD(P)H:quinone oxidoreductase 1; B[a]P, benzo[a] pyrene; P450, cytochrome P450 enzyme.

evidence that phase II enzymes involved in metabolism pathways have a profound effect on carcinogenesis.

It is hypothesized that the primary mechanisms for cancer risk reduction by consumption of cruciferous vegetables are phase I enzyme inhibition, which results in reduced activation of carcinogens, and phase II enzyme induction, which causes better excretion of reactive intermediates (12). Induction of phase II enzymes by cruciferous vegetables is mainly through activation of the NF-E2-related factor 2 (Nrf2)/antioxidant response element pathway by isothiocyanates, a family of anticarcinogenic compounds in cruciferous vegetables. Phase II enzyme induction is observed only in Nrf2 wild-type mice, and not in Nrf2-null mice (13). As shown by Xu et al. (14) in a 7,12-dimethylbenz(a)anthracene– and 12-O-tetradecanoylphorbol-13-acetate–induced skin carcinogenesis model, the chemopreventive effect of isothiocyanates against carcinogens is also potent in Nrf2 wild-type mice, in which isothiocyanate treatment significantly reduced skin cancer incidence by 3-fold, with no effect in Nrf2-null mice.

Understanding the biochemical underpinnings of preventive effects of cruciferous vegetables or their bioactive components is further complicated by the overlapping substrate specificities of the α, μ, π, and θ isoforms of GSTs. All are capable of catalyzing conjugation reactions of isothiocyanates with glutathione, although with varying efficiency. Therefore, when one isoform is not sufficient, other isoforms may be up-regulated to compensate for the reduced activity of the insufficient one. Furthermore, isothiocyanates are metabolized via the mercapturic acid pathway, which requires not only GSTs but also r-glutamytranspeptidase, cysteinylglycinase, and N-acetyltransferase, which also usually vary in efficiency.

To date, the main focus of nutrigenetics in relation to cruciferous vegetable intake has been on the GSTs. However, cruciferous vegetables also induce the activity of other phase II conjugating enzymes such as UGTs. In this issue of the journal, Navarro and colleagues in the Lampe group (15) report dose-dependent relationships between levels of bilirubin, a marker of activity of glucuronidation by UGT1A1, and diets containing cruciferous vegetables. Their controlled feeding study in healthy nonsmoking participants ages 20 to 40 years found that these relationships were modified by genotypes for UGT1A1 and, to a lesser extent, by genotypes for GST. UGT1A1 is expressed predominantly in the liver and catalyzes the glucuronidation of a wide variety of substrates. The discovery of a UGT1A1 role in the metabolism of the chemotherapy drug irinotecan led to the extensive study of this enzyme in relation to drug metabolism and cancer treatment outcomes. In numerous studies, a common dinucleotide (TA) repeat polymorphism in the UGT1A1 promoter has affected severe irinotecan-related toxicity (16). The clinical relevance of this example of pharmacogenetics is clear: The Food and Drug Administration approved the UGT1A1 pharmacogenetic test and changes in the irinotecan package insert that recommend UGT1A1 genotyping, with reduction of the starting dose of irinotecan for homozygous risk-allele carriers. The concept that specific therapeutic agents have differential effects on treatment-related toxicities and efficacy is now widely accepted, and package inserts for several drugs in addition to irinotecan now recommend dose adjustments based on genotypes. Evidence also indicates that other chemopreventive agents, such as aspirin and celecoxib, have differential effects based on genotypes (17, 18).

The growing field of nutrigenetics promises to clarify the often confusing epidemiologic literature on diet and cancer risk. Perhaps the link that is needed for translating observational studies into positive chemoprevention trials is mechanistic studies such as those conducted by the Lampe group (15). This group has conducted a series of rigorous feeding studies in human participants, first showing that cruciferous vegetable intake increased UGT1A1 activity, but only among participants with UGT1A1 genotypes encoding lower transcription (19, 20). In the current study, they further show that vegetable-containing diets result in a dose-response reduction in levels of bilirubin, a measure of UGT1A1 activity, with bilirubin levels higher among participants with *28/*28 UGT1A1 genotypes.

These types of studies provide important information on the mechanisms of action of cruciferous vegetables and, more importantly, have significant implications for the interpretation of epidemiologic studies and their translation into well-designed chemoprevention trials. Clearly, as shown in the Navarro et al. article in this issue of the journal, the biochemistry underlying relationships between diet and cancer is extremely complex, and the response to specific nutrients and food components will likely vary according to individual genotypes. Furthermore, it is unlikely that studying the modification of agent effects by one or two genes is sufficient. Entire metabolic pathways should be considered, as should be other exposures that may induce or inhibit enzymatic activity, in assessing the chemopreventive potential of foods or their components. An observational study by Touvier et al. (21) clearly illustrated the importance of other exposures in modifying intervention efficacy; vitamin supplementation with β-carotene reduced the risk of lung cancer among nonsmokers and increased this risk among smokers. An accompanying editorial by Mayne and Lippman (22) noted the important implications of these findings for chemoprevention trials. These observational results are consistent with prospective clinical subgroup data from a placebo-controlled randomized trial, which showed statistically significant harm among current smokers and benefit among never smokers who received...
a retinoid (23). It is intuitive that rigorous, well-controlled feeding studies may be a necessary bridge between nutritional association studies and clinical trials. Understanding the relationships between nutrients and specific enzymes at the biochemical level and the role of genetic variability in modifying these relationships may allow the targeting of intervention studies at those high-risk cohorts most likely to benefit.

Lessons learned from the controlled feeding studies of the Lampe and other groups should be applied in designing future chemoprevention trials, which will require incorporating the expertise of biochemists, nutritionists, and molecular biologists. Because of the tsunami of emerging genomic information, it is important that investigators remain mindful of the biochemical complexity underlying relationships between exposures or genotypes and carcinogenesis. As shown by the Navarro work, genotypes are not static variables, and their relationships with disease risk will likely be modified by the effects of other variants in entire genetic pathways, key exposures evaluated, and other exposures that may modify relationships through inhibition or induction of enzyme activity. There is increasing marketing of “genotype chips” designed for individual cancer risk assessment, and there is emerging use of nutrigenetics to provide individual dietary recommendations based on genetic profiles. As illustrated in the work of Navarro et al., associations between diet, genetics, and cancer risk are not simple or linear, and it is naïve to think that individualized dietary recommendations can be based on one’s genome and the results of epidemiologic studies. For public health measures, it is now justifiable to recommend moderate consumption of a healthy diet, high in fruits and vegetables and low in meat and fat, for the benefit of entire populations. Specific recommendations for cancer prevention based on individual genotypes, however, will require many more years of research such as the rigorous studies reported by Navarro et al. in this issue of the journal.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References