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EVIDENCE FOR THE RELATIONSHIP BETWEEN DIET AND CANCER

S.A. Ross

Nutritional Science Research Group, Division of Cancer Prevention, National Cancer Institute, EPN 3157, National Institutes of Health, Department of Health and Human Services, 6130 Executive Blvd., MSC 7328 Bethesda, MD 20892-7328, USA

The relationship between diet and cancer has advanced in recent years, but much remains to be understood with respect to diet and dietary components in cancer risk and prevention. Evidence from clinical trial outcomes, epidemiological observations, preclincial models and cell culture systems have all provided clues about the biology of cancer prevention. Sequencing of the human genome has opened the door to an exciting new phase for nutritional science. There are also many advances in our understanding of the control of gene expression in eukaryotic cells that might impact cancer development, including mechanisms regulating chromatin structure and dynamics, epigenetic processes (DNA methylation, histone posttranslational modification), transcription factors, and noncoding RNA and evidence suggests that environmental factors such as diet influence these processes. Unraveling the effects of bioactive food components on genes and their encoded proteins as well as identifying genetic influences on dietary factors is essential for identifying those who will and will not benefit from intervention strategies for cancer prevention. Additional research needs concerning diet and cancer prevention include: identification and validation of cancer biomarkers and markers of dietary exposure; investigation of the exposure/temporal relationship between food component intakes and cancer prevention; examination of possible tissue specificity in response to dietary factors; and examination of interactions among bioactive food components as determinants of response. Other emerging areas that require greater attention include understanding the link between obesity, diet and cancer, the interaction between diet and the microbiome, as well as how bioactive food components modulate inflammatory processes. Importantly, for the future of nutrigenomics, the "omics" (e.g., genomics, epigenomics, transcriptomics, proteomics, metabolomics) approach may provide useful biomarkers of cancer prevention, early disease, or nutritional status, as well as identify potential molecular targets in cancer processes that are modulated by dietary constituents and/or dietary patterns.
Key Words: **diet, cancer, nutrigenomics.**

There have been several advancements in our understanding of cancer at the molecular level. Growth factors, hormones, cytokines, oncogenes, viruses, bacteria, and carcinogens are among the factors that have been identified to initiate and promote cancer [1]. Many of the subcellular mechanisms that promote hyperproliferation, invasion, angiogenesis and metastasis have also been determined. The structure of the entire human genome consisting of almost 25,000 genes as well as the characterization of some of the genes that mediate tumorigenesis is also now evident. Furthermore, there are many advances in our understanding of the control of gene expression in eukaryotic cells that might impact cancer development, including mechanisms regulating chromatin structure and dynamics, epigenetic processes (DNA methylation, histone posttranslational modification), transcription factors, and noncoding RNA. In spite of this significant increase in knowledge about cancer, the application of these advancements to cancer prevention and treatment practice needs greater attention. Moreover, the incidence of certain cancers is projected to increase in the coming decades due to growing and aging populations in many countries. Thus, there is a need for effective preventive strategies for cancer. Importantly, dietary habits are recognized to be important modifiable factors influencing cancer risk and prevention. The World Cancer Research Fund/American Institute of Cancer Research (WCRF/AICR) summary of the available epidemiological evidence on food, nutrition, physical activity, and the prevention of cancer clearly support the suggestion that cancer incidence and death are potentially avoidable by modification of the diet as well as by physical activity [2]. In addition to such observational evidence, both *in vitro* and *in vivo* studies have suggested that several bioactive food components, such as phytochemicals found in plants [3] and fungochemicals found in mushrooms [4], are likely to alter susceptibility to cancer. In fact, both essential nutrients and non-essential bioactive food components, have been implicated in altering many of the pathways of cancer, including apoptosis, cell cycle control, differentiation, inflammation, angiogenesis, DNA repair, and carcinogen metabolism [5]. Findings from either human epidemiological or human and animal experimental studies suggest that the study design (e.g., cancer site, model system or population, food constituent and/or total diet composition) may be an important determinant of the direction and magnitude of the response, but variation in biological response may also be involved. Nutritional genomics is an emerging multidisciplinary science that recognizes the potential of nutrients to act as signals to influence cell behavior and also should provide insights about variation in response. For example, variation in genetic influences on the diet due to gene polymorphisms (nutrigenetics) and/or dietary influences on gene expression (nutritional transcriptomics), on DNA methylation and other epigenetic events (nutritional epigenetics) may account

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*Correspondence: E-mail: rosssha@mail.nih.gov
Abbreviations used: AMP – adenosine monophosphate; AMPK – AMP-activated protein kinase; BMI – body mass index; ER – estrogen receptor; GWAS – genome-wide association studies; MTHFR – methylene tetrahydrofolate reductase; PLP – pyridoxal-phosphate; RFS – relapse-free survival; SELECT – Selenium and Vitamin E Cancer Prevention Trial; SNP – single nucleotide polymorphisms; VDR – vitamin D receptor; WHEL – Women's Healthy Eating and Living; WINS – Women's Intervention Nutrition Study.

for inconsistencies from study to study. Unraveling the effects of bioactive food components on genes and their encoded proteins as well as identifying genetic influences on these dietary factors is essential for identifying those who will and will not benefit from intervention strategies. Dietary recommendations will continue to be made at the population level, but the possibility for certain dietary recommendations to be customized to individual needs is an expectation of the future. Advances in the comprehension of the human genome and its regulation hold promise for such personalized nutrition. However, the diversity of human genetic backgrounds, individual nutrition (e.g. differences in food composition, influences of culture and food preparation, and food processing) and the heterogeneity of pathways to disease and health will certainly present challenges to personalized nutrition.

Diet and cancer prevention clinical intervention studies. Evidence from clinical interventions for the relationship between diet and cancer has been mixed. Many of the placebo-controlled clinical trials, whether carried out with carotenoids [6, 7], folic acid [8], selenium [9] or other agents, have resulted in either null or inconclusive outcomes. A recent example is The Selenium and Vitamin E Cancer Prevention Trial (SELECT) which recruited over 35,000 men age 50 and older to determine if two dietary supplements (200 μg/day of l-selenomethionine and 400 IU/day of all-rac-alpha-tocopheryl acetate taken individually or together versus placebo) can protect against prostate cancer [9]. SELECT was stopped in 2008 following a minimum of 7 years of supplementation when preliminary analyses showed no effect of selenium or vitamin E on the incidence of prostate cancer, and because there was a statistically non-significant increase in the number of prostate cancer cases among the men taking only vitamin E. In addition there was a small, but not statistically significant increase in the number of cases of adult onset diabetes in men taking only selenium. These were disappointing results because of the many years of positive findings from Phase I and II clinical trials indicating that both selenium and vitamin E had a strong potential for prostate cancer prevention. SELECT analyses for secondary endpoints will continue as data is finalized from study centers and other clinical studies are ongoing to help explain the findings. For example, a clinical trial to determine if different forms (selenomethionine and selenium yeast) and doses of selenium tailored as a function of age are effective in inhibiting oxidative stress and other markers of risk for prostate cancer in healthy African American and white American men is being supported by NCI [*http://projectreporter.nih.gov/project_info_description.cfm?aid=7878646&icde=4419435*].

Evidence from epidemiologic cohort studies supported the initiation of many of the dietary supplement trials of specific nutrients for cancer prevention, but it is now thought that reducing these findings to specific nutrients has not generally worked in randomized control trials. The results suggest several interacting factors might be important in preventing cancer, including relative macronutrient density, food matrix, and complex mixtures of bioactive food components (not limited to micronutrients), as well as nutrient form and dose as described above. Another speculation is that specific nutrient supplementation may be effective only in a population that is deficient in that nutrient, which was the case in the generally selenium deficient population of the Nutritional Prevention of Cancer study [10] but not in the SELECT population which was relatively selenium replete.

Results of the General Population Trial in Linxian, China demonstrated that individuals who received a supplement containing beta-carotene, vitamin E and selenium, had a 13% reduction in cancer mortality [11]. This study was conducted in a borderline-deficient population for various nutrients, thus, again it may be that supplementation is most likely to be beneficial for individuals who are low or deficient at baseline. In a recent publication of this cohort, post-intervention follow up indicated that the beneficial effects of selenium, vitamin E, and beta-carotene on mortality were evident up to 10 years after cessation of the supplementation program [12]. Interestingly, the benefits were greater in individuals who were < 55 years at the beginning of the intervention. Cancer risk appeared to increase in those who started supplement usage when 55 years of age or older. These findings suggest that sustained exposure may not always be necessary to bring about a desired outcome. It may also be that the observed response in risk as a function of age reflected differences in the frequency of neoplastic conditions.

The Aspirin/Folate Polyp Prevention Study investigated the effects of folate supplementation (at 1 mg/d folic acid, the synthetic form of folate) on the development of colorectal adenomas [8]. Overall, the investigators reported no effect of folic acid supplementation on the development of adenoma, the primary outcome. Unfortunately, in subgroup analyses they found an increased risk of advanced lesions at the second follow-up. Also of concern was the finding that the risk of cancers other than colorectal cancer were significantly increased in the intervention, which was thought to be largely due to prostate cancer. It was hypothesized that an explanation for the increased risk of advanced and multiple adenomas in the intervention group was that undetected early precursor lesions were present in the mucosa of these patients (who are at increased adenoma risk), and that folic acid promoted growth of these lesions [13]. This hypothesis is consistent with experimental studies showing increased colorectal neoplasia when folic acid is administered after lesions are present. Thus, a greater understanding of the temporal relationships between diet and disease are needed to determine appropriate prevention strategies.

In addition to dietary supplement intervention studies dietary behavior/lifestyle interventions have also been examined. For example, studies to determine the efficacy of dietary change in reduction of breast cancer recurrence have also been conducted. Examples of such trials are the Women's Healthy Eating and Living (WHEL) [14] and Women's Intervention Nutrition Study (WINS) [15]. Both studies achieved significant reductions in energy from fat, and the WHEL Study achieved large increases in the consumption of vegetables, fruit and fiber. The WHEL study investigators observed no

significant association between diet and prognosis while a secondary analysis suggested that the dietary intervention reduced distal recurrences among the subgroup without hot flashes identified at baseline [16].

WINS examined postmenopausal women only and reported a 24% increase in relapse-free survival (RFS) in postmenopausal women with primary early-stage resected breast cancer who received standard cancer management. Of interest is that after eight years, the increase in RFS was greater in women with estrogen receptor (ER)-negative disease than women with ER-positive disease. This finding was supported by both the Women's Health Initiative [17] and should be considered for further investigation because of the burden of ER-negative breast cancer. Both the WHEL and WINS studies suggested that dietary pattern may be effective within a subgroup of people studied [16]. Additional investigation of these sub-groups is warranted.

Controlled interventions may provide unexpected information because of the quantities of the test agent examined, the duration of the intervention or the subjects examined. Furthermore, variation in response within the cohort may reflect timing of the amount and duration of exposure to a specific bioactive food component and to its interactions with multiple food constituents, environmental factors, the genetics of the host, or a combination of these factors. Recently, basic nutrition studies have been deemed critical to determine novel cancer prevention strategies. In this regard, several preclinical models are currently available which can be used to determine the mechanism of action as well as efficacy of diet and bioactive food components [1]. Nutritional genomics and omics approaches are also likely to assist in elucidating mechanisms, molecular targets and identifying appropriate intervention strategies.

Obesity, diet and cancer. During the past 20 years there has been a dramatic increase in the prevalence of overweight and obesity in the United States and many other countries. This increase impacts many conditions such as hypertension, cardiovascular disease, type 2 diabetes mellitus, and some cancers. Obesity is a multi-factorial disorder affected by multiple genetic and environmental factors, in particular diet, nutrients and their interrelationships. Epidemiological evidence suggests that increased body mass index (BMI) is associated with an increased risk for cancers of the esophagus, pancreas, colon and rectum, endometrium, kidney and breast (in postmenopausal women), among others [18]. The biological mechanism that explains how obesity increases cancer risk may be different for different cancers and exact mechanisms are not known for any of the cancers. The metabolic consequences of obesity include elevated levels of IGF-1, insulin, adipokines, and pro-inflammatory cytokines and these alterations likely provide clues for the relationship between obesity and cancer [19]. For example, these metabolic changes result in activation of signaling pathways that culminate in activation of the mammalian target of rapamycin (mTOR) and downstream proliferation and survival signals; altered steroid hormone metabolism, resulting in increased estradiol and other estrogen metabolites; and increased inflammation and oxidative stress, including activation of cytokine signaling [20]. Other evidence indicates that increased risk of carcinogenesis is associated with decreased activity of adenosine monophosphate (AMP)-activated protein kinase (AMPK) which is a major metabolic-sensing protein implicated in the prevention of metabolic disorders [21]. Activation of AMPK by bioactive food components is currently being investigated for the prevention of several diseases, including cancer. For example, the anticancer effects of the tea polyphenol (-)-epigallocatechin-3-gallate [22] and the phytoalexin resveratrol found in grapes [23] have recently been thought to be mediated via activation of AMPK. Thus, in addition to novel physical activity, behavioral and dietary intervention studies that focus on understanding the prevention of obesity, a current focus of nutrition, obesity and cancer research concerns the effects of diet composition and dietary constituents on the dysregulated signaling pathways described above.

Nutrigenetics. Genetic differences in taste preference, food tolerance, and dietary constituent absorption and metabolism all potentially influence the effect of diet and bioactive food components on cancer risk. Among the various common types of alterations in DNA sequence, single nucleotide polymorphisms (SNPs) have been studied the most. If a SNP induces a modification of one important amino acid in the encoded protein sequence, this can alter the protein function/activity and thus the pathway or process involved. A modification in the gene promoter sequence can alter the promoter activity and thus the level of gene transcription, resulting in altered encoded protein level and linked function. In other cases, no effect on protein level or activity can be observed. Thus, the functional impact from SNPs on the encoded protein could be variable. The strength of the biological impact will also depend on heterozygosity or homozygosity of the variant.

While there is a good deal of evidence that the frequency of functional polymorphisms may influence the response to a variety of dietary components, there is a need to validate and verify these findings. Most findings are associated with single observations in an epidemiological context and therefore need to be substantiated for their relevance and physiological significance in other settings. Additionally, thought needs to be given to the interaction of multiple genes in order to understand what is occurring within cells and ultimately being expressed in terms of cancer development and prevention. Ulrich and collaborators have highlighted this point for polymorphisms in folate-metabolizing enzymes that may be linked to cancer risk [24]. These investigators encourage the use of a pathway-based approach to data analysis to help differentiate the independent and combined effects of dietary intakes and genetic variability in folate metabolism. The use of haplotypes, which are a set of closely linked genetic markers present on one chromosome which tend to be inherited together, will likely offer a helpful solution for screening large populations. The importance of using haplotypes versus SNPs to examine the vitamin D receptor gene (*VDR*) gene has been reported [25]. In this example,

two subjects (A and B) have identical genotypes at three polymorphisms in the *VDR* (the *Cdx2*, the *FokI*, and the *Bsm–Apa–Taq* 3'UTR polymorphisms) but only differ in their particular combinations of alleles on chromosomes (i.e., their haplotypes). The result for subject B is that less «high-activity» VDR proteins (i.e., having the «F» allele) are expressed, which is expected to lower responses to vitamin D. Interestingly, if only one of the polymorphisms was tested, this difference would not have been perceived. Furthermore, if only the three individual polymorphisms were analyzed and the haplotypes not taken into account, these effects would also not have been recognized. Thus, not controlling for the underlying complexities in *VDR* polymorphisms, i.e., by not analyzing multiple polymorphisms and analyzing their haplotypes, can also help to explain contradictory results between studies.

Genome-wide association studies (GWAS) compare the complete DNA of people with a disease or condition to the DNA of people without the disease or condition and have recently been employed to identify genomic markers associated with different disease phenotypes. Unfortunately, replication in different populations to validate results has been a challenge and loci identified through such studies have been found to explain only a small portion of variability for a trait. Investigators are just beginning to model environmental factors, including diet, in GWAS [26]. These studies should help to provide an understanding of the complex interplay of genetic and environmental factors affecting human quantitative traits. Inclusion of environmental factors represents a much needed next step in the quest to model the complete environmental and genetic architecture of complex traits. An interesting recent example for a GWAS, which has relevance for nutritional status, concerns associations between genetic variation and plasma one-carbon metabolites [27]. In this study, genome-wide significant associations for plasma homocysteine, plasma vitamin B_{12} and plasma pyridoxal-phosphate (PLP) were determined. For plasma vitamin B_{12}, the investigators replicated an association for *FUT2* and identified genome-wide significant SNPs in biological candidate genes: *TCN1*, a vitamin B_{12} binding protein; *MUT*, which converts methylmalonyl CoA to succinyl CoA; and *CUBN*, the receptor for intrinsic factor-vitamin B_{12} complexes. For plasma homocysteine, they observed genome-wide significant associations with the 5,10-methylene tetrahydrofolate reductase (*MTHFR*) functional SNP Ala222Val and a possible new independent locus 102 kb upstream of *MTHFR*. For plasma pyridoxal 5'-phosphate (PLP, the active form of vitamin B6), the investigators noted genome-wide significant associations in *alkaline phosphatase*. These data reveal new biological candidates and confirm prior candidate genes for plasma homocysteine, plasma vitamin B_{12} and plasma PLP.

Copy number variation- a term used to describe gains and losses of segments of DNA- is another possible genetic difference influencing variation in biological responses and risk of disease. An example describing a relationship between diet and copy number variation is for the amylase gene. Investigators compared the copy number for the salivary amylase gene (*AMY1*) in individuals from populations consuming high-starch diets to those with traditionally low-starch diets [28]. They found higher *AMY1* copy numbers and protein levels in populations that traditionally consume high starch diets. This example of positive selection on a copy number–variable gene is likely the first discovered in the human genome. Research approaches and tools are now available to enable characterization of the full extent of copy number variation in the human genome and their contribution to human variation and disease.

Nutritional epigenetics. The impact of epigenetic mechanisms in the etiology of cancer and other chronic diseases has been increasingly recognized in recent years. Epigenetics refers to the study of mitotically and/or meiotically heritable changes in gene function that are not attributable to a change in the DNA sequence. Evidence suggests that diet and other environmental factors may be significant regulators of epigenetic events. Bioactive food components have been shown to exert cancer protective effects through modulation of epigenetic mechanisms, such as DNA methylation of CpG islands in promoters and other regions of the genome, chromatin silencing complexes, post-translational modifications of histone tail domains, and regulation of non-coding RNAs. Moreover, dietary alteration of epigenetic events has been associated with modulation of several cellular processes associated with carcinogenesis, including differentiation, inflammation, apoptosis, cell cycle control/proliferation, carcinogen metabolism, and angiogenesis, among others.

A classic example for the influence of diet in DNA methylation and cancer is the finding that dietary methyl (folate, choline and methionine) deficiency has been shown to alter hepatic DNA methylation patterns and induce hepatocarcinogenesis in the absence of a carcinogen in rodents [29].

Researchers recently evaluated whether diet and multivitamin use influenced the prevalence of gene promoter methylation in cells exfoliated from the aerodigestive tract of current and former smokers [30]. In this study, members (n = 1,101) of the Lovelace Smokers Cohort completed the Harvard Food Frequency Questionnaire and provided a sputum sample that was assessed for promoter methylation of eight genes commonly silenced in lung cancer and associated with risk for this disease. Methylation status was categorized as low (fewer than two genes methylated) or high (two or more genes methylated). Significant protection against methylation was observed for consumers of leafy green vegetables and folate as well as with current users of multivitamins. These findings support the concept that novel interventions to prevent cancer could be explored based on the ability of diet and dietary supplements to affect reprogramming of the epigenome.

Epigenomic approaches are likely to assist in characterizing genome-wide epigenetic marks that are targets for dietary regulation. The ability to characterize cell and context specific epigenomes (be it profiles of DNA methylation or histone modifications) will greatly impact the ability to determine, on a global level, how diet

impacts differential epigenetic effects on normal *versus* cancer cells. This information will also provide the tools to illuminate epigenetic changes resulting from dietary exposures during critical periods of prenatal and postnatal development, adolescence, and senescence, as well as examine the potential impact of diet on transgenerational transmission of epigenetic changes. In addition, relationships between genetics and epigenetics may provide further insights about transcriptional regulation during carcinogenesis and how dietary factors participate in these interactions. Moreover, the identification and characterization of novel epigenetic marks and mechanisms with the capacity to regulate gene expression are likely to surface over the next few years.

Nutritional transcriptomics. Modulation of genomic and epigenomic processes do not fully account for the influence that dietary factors can have on phenotype since changes in the rate of transcription of genes (transcriptomics) may also be important. Transcriptomics allows for genome-wide monitoring of the simultaneous expression of tens of thousands of genes as well as a comparison of relative expression between these genes. Messenger RNA microarray technologies provide an important tool to discover gene expression changes that are linked to cellular processes, however such responses are very likely to be cell type specific and may vary between healthy and neoplastic conditions, as well as during cancer progression. Several bioactive food components have been reported to significantly influence gene transcription and translation in a concentration and time dependent manner. These changes may be key links in the ability of food components to influence one or more biological processes including cellular energetics, cell growth, apoptosis, and differentiation, all of which are important in regulating cancer risk and consequences.

Adaptation to excess exposure to foods and their components occurs through shifts in absorption, metabolism or excretion. Thus, the quantity and duration of exposure must be considered when evaluating the response of gene expression patterns. Over-interpretation of the physiological significance of a gene expression pattern is possible because microarray technologies provide only a single snapshot in time. While mRNA microarray technology continues to be a powerful tool for examining potential sites of action of food components, their usefulness for population studies remains uncertain.

One study suggests the feasibility of using gene expression changes in human prostate epithelium as a measure of response to a dietary intervention [31]. A low-fat/low-glycemic load diet, and its related weight loss, was associated with multiple gene expression changes in human prostate epithelium following a six-week diet. Gene expression changes were discovered in cell migration and tissue remodeling, including matrix metalloproteinase-7 (MMP7) (also called matrilysin), CXCR4, CXCL2, lumican, and SPARC-like 1 (Secreted Protein, Acidic and Rich in Cysteines-like 1). Other genes that were modified in expression involved in intracellular signal transduction, such as the immediate early response genes 2 and 3, the dual specificity phosphatase 1, and the v-ets oncogene homologue. Expression of insulin-like growth factor-II receptor transcripts increased, perhaps due to a positive feedback of the low-glycemic load diet. Genes that were down-regulated include prostate-specific membrane antigen and peroxiredoxin 1, which may play an antioxidant protective role in cells. These results provide important information for future studies that aim to examine the impact of diet, obesity in prostate carcinogenesis and/or progression.

As an example of a biomarker investigation, researchers found that a small group of women at increased risk for breast cancer who followed an extreme 900 calorie per day diet had reduced gene expression of stearoyl-CoA, a gene involved in cancer growth, compared to a control group on a 2000 calorie per day diet [32]. They also found changes in blood biomarkers (using a metabolomic profiling approach) for breast cancer risk (e.g., insulin, leptin). The researchers concluded that the reduced expression of genes in lipid metabolism and glycolytic pathways were detectable in breast tissue following dietary energy restriction and that these may represent promising molecular targets for dietary energy restriction mimetics or during dietary intervention studies. Thus, transcriptomics approaches can assist in identifying and characterizing molecular biomarkers. Future studies of this nature may provide insight into the molecular mechanisms underlying the associations of diet and obesity with the development or progression of certain cancers. Much of the current evidence, however, suggests that mRNA abundance is not always proportional to protein activity and thus cannot substitute for functional analyses of candidate genes. While the transcriptional profile can be useful in predicting metabolic stress, simpler indicators may suffice. It is possible that more select gene expression microarrays may be useful if targeted to some cellular process. At this point, however, it seems prudent to evaluate the costs and benefits of transcriptomics technologies before including this research approach into certain study designs, such as large population studies.

SUMMARY

Although our understanding of the relationship between diet and cancer has advanced in recent years, much remains to be revealed with respect to diet and dietary components in cancer risk and prevention. Evidence from clinical trial outcomes, epidemiological observations, preclincial models and cell culture systems have all provided clues about the biology of cancer prevention. Emerging areas such as the interaction between diet and the microbiome as well as how bioactive food components modulate inflammatory processes require greater research attention. Issues remain about the quantity of dietary components and the optimal dietary pattern needed to bring about a biological effect, as well as the timing of exposure and other variables (chemical form, duration of exposure) that can influence the response. Interactions between the different components within a food may explain why isolated components do not always result in the same biological outcomes as does the intact food.

Likewise, interactions among foods and their constituents may contribute to the overall relationship between eating behaviors and cancer. Importantly, for the future of nutrigenomics, the «omics» (e.g., transcriptomics, proteomics, metabolomics) approach may provide useful biomarkers of cancer prevention, early disease, or nutritional status, as well as identify potential molecular targets in cancer processes that are modulated by dietary constituents and/or dietary patterns.

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