Diet, Nutrition, and Cancer Epigenetics

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Abstract

The search for a connection between diet and human cancer has a long history in cancer research, as has interest in the mechanisms by which dietary factors might increase or decrease cancer risk. The realization that altering diet can alter the epigenetic state of genes and that these epigenetic alterations might increase or decrease cancer risk is a more modern notion, driven largely by studies in animal models. The connections between diet and epigenetic alterations, on the one hand, and between epigenetic alterations and cancer, on the other, are supported by both observational studies in humans as well as animal models. However, the conclusion that diet is linked directly to epigenetic alterations and that these epigenetic alterations directly increase or decrease the risk of human cancer is much less certain. We suggest that true and measurable effects of diet or dietary supplements on epigenotype and cancer risk are most likely to be observed in longitudinal studies and at the extremes of the intersection of dietary risk factors and human population variability. Careful analysis of such outlier populations is most likely to shed light on the molecular mechanisms by which suspected environmental risk factors drive the process of carcinogenesis.

Contents

INTRODUCTION

Genes play a large role in determining risk for most common diseases (e.g., 31, 44, 70); environmental factors, including diet, also play an important role (e.g., 61, 68, 113). The connection between diet and human disease has a long history in epidemiology (57, 88, 94, 102), but the mechanisms by which dietary factors might increase or decrease disease risk are far from certain. The realization that individual dietary components can alter the epigenetic state of genes and that these epigenetic alterations and their concomitant changes in gene expression might be the molecular pathway by which diet alters disease risk is a more modern notion, driven largely by studies in animal models (1, 83, 93, 109, 115). Most studies attempting to link dietary components to epigenetic alterations have targeted the one-carbon metabolic pathway, directly or indirectly, because of its central role in providing methyl donors for both DNA and histone methylation reactions. Components of one-carbon metabolism, including folic acid, betaine, and choline, can alter the methylation levels of individual genes, and these alterations are associated with changes in gene expression and overt phenotype (1, 83, 93, 109, 115). In fact, many dietary components have the potential to influence the biochemistry of methylation; so far, however, most of the measurable effects have been observed in animal models operating at the extremes of exposure regimes that are of questionable significance to human health. For example, the dietary exposures demonstrated to have the largest measurable effects on the epigenome have been associated most often with effects on offspring after exposure in utero (1, 83, 93, 110, 115). Although some human in utero exposures, such as episodic famine (45, 98) or seasonal food shortages (111), have approximated extreme exposure regimes, they are generally rare and difficult to reproduce. Other, less extreme dietary exposures, including high-fat Western diets and calorie excess, have also been associated with epigenetic modifications (1, 39, 83, 93, 109), and these exposure regimes are much more likely to be prevalent in human populations and to be relevant to human health. In this regard, body fatness, abdominal fatness, and adult weight gain are three of only a small number of dietary exposures for which the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR) have found convincing epidemiologic evidence for an association with multiple cancers (116) and for which an additional significant risk factor is age. Also abundant is similar evidence that links calorie excess and other age-related diseases, including cardiovascular disease (9, 25) and type 2 diabetes (2). If epigenetics provides a mechanistic intersection between

an individual's genes and the environment, then identifying ways to control the molecular traffic through that intersection raises the possibility that individuals might take specific direct action to affect this process and alter their risk of disease (61, 113).

At this juncture, nutritional epigenetics is a very young science in which there are many questions and conflicting observations. Many recent reviews have addressed epigenetics, diet, nutrition, and other lifestyle factors (5, 13, 14, 55, 61, 113), so we do not attempt to be comprehensive in citing the literature. Instead, we concentrate our efforts on the subjects we feel are most likely to yield information that will prove useful in the clinical arena as well as in public health. In this respect, cancer biology is likely to be at the forefront of understanding the connections between nutrition, diet, and epigenetic pathways, just as it has been at the forefront in using genetic information to tailor cancer treatments and improve outcomes (20, 69).

HOW DIET AND NUTRITION HAVE BEEN LINKED TO CANCER

In this review, we discuss briefly the epidemiologic data on diet and cancer, the link between diet and epigenetic modifications, and why DNA methylation is likely to be the best measure of epigenetic change in molecular epidemiologic studies. We also highlight the likely importance of and interaction among dietary factors, particular metabolic pathways and chronic inflammation. Finally, we argue that mechanistic insight into how diet and nutrition affect cancer risk is most likely to come from the study of individuals at the extremes of dietary exposures and at the extremes of DNA methylation alterations.

Migration Studies on Human Populations

Epidemiologic studies have long suggested links between diet, nutrition, and many forms of cancer. Perhaps the most provocative early data that suggested a true link between diet and an individual's risk of cancer were from studies in the 1970s and 1980s, which compared cancer incidence in immigrants to the United States and their native-born offspring with the incidence in their country of origin (72, 96). These studies showed multiple differences in site-specific cancer incidence. Upon moving to the United States, Japanese immigrants showed a substantial increase in colorectal cancer and less dramatic increases in breast and ovarian cancers (96), mirroring the site-specific incidence of the European-derived resident population of the United States. The authors hypothesized that these changes could be attributed to the consumption of "fats and other dietary components" (96). Moreover, there appeared to be a migratory exchange of cancers of the type more common in the country of origin for cancers of the type more common in the adopted country. The Japanese, for example, witnessed a steep decline in the incidence of stomach, liver, and esophageal cancers upon moving to the United States, concurrent with the observed increases in breast, ovarian, and colorectal cancer (96). The decline in stomach cancer, in particular, was also hypothesized to be due to changes in diet (96). In an unfortunate twist on the migration of Japanese people to places where Western diets are consumed, Western diets have increasingly migrated to Japan, with a corresponding increase in the incidence of colon cancer (94).

Prospective and Case-Control Studies

As a result of such migration studies, many subsequent studies have searched for an association between healthy or prudent diets (characterized, generally, as rich in fruits and vegetables and whole grains low in intake of fats and red and processed meats) and lower cancer risk. Epidemiologic data from individual studies in which diets were categorized by food frequency questionnaires

generally found that Western diets were associated with higher risk of colon and breast cancer (48, 57, 75, 89, 102) in comparison with prudent or whole-foods diets, but the effects were often small and difficult to reproduce. For these reasons, meta-analyses and systematic literature reviews give a more robust picture of the effects of individual dietary components on cancer risk.

Perhaps the largest systematic review of the literature on associations among diet, nutrition, and cancer risk has been done by the WCRF and the AICR (117). An enormous amount of literature (more than 7,000 publications) on the effects of food, nutrition, and physical activity on the risks of 16 different cancers has been systematically reviewed and the evidence summarized and distilled into a very impressive graphic (116) that depicts whether there is probable or convincing evidence for an association, positive or negative, between particular factors and each type of cancer.

Given the thoroughness of the systematic literature reviews presented at the WCRF website and the organization's Continuous Update Project, which tracks all relevant randomized controlled trials and cohort studies, we refer the reader to this resource for detailed reports and methods used to review the literature. As might be expected from the organization's focus on diet, dietary supplements, physical activity, and cancer prevention, the cancer with the largest number of identified risk factors is colorectal cancer; red meat, processed meat, and alcoholic drinks have been associated with increased risk, and dietary fiber, garlic, and milk/calcium supplements have been associated with decreased risk (116). Physical activity is also judged to decrease the risk of colorectal cancer and, conversely, two measures of excess adiposity are judged to increase risk (116).

As an indirect integration of all components of an individual's diet and level of physical activity, the metric associated with the largest number of cancers is "body fatness," which increases the risk for cancers of the esophagus, pancreas, gallbladder, colon and rectum, breast (postmenopausal), endometrium, and kidney (116). Conversely, body fatness is associated with a decreased risk of premenopausal breast cancer (116).

Consistent with the early suggestion of decreased cancer risk for prudent diets (27, 114), the dietary component associated with a decreased risk for the largest number of cancers is the consumption of fruits, which decreases the risk for cancers of the mouth, pharynx and larynx, esophagus, lung, and stomach (116). Consumption of nonstarchy vegetables also decreases risk for all these cancers, except cancer of the lung (116). Fermentation of these apparently cancer-preventing fruits and vegetables into alcoholic beverages (and their consumption) is the single dietary factor associated with an increased risk for the largest number of cancers, including mouth, pharynx and larynx, esophagus, liver, colon, and breast (116).

The overall goal of systematically analyzing all these cohort and prospective studies on diet, lifestyle, physical activity, and cancer is to influence human choice and behavior such that cancers are prevented rather than treated. The International Agency for Research on Cancer (33) has attempted to quantify further the old adage that "an ounce of prevention is worth a pound of cure" by estimating what percentage of different cancers might be prevented by appropriate food, nutrition, physical activity, and body fatness in the United States, the United Kingdom, Brazil, and China (33). There are, of course, many caveats and assumptions inherent in such calculations, but one cannot help but be encouraged by the conclusions that two-thirds to three-quarters of some cancers (mouth, pharynx, larynx, esophagus) and as much as one-quarter of all cancers might be prevented by prudent human behavior and proper diet and lifestyle (33). Such reductions in the incidence of cancer would be comparable to, or surpass, the overall effects of the very best treatments available.

If these estimates of preventable cancers are even moderately accurate, the argument that environmental factors, many of which are found in our food and/or influence our weight (61), can modulate our genetic risk of cancer becomes highly tenable. The mechanisms by which they do so, then, become of interest for both theoretical and practical reasons. However, we must offer

a word of caution about these sunny interpretations of the epidemiologic studies: Randomized clinical trials testing the efficacy of dietary supplements that might have been reasonably expected to decrease the incidence of cancer, given the reproducibility of the observational studies on epidemiologic associations, have failed to show the expected reduction in cancer incidence or, disturbingly, have suggested an increase in the incidence of disease (19, 34, 36, 37, 63, 64, 92, 122). The reasons for these contradictory results are unclear. Possible confounders include the likelihood that an excess of high-risk patients were enrolled in the trials or that the length of the intervention was insufficient. In any case, the notion that one might exert some influence on one's risk of cancer by lifestyle modification is a powerful motivation for many individuals as well as for health care organizations and the agricultural and pharmaceutical industries.

ASSOCIATIONS WITH EPIGENETICS

Because the goal of this review is to examine the evidence for causal links between diet and epigenetic alterations and epigenetic alterations and cancer, we must note at the outset that the link between epigenetic alterations and cancer is the more convincing of the two. Studies have described many epigenetic alterations between human cancer cells and their normal counterparts. That many of these alterations are directly related to the cancer phenotype is demonstrated most convincingly by nuclear transfer studies in the mouse (12, 46) in which melanoma cells (46) can be reprogrammed into embryonic stem cells that can further differentiate into most, if not all, cell types in chimeric mice. These data suggest strongly that many of the cancer-associated changes to the epigenome can, when reversed, result in a noncancer cell. Thus, diet-associated alterations to the epigenome have become the object of an intense search.

Practical Epigenetics

With respect to how one might measure the potential effects of diet on the epigenome and the effect of the epigenome on cancer risk, three classes of epigenetic molecules might be able to make these distinctions: DNA methylation, modifications of histones and other chromosomal proteins, and noncoding RNAs, including miRNAs (microRNAs) and long-noncoding RNAs. However, and with special relevance to environmental effects on the epigenome, all three measures are not likely to be equally capable of distinguishing the epigenetic differences between individuals that may be of clinical interest with regard to diet/nutrition and other environmental exposures. The scope of the problem lies in the observed level of interindividual variation, the expected effect size of the disease-associated variable or exposure, and the precision and throughput with which the epigenetic measurements can be made. These considerations make DNA methylation the most likely candidate to be a biomarker of environmental exposures. DNA is a highly stable molecule; levels of interindividual variation in global or site-specific methylation do vary but are constrained (i.e., methylation at any one site can vary, as a fraction of molecules measured, between 0 and 1); high-precision, highly reproducible techniques are available with the capacity for high throughput (10, 29, 50, 54, 58, 112, 123); and these techniques can distinguish differences in population means of the expected small magnitude in samples of moderate size. Thus, interindividual variation is low enough and the precision of the DNA methylation measurement is high enough that DNA methylation can likely be used to distinguish the effects of diet/nutrition on epigenotype, even if those effects are expected to be small in magnitude. With current technologies, the same cannot be said for histone modifications or even for gene expression arrays interrogating long noncoding RNAs or miRNAs. This truth is evident from the number of individuals/samples found in public databases (more than 8,000 individuals using the Illumina 450K array) using DNA methylation

arrays (65) versus the number of individuals profiled by ChIP Seq (e.g., 285 samples are available for H3K27me3 on the National Center for Biotechnology Information epigenomics website, **http://www.ncbi.nlm.nih.gov/epigenomics/**).

Epigenetic Alterations and Cancer

Since Feinberg & Vogelstein's (32) original observation of gene-specific hypomethylation in primary colon tumors, compared with normal tissue, hundreds of reports have detailed DNA methylation alterations in almost every human tumor. In fact, the degree to which methylation alterations take place distinguishes a class of tumors [CpG island methylator phenotype (CIMP+) (100)] with distinct molecular properties and clinical outcomes that exist in a wide variety of cancers. In fact, many of the alterations found in CIMP+ tumors are common to different cancers, suggesting some mechanistic commonality to the process (82). The possibility that alterations in site-specific or global methylation affect tumor phenotype and patient outcomes is so compelling that genome-wide methylation profiling has been performed on hundreds of tumors in The Cancer Genome Atlas (TCGA; **http://cancergenome.nih.gov/**).

The Effects of Nutrition on Epigenetic Regulation

Although nutrition and dietary factors have been associated with cancer risk, the conjecture that epigenetics, writ broadly, serves as the mechanistic link between the two is far from certain. Animal studies have demonstrated strong associations between multiple dietary factors and significant alterations to the epigenome (e.g., 23, 39, 41, 49, 84, 107, 109), many of them effects of maternal nutrition on methylation state in the offspring (1, 16, 24, 66, 83, 93, 115); human studies, however, have yielded inconsistent results (6, 7, 18, 23, 45, 53, 71, 73, 78, 85, 91, 99, 111, 120, 121). For the purposes of this discussion, we assume that nutrition/dietary components are likely to have an effect on an individual's risk of cancer and that the mechanism by which cancer risk is affected is likely to be through epigenetic modification of an individual's genome. The precise molecular mechanisms by which this is achieved are incompletely understood, but reasonable assumptions, rooted in decades of classical physiology and biochemistry, point to dietary effects on the one-carbon metabolic pathway as one potential link (**Figure 1**). Dietary folate, B vitamins, choline, betaine, and other reactants may influence the methyl donor pool and, ultimately, levels of DNA and histone methylation. The hypothesis that nutrition and diet also have other, indirect, effects that also influence the establishment or maintenance of epigenetic modifications, via inflammatory pathways or other stress responses (reviewed in 101, 103) for example, is of great interest and importance. In this regard, both calorie restriction (22) and calorie excess have effects on DNA methylation, and both are thought to have opposite effects on the rate of biological aging. As mentioned above, calorie excess [using high body mass index (BMI) as a proxy for calorie excess] is a risk factor for several types of cancer, and multiple DNA methylation alterations are associated with BMI, per se (26, 47).

An additional all-important but unstated assumption implicit in the hypothesis that nutrition and dietary components influence cancer risk by altering the epigenome (and all hypotheses involving a role for the environment in shaping the epigenome) is that an individual's environmental exposure history may be recorded as epigenetic alterations in the cellular genome of normal tissues. Unless such changes are very transient (and thus do not qualify as epigenetic alterations in the original sense of the term), the existence of such a "molecular fossil record" (104) of individual environmental exposures has the potential to be both diagnostic and prognostic in any disease in which gene–environment interactions are thought to be significant (**Figure 2**), including many

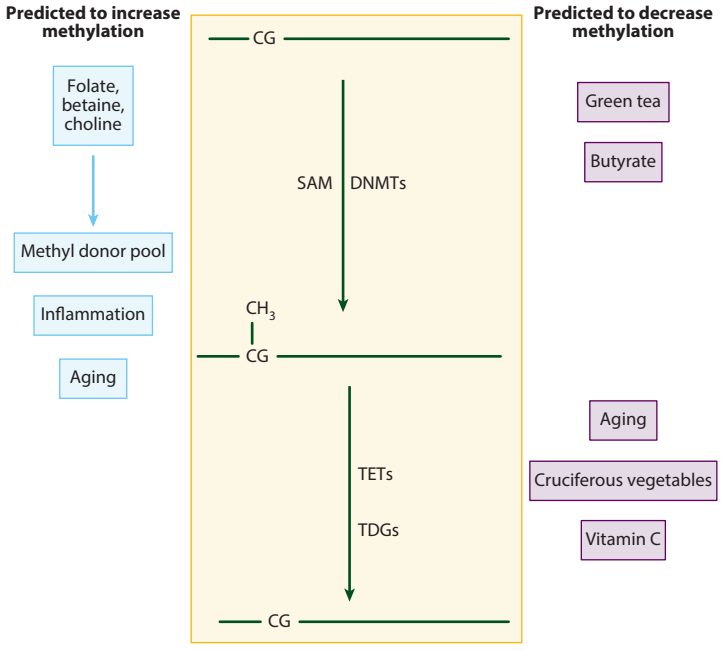

Figure 1

The center portion of the diagram (*top to bottom*) depicts the mechanism of cytosine methylation from an unmethylated CpG dinucleotide by DNA methyl transferases (DNMTs), using S-adenosyl methionine (SAM) as a methyl donor. 5-Methyl cytosine may be demethylated through the action of dioxygenases (TETs) and thymine deglycosylases (TDGs). Demethylation may also occur by DNA replication in the absence of maintenance DNMTs. Depicted are factors that have been implicated in global or site-specific increases (*left side*) or decreases (*right side*) in DNA methylation (see text).

cancers (e.g., 15, 87). The differential accumulation of epigenetic load by different individuals is expected to mirror the risk for disease. If suitable diagnostic/prognostic biomarkers can be developed, those at highest risk may be identified for targeted intervention to reduce their risk.

MECHANISMS OF INTERACTION

Epigenetic modifications have been shown to be altered by manipulations that might be expected to affect the methyl donor pool directly, as well as physiological stressors that operate indirectly.

Direct Mechanisms: Metabolic Pathways

The earliest demonstrations that dietary supplementation with one-carbon pathway reactants could influence phenotype came from mouse models in which coat color of offspring could be altered by maternal diet supplementation with betaine, choline, and folic acid (115). This variation in phenotype was subsequently shown to be correlated with DNA methylation levels at the A^{vy} promoter (110). Many additional studies in animal models have shown correlations between maternal diet and DNA methylation levels or histone modifications in offspring (1, 24, 66, 93). Correlations between epigenetic modifications and individual diet (as opposed to maternal diet during gestation) have also been provided by animal models (23, 39, 41, 49, 62, 84, 107, 109), in some cases leading to mechanistic interpretations amenable to dietary intervention (28).

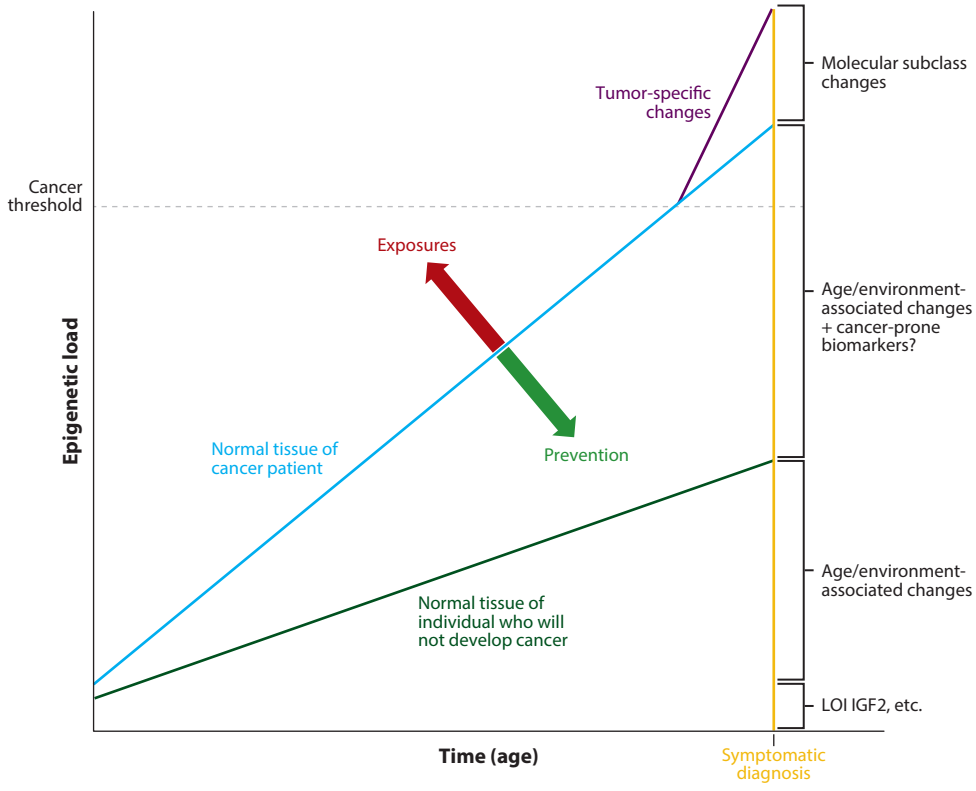

Figure 2

The molecular fossil record hypothesis for age- and environment-related epigenetic modifications and their relationship to cancer. Each individual is assumed to have been born with some level of epigenetic (and genetic) risk for cancer [e.g., loss of imprinting (LOI) at *IGF2*]. As individuals age, age-related (e.g., methylation loss through stem cell divisions, spontaneous deamination) and environmental exposure-related (e.g., folate supplementation, inflammation; see **Figure 1**) alterations occur, increasing epigenetic load. Individuals who accumulate epigenetic load at a slower rate are unlikely to develop cancer, whereas others accumulate changes at a rate sufficient to cross the threshold required for tumor promotion. Dietary factors may increase (exposures) or decrease (prevention) the rate of accumulation of epigenetic load.

Given the initiation of a US nationwide program for the fortification of foods with folate, beginning in 1996 (106), the focus of many human studies has been on determining whether folic acid (the synthetic form of folate) levels are correlated with DNA methylation. Results have been equivocal. When global DNA methylation has been analyzed for a correlation with folate levels in peripheral blood, there has been little to no support for a correlation (95, 121). However, Ulrich et al. (105) found that when the population was stratified into upper and lower halves, *LINE1* methylation was higher in the high-folate group. Women with supraphysiological serum concentrations of folate (>200.6 ng/ml) were also more likely to have highly methylated (highest tertile) *LINE1* elements in peripheral blood mononuclear cells than were women with lower circulating folate levels (79). Even assessing whether folate fortification of the food supply affected global DNA methylation levels has proven confusing. Women in the highest red blood cell (RBC)–folate tertile had higher DNA methylation levels than did women in the lowest tertile in the prefortification period, but lower global methylation levels in the postfortification period (7). Measurements of gene-specific methylation levels for a correlation with serum folate measurements have also

yielded conflicting results. Wallace et al. (108) found that folate levels in RBCs correlated with gene-specific methylation levels in the colon (*ESR1* and *SFRP1*) but not global methylation in the colon as measured by *LINE1* levels (35).

There are also numerous observations that link epigenetic changes to many kinds of human cancer. Given this seemingly straightforward path between one-carbon pathway supplements and epigenetic changes and epigenetic changes and cancer, one would suppose that evidence linking one-carbon pathway supplements to an increased or decreased risk of cancer would be clear and strong. Unfortunately, this is not the case. Of the 16 cancers for which the WCRF/AICR has monitored the impact of foods containing folate (see **Figure 1**), a "probable" decreased risk designation has been assigned to only one (pancreatic cancer) (116).

Although components of one-carbon metabolism are the most intensively studied dietary intervention with an effect on epigenetics, many others have been described that could contribute to epigenetic variation. In the DNA methylation pathway, the most straightforward is vitamin C, which is a cofactor for the TET family of enzymes that mediate the formation of hydroxymethylation and eventual DNA demethylation. The link between vitamin C and DNA methylation can be readily seen at high doses (11, 119), but it remains to be established if it is relevant at more physiologic levels. Another well-documented pathway affected by nutrition is histone acetylation; indeed, chemical/nutritional effects on histone acetylation may be important to physiologic regulation in selected instances. For example, the formation of queen bees depends on their prolonged exposure to royal jelly, a large component of which is a histone deacetylase inhibitor (90). Butyrate is both an energy source and a histone deacetylase inhibitor, thus serving as a signaling metabolite in mammalian cells (74). Royal jelly and butyrate are both used as dietary supplements in humans, and their long-term use may have epigenetic effects as a consequence. Indeed, cruciferous vegetables and green tea extracts also contain chemicals with histone deacetylase inhibitory activity (81). Finally, heavy metals such as arsenic and cadmium also affect epigenetic regulation, possibly through histone methylation (17). For all these examples, however, we still lack clear evidence for measurable effects of exposures on human epigenetic variation.

Indirect Mechanisms: The Role of Inflammation

Cancer largely affects the elderly, and the idea that epigenetic changes accumulating during an individual's lifespan may play a role in the development of cancer has been put forward on multiple occasions (3, 38, 51). There are likely several mechanisms or physiological states that indirectly affect the rate at which DNA methylation changes occur as we age. In addition to recent studies that have demonstrated a relatively strong link between increasing BMI and increased DNA methylation age (47), research has shown that the single dominant factor modulating age-related methylation is chronic inflammation. In the colon (52), esophagus (30), stomach (67), and liver (86), chronic inflammation is associated with substantially increased methylation in apparently normal tissues. In a gerbil model of *Helicobacter pylori* stomach infection, methylation increases after infection-acquired chronic inflammation (77), and even though bacterial eradication reduced methylation, levels did not return to baseline. In a mouse model of inflammatory bowel disease, inflammation was associated with a marked increase in the methylation of genes targeted by polycomb in embryonic stem cells (42). A more recent study using the azoxymethane/dextran sulfate sodium (AOM/DSS)-induced colitis mouse model suggests that DNA methylation changes occur early and do not require an overtly active inflammatory process (56). DNA methylation differences, including those in inflammation-related genes, were observed in both SCID (severe combined immunodeficiency) mice (which lack functional T and B cells) and their wild-type counterparts within 8 days of DSS treatment, which supports the notion that the DNA methylation changes take place

early in the process. Thus, a model emerges whereby methylation drift accumulates with age, and the rate of drift is accelerated by chronic inflammation (and possibly other exposures) (51).

Given the strong link between inflammation and epigenetic changes, it becomes possible if not outright plausible that nutrition also affects epigenetics indirectly by triggering or alleviating chronic inflammation. Numerous epidemiologic studies have documented a link between Western diets and biomarkers of inflammation. This link could occur directly through metabolites in the diet (43, 97) or indirectly through modulation of the microbiome (8). Direct evidence for a methylation/aging/nutrition axis remains scarce but is nevertheless interesting to consider. As mentioned earlier, a recent study showed that RBC folate levels, a measure of chronic dietary exposure, did not correlate with repeat element methylation (a surrogate for global methylation) but did positively correlate with age-related methylation (35). The top and bottom quartiles in RBC folate had the same difference in age-related methylation as shown by 10 years of age. Folate ingestion was also shown to induce an inflammatory-like gene expression profile in the colon (80), and it is therefore possible that the observed association with RBC folate is related in part to inflammation. Patients with inflammatory bowel disease (IBD), ulcerative colitis (UC), or Crohn's disease (who are at dramatically increased risk of colorectal cancer) exhibit multiple DNA methylation differences in their normal colon mucosa compared with individuals who do not have chronic inflammation (21, 52, 76). A potential link also exists between diet, inflammation, epigenetic alterations, and cancer because UC patients are often folate deficient at diagnosis (4, 118) and most such patients are treated by folate supplementation (40, 59, 60).

Given the sometimes strong/sometimes tenuous associations between inflammation and cancer, epigenetics and cancer, diet and cancer, and diet and epigenetics, few reviewers could resist the temptation to fashion a global hypothesis in which all the associations are converted to causes and effects. In this worldview, there are two likely alternatives: Either dietary factors result in cancer-causing epigenetic changes indirectly, through the inflammatory response pathway, or dietary factors directly cause epigenetic changes, leading to cancer. The diet–DNA methylation link and a potential link between environmental chemical exposures and altered epigenetics in humans will be resolved only by extremely precise studies of carefully selected loci in large populations.

SUMMARY AND ASSESSMENT

Given the large body of epidemiologic data associating diet with cancer, the many animal studies that have demonstrated direct links between specific dietary components and epigenetic changes, and the large number of epigenetic changes associated with cancer, one cannot help but feel somewhat disappointed that randomized clinical trials and large observational studies in humans have failed to show clear and consistent effects of diet or dietary supplements on epigenetic parameters or cancer incidence in all but a few cases. In some respects, such results are not surprising given the heterogeneity of the human population for all the variables that might influence epigenetic variation and cancer incidence. In addition, the complexity of the biochemical pathways leading to epigenetic modifications (**Figure 1**) suggests a robust homeostatic response to disruption by manipulating the supply of individual components. We suggest that true and measurable effects of diet or dietary supplements on epigenotype and cancer risk are most likely to be observed at the extremes of the intersection of dietary risk factors and human population variability: in individuals who are malnourished (i.e., as a result of famine, alcoholism, or drug addiction), who suffer from chronic inflammation (i.e., IBD, dialysis), or who experience chronic exposures to candidate epigenotype disruptors (i.e., folate oversupplementation or so-called obesogens). In this regard, the recent identification of epigenetic changes associated with increasing BMI (26, 47) is heartening for the possibility that relevant extremes of exposure may be more common than assumed.

These extremes of exposure may result in a high frequency of individuals who have dramatically altered epigenomes/outliers, which in turn would provide a population in which careful study of the altered genes and pathways offers insight into the mechanisms by which diet is linked to cancer. The reciprocal approach may also have value. Identification of cancer patients who have outlier levels of epigenetic alterations at multiple genes (i.e., the outliers of the outliers) may distinguish patients in whom particular exposures may be common, similar to the way that CIMP+ tumors are associated with particular anatomical sites or particular outcomes. Given that outlier individuals are uncommon, by definition, sufficient numbers of such individuals may require nonrandom recruitment of special populations or careful analysis of carefully selected subpopulations from much larger studies. Although there are dangers in generalizing results from selected populations to the population at large, the history of cancer genetics research is rife with examples of findings from rare patients being generalized to the larger population. There is no reason to believe that the same will not hold true in the history of cancer epigenetics research.

SUMMARY POINTS

1. The WCRF and the AICR have found convincing evidence that particular dietary components and cumulative dietary effects, such as obesity, are associated with several cancers.
2. The connection between specific dietary factors and epigenetic alterations is clear in some animal models, but data in human populations are inconsistent.
3. Dietary factors are likely to interact, either directly or indirectly, with the epigenome to accelerate or decelerate age-related epigenetic changes in cancer-associated genes.
4. Randomized clinical trials have generally failed to show clear and consistent effects of diet or supplements on cancer risk because of high phenotypic variability in response.

FUTURE ISSUES

1. Measurable effects of diet or dietary supplements are most likely to be observed at the extremes of dietary risk factors and population variability.
2. Careful attention should be given to inclusion of outlier phenotypes in diet/nutrition-associated cancers.

DISCLOSURE STATEMENT

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