A Review of the Literature: Nutriepigenetics and Potential Role of Folate on Cancer Risk and Prevention

INTRODUCTION

With the continuing advancements in our understanding of the human genome, new techniques and methods are being employed to alter genetic expression. The study of the modification of gene expression without changing the gene is known as epigenetics[10]. Epigenetic changes include DNA methylation and histone modification, both of which regulate chromatin remodeling to ensure gene expression regulation and stable gene silencing[7]. Since epigenetic alterations occur during early stages of carcinogenesis and have been identified as possible initiators of cancer development, epigenetic alterations have become a great interest in both the realms of nutritional sciences and cancer, as nutrition plays a role in the ability to modify gene expression as well as protect the genome from damage[3,8].

Cell cycle regulation, DNA damage repair potential, induction of apoptosis, inflammatory response signaling, and cell growth differentiation and control are some of the many important deregulated cellular functions and pathways during carcinogenesis. Epigenetic alterations, including promoter hypermethylation and global hypomethylation can lead to tumor suppressor gene silencing and chromosomal instability, respectively, contributing to carcinogenesis[3]. It is hypothesized that either histone modifications affect chromosome function through the alteration of histone electrostatic charge, resulting in altered histone structure or DNA binding; or that modifications act as binding sites for protein recognition motifs[1,10]. Several dietary components including folate, polyphenols, selenium, retinoids, isothiocyanates, and fatty acids have shown anti-carcinogenic epigenetic potential by disrupting many of the deregulated processes such as tumor suppressor gene promoter hypermethylation, histone onco-modifications, as well as global DNA hypomethylation[9].

Statement of the Controversy

Despite the potential for nutriepigenetics to be used as targets in cancer prevention and management, the full capabilities of this type of treatment still remains relatively undiscovered. Currently, much of the research is derived from *in vitro* and in *vivo studies*, with *in vivo* animal studies being favored due to the ability to control and select for minimal genetic variations and modulate dietary intake, whereas human cancer cases are difficult to control for diet and a patient's specific genotype that might be contributing to cancer risk[3,8]. This challenge highlights "the need for highly controlled genotypes and environmental conditions that allow for identifying different regulatory patterns based on diet and genotype" according to the Journal of Environmental Health Perspectives[8]. Utilizing nutriepigenetics as a viable treatment for cancer risk and prevention remains contested, as outcome is largely based on a case-by-case basis, and is highly dependent on the specific gene, target organ, cell type, as well as dosing and timing of specific nutrients[6,9].

Bioactive food components, nonessential molecules present in foods that exhibit the capacity to moderate metabolic processes, have been shown to modulate DNA and histone methylation, as well as cancer susceptibility[14]. Dietary components such as folate are involved in one-carbon metabolism and are vital in DNA methylation due to their influence on the resupply of methyl groups in methylation metabolic pathways[6,14]. Folate, in the form of 5'-methylTHF, is involved in the remethylation of homocysteine to methionine, a precursor to the primary methyl group donor for most methylation reactions, S-adenosylmehtionine(SAM)[6,14].

The purpose of this thesis paper is to examine the current state of nutriepigenetics in cancer risk reduction and prevention. The paper will review the role the bioactive food component folate

has in DNA methylation and histone modification, and will explore the strengths as well as limitations of the current research.

LITERATURE REVIEW

DNA methylation is a key epigenetic component that modifies DNA directly and regulates gene integrity and expression through the methylation of CpG dinucleotide residue cytosine bases at the 5' carbon end[5,7]. Within certain genes, CpG dinucleotides are also sites for mutation in human cancers, accounting for up to 30% of all germline point mutations[6]. With age comes the occurrence of genomic DNA hypomethylation, gene and tissue specific promoter DNA hypermethylation, as well as declined folate status, and leaves telomere sequences prone to DNA strand breaks, potentially shortening telomere length. This shortening is associated with reduced life span, and is a proposed mechanism of age-related diseases such as cancer[5]. This link between age and cancer is purported due to hypomethylation and hypermethylation occurring in both states. Decreased DNA methylation is believed to promote chromosomal instability, loss of imprinting in transcription, as well as up-regulation of silent genes which is thought to lead to carcinogenesis and tumor development.

Even *in utero*, DNA methylation patterns established by maternal diet play a role in protection of pediatric disease development and later life health, as shown by maternal mice methyl group diet supplementation permanently altering offspring phenotype through elevated methylation at the promoter CpG site[6]. Folate aids in the regeneration of DNA methylation methyl groups through one carbon metabolism, with early exposure being hypothesized to prevent tumors through its provision of methyl groups in sustaining normal methylation patterns and DNA repair[2]. Low folate status however decreases levels of the methyl donor S-adenosylmethionine (SAM), while also increasing levels of methyl-transferase inhibitor S-adenosylhomocysteine (SAH)[2,5,6,9].

An *in vitro* study of untransformed mammalian cell lines, a mouse fibroblast and hamster ovary, as well as human colon adenocarcinoma cell lines (HCT116/Caco-2) explored the effect of folate on SAM and SAH concentrations. Folate alone was adequate to reduce SAM pools, leading to an increase in SAH levels, which is believed to lead to reduced methyltransferase activity. It was found however that SAM: SAH ratios were actually significantly higher in the folate depleted mouse fibroblast and HCT human cell lines[16]. Furthermore, only the untransformed cell lines showed significant change in genomic DNA methylation in folate deficient cells, with a 28% reduction in DNMT activity in the fibroblast cells but no significant change in the ovary cell line. These results support that folate deficiency results are dependent on cell type, and that genomic DNA methylation may be independent of SAM:SAH ratios.

Epidemiologic studies have shown an inverse relation between folate status and the risk of cancers such as lung, esophageal, stomach, colorectal, pancreatic, breast, as well as neuroblastomas and leukemia[6]. Of these cancers, colorectal cancer management has shown promise, as results from the epidemiologic studies report a 20-40% risk reduction in participants with high dietary intake of folate compared to those with low folate status. A number of small intervention studies have illustrated the improvement and reversal of surrogate endpoint biomarkers of colorectal cancer with folate supplementation[6]. Data from animal studies also support a negative correlation between folate status and colorectal cancer risk, as folate supplementation was linked with restored methylation status in colon cancer cells[6,9]. Despite this correlation, moderate and sustained folate deficiency alone did not induce significant DNA hypomethylation in DMH, a carcinogen to promote rat colon carcinogenesis. It was however observed that a significant hypomethylation of p53 tumor suppressor gene occurred at some exons in the DMH rat colon, suggesting that effect of folate status on DNA methylation patterns may be

site and gene-specific[5,6]. Inconsistent changes in p53 methylation in rat colon due to folate status also suggests that methylation changes in the are dependent on total methyl donor supply rather than folate alone[6].

Further research on the effects of folate status on DNA methylation in *in vitro* systems support the assertion that DNA methylation changes are site and gene specific, as well as the direction of these changes being potentially dependent on cell and target organ specific. Human clinical trials have shown reduced DNA methylation in leukocytes in response to folate depletion that was restored after subsequent folate supplementation, while several other studies have observed no significant correlation between lymphocyte DNA methylation and folate and homocysteine concentrations. At least two studies identified a positive correlation between colonic DNA methylation and folate concentrations as well as a negative correlation with homocysteine levels in individuals with and without colon adenomas and adneocarcinomas[6]. Further human intervention studies focusing on folate supplementation remains divided, as sustained supplementation of folate 12.5-25 times the daily requirement significantly increased colonic DNA methylation in individuals with resected colorectal adenoma, while showing no significant effect on patients with chronic ulcerative colitis who received the same dosing[6]. These human studies further support the hypothesis of the correlation between folate status and genomic DNA methylation being dependent on site and tissue, as well as the level of folate supplementation and depletion. It still remains inconclusive on the extent of folate deficiency and the statistical significance in DNA methylation reduction, both on a genomic and site/gene specific level[2,6].

Fragile histidine triad (FHIT), is a tumor suppressor gene that is commonly silenced in cervical cancer. Promoter hypermethylation has been observed in this cancer, leading to FHIT silencing. In order to evaluate the influence of folate in FHIT expression, a cross-sectional study

was conducted on 250 women, including those with normal cervix cells, neoplasm grade 1/2, as well as squamous cell carcinoma[15]. Two different cell lines were treated with increasing levels of folate, ranging from 0 to 1000 micrograms. It was found that folate status was significantly reduced the more severe the cervical cancer was. Furthermore, FHIT promoter methylation was significantly higher in all cervical cancer groups. It was shown that apoptosis rate and proliferation inhibition rate was positively correlated with folate concentration, while no significant differences were seen between the two cell lines. Western blotting also showed that promoter methylation weakened with higher folate concentrations. Protein expression of FHIT was significantly higher between 0 and 100 micrograms of folate supplementation, while no significant change was seen between 100 micrograms and higher folate concentrations. This cross-sectional study supports folate's role in modulating cervical cancer risk, as folate was found to be a significant factor in FHIT gene hypermethylation[15].

Prostate cells have also been shown to be sensitive to folate, as seen through an *in vivo* study on 100 microgram folate depletion in 3 mouse prostate cancer cell lines, benign, tumorigenic, and metastatic. An analysis of SAM and SAH pools showed a significant decrease in SAM:SAH ratios in the benign and tumorigenic but not the metastatic cell line. The benign and tumorigenic cell lines also showed reduced intensity of the CpG island, suggesting promoter hypermethylation in 100 micrograms of folate deficiency. These epigenetic changes were correlated with increased anchorage dependent growth in prostate cells[13].

A cohort study of 1101 members of the Lovelace Smokers cohort conducted a food questionnaire and sputum sample, identifying 8 genes commonly methylated and subsequently silenced in lung cancer risk and progression. It was found that individuals with high folate and

multi-vitamin (with folate present) intake showed significant reduction in promoter methylation status, suggesting folate acts as a significant protector against gene methylation in lung cancer[17].

DNA damage, while a relatively common occurrence in cells, still holds the potential to lead to genetic mutations and cancer. Histone proteins are crucial components of eukaryotic chromatin, acting as spools around which DNA winds, with post translational modifications on their tails theorized to be vital in the DNA repair and damage response[1]. The four core histones, H2A, H2B, H3, and H4 form the nucleosome core, and feature DNA-binding motifs that recognize specific sequences of DNA[1,11]. DNA interaction with histone is mediated by the charges of the DNA backbone and charged arginine and lysine residues of histones, with modifications to the histone altering the histone residue charges, thus controlling chromatin compaction[1,10]. Modifications also serve as high affinity binding sites for protein specific binding domains, and it is these modifications to the histone that are involved in tumorigenesis and cancer progression. In most lung tumors, the histone demethylase KDM2A is overexpressed, and contributes to tumor formation and metastasis[11]. Histone methylation is a common modification linked to the DNA damage response, and it is histone methyltransferases and demethylases, in conjunction with their targeted methylation that is responsible for this response[1]. Histone methylation occurs at specific sites, including H3K9 methylation's role in genome stability, H3K36 methylation in recruitment of DNA repair factors through the facilitation of conjoining DNA ends, H3K79 methylation recruitment of and localization 53BP1 to DNA break sites, and H3K27/H3K4 methylation which block DNA break site transcription and initiate DNA repair[1,2,10]. Demethylation of modified histones is largely carried out by lysine demethylase (LSD1), which removes methyl groups from methylated lysines at position 4 of histone 3, regulating gene expression. LSD1 also acts as a folate-binding protein, as folate, in the form of THF binds to LSD1 to protect it from damage[11].

Preliminary studies revealed mice fed folate deficient diets and resulting low folate status in the liver was associated with an increase in the methylation of lysine 4, a possible result of decreased levels of LSD1. Also, western blotting showed a significant change in H3K4me2, a substrate for LSD1, with reduced folate intake, further supporting the positive correlation between folate status and LSD1 activity[11]. Due to the regulatory gene expression role of LSD1, and its utilization of THF, a folate co-enzyme, to protect enzymes from damage, folate deficiency is thought to have an inhibitory effect on LSD1[11]. This correlation could have far-reaching epigenetic effects, as LSD1 has been found to demethylate non-histone proteins as well. This can most notably be seen in a substrate of LSD1, DNMT1, the enzyme responsible for maintaining DNA methylation during replication. LSD1 knockout in mice has been shown to lead to decreased levels of DNMT1, resulting in global DNA methylation loss[12]. If folate deficiency is linked to decreased LSD1 activity, which is associated with DNA methylation activity, disruption of histone modifications can be further implicated in carcinogenesis through their ability to induce irregular gene expression and impair DNA damage repair[9,12].

In contrast to genetic changes in cancer, the effects of epigenetic changes are potentially reversible, allowing for the utilization of dietary compounds in cancer risk reduction and prevention. Given the fact that epigenetics plays a vital role in gene regulation and expression, folate has been a focus of this research due to its potential ability of modifying DNA and histone methylation, two important factors in cancer development. The combined evidence from animal, in vitro, as well as human studies suggests that folate's effect on these epigenetic changes is largely dependent on cell type, cancer severity, target organ, and are gene and site specific[3,5,6,15,16,17]. While animal and *in vitro* studies have provided significant information about mechanisms of epigenetic regulation, it is still unclear about how applicable the research from those studies to human health

are, or if some discrepancies exist between *in vitro* and human studies with regards to human health and biology, as methylation status due to folate in rodent livers remains inconclusive[6]. The few human intervention and *in vivo* studies available have shown consistent significant effects of folate status on DNA methylation patterns in cervical, lung, and prostate cancer. While this effect still remains dependent on the cell type and specific target gene, this highlights the viability of folate in epigenetic changes and cancer risk reduction, as well as the importance for further study into folate's significant cancer targets.

As the epigenome and its specific pathways remain largely unknown, challenges remain for future nutriepigenetic research. Timing and dosing of dietary intervention is critical in the efficacy of its epigenetic regulation. Intervention *in utero* as well as in early life show the most promise in cancer risk reduction, but remains largely untested throughout all ages. Although folate intake has been positively correlated with genomic DNA methylation and negatively correlated with promoter methylation, the length of time needed for sustained epigenetic changes remains unknown, as many effects on DNA and histone methylation have shown transient results[1,6,12]. Furthermore, it was shown that folate alone was insignificant in modulating DNA methylation in certain cell lines and cancer types, suggesting DNA methylation may circumvent SAM: SAH pathways, and greater efficacy may be found when combining different bioactive food compounds in human intervention. Further understanding of the role nutrients play on epigenetic changes can be used to further develop epigenetic drugs that mimic these effects. A focus on the complete mapping of the epigenome can also aid in the development of biomarkers for cancer monitoring and risk reduction, as identified histone modifications and DNA methylation changes have already begun to be utilized as markers for cancer monitoring[10]. While the role of folate in DNA methylation and histone modification remains controversial, it illustrates the potential ability to

reverse epigenetic changes, and through further research of the true impact of nutrition on epigenetics, may serve as an important tool in predicting individual cancer risk, as well as providing natural therapies for combatting cancer.

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