Advances in Nutritional Epigenetics—A Fresh Perspective for an Old Idea. Lessons Learned, Limitations, and Future Directions

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ABSTRACT: Nutritional epigenetics is a rapidly expanding field of research, and the natural modulation of the genome is a non-invasive, sustainable, and personalized alternative to gene-editing for chronic disease management. Genetic differences and epigenetic inflexibility resulting in abnormal gene expression, differential or aberrant methylation patterns account for the vast majority of diseases. The expanding understanding of biological evolution and the environmental influence on epigenetics and natural selection requires relearning of once thought to be well-understood concepts. This research explores the potential for natural modulation by the less understood epigenetic modifications such as ubiquitination, nitrosylation, glycosylation, phosphorylation, and serotonylation concluding that the under-appreciated acetylation and mitochondrial dependant downstream epigenetic post-translational modifications may be the pinnacle of the epigenomic hierarchy, essential for optimal health, including sustainable cellular energy production. With an emphasis on lessons learned, this conceptional exploration provides a fresh perspective on methylation, demonstrating how increases in environmental methane drive an evolutionary down regulation of endogenous methyl groups synthesis and demonstrates how epigenetic mechanisms are cell-specific, making supplementation with methyl cofactors throughout differentiation unpredictable. Interference with the epigenomic hierarchy may result in epigenetic inflexibility, symptom relief and disease concomitantly and may be responsible for the increased incidence of neurological disease such as autism spectrum disorder.

KEYWORDS: 5-methyltetrahydrofolate, acetylation, autism spectrum disorder, carbon, DNA, environment, epigenetics, evolution, folic acid, future directions, gene expression, glycosylation, histone, limitations, methane, methylation, natural selection, neural tube defects, nitrosylation, nutrition, nutritional epigenetics, one carbon etabolism, phosphorylation, pollution, single nucleotide variant, ubiquitination, methylenetetrahydrofolate reductase, MTHFR

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Introduction

Our dietary choices, lifestyles, and environment broadly impact the epigenome, its actions and build the foundations for optimal health and longevity. The study of environmental and nutritional impacts upon the epigenome, throughout replication, development, evolution and life stages can be defined as nutritional epigenetics.[1]

It has been well documented that dietary and lifestyle choices within populations influence human gene expression and the consumption of vitamins and minerals through supplementation and fortification have demonstrated modulation of gene expression through the interaction at the level of the epigenome.[2]

Over the recent decade, B vitamins have been a strong nutritional epigenetic research focus for the manipulation of methylation and the prevention of neural tube defects.[3] Today; gene-editing techniques lead the research focus showing great promise for chronic disease management.[4] However, this technique brings forth several ethical issues and possesses unknown evolutionary consequences.[5] Therefore, natural modulation of the epigenome may be a preferable, non-invasive, sustainable, personalized alternative for chronic disease management.

This research provides a fresh perspective on the function of methylation, explores the potential for the less understood epigenetic modifications, and evaluates nutritional epigenetics for the management of chronic diseases.

Epigenetics

The perception of epigenetics and its meaning has changed significantly over the past half-century.[6] In 1942, Embryologist Conrad Waddington proposed the name epigenotype to describe a complex of developmental processes that occur between genotype and phenotype.[7] In the early 1960s, Doskočil & Šorm identified the distribution of 5-methylcytosines of deoxyribonucleic acids (DNA)[8] and researchers of the Rockefeller Institute began studying the structure, function and modulatory effects of histones on the regulation of ribonucleic acid (RNA) synthesis.[9] Thirteen years later, Holliday and Pugh described DNA modification by methylation in development,[10] and following this, epigenetic research exploded.

In October 1990, the Human Genome Project commenced and was completed by April 2003. The $2.7 billion project gave researchers the ability to begin piecing together nature's genetic blueprint.[11]

The modern understanding of epigenetic mechanisms is the regulation of gene expression, which results from modifications in chromatin structure, histone tails, and nucleotides without an alteration in the DNA sequence; meaning an inheritable change in phenotype without a change in genotype.[6] Until recently, the exploration of epigenetic mechanisms had its limitations due to the inaccessibility and affordability of genomic sequencing technologies.[11] Recent advances in technology have reduced the cost of human genome sequencing by 99.99%,[11] making genomic research accessible and allowing the exponential expansion of research in the industry, including nutritional epigenetics.

Genetic differences and epigenetic inflexibility resulting in abnormal gene expression, differential or aberrant methylation patterns account for the vast majority of diseases including cancer,[12] autoimmune disease,[13] obesity,[14] metabolic diseases,[14] and complex multi-system conditions such as fibromyalgia[15] and chronic fatigue syndrome.[16]

The Epigenome

Shortly after discovering the cell specificity of histones in 1950;[17] Allfrey and colleagues discovered structural modifications of acetylation or methylation to histones, regulated RNA synthesis.[9,18] Within the genome, a histone octamer is at the core of the chromatin's nucleosome and the nucleosome is complete when coiled in DNA.[19] The histone tails that protrude from the nucleosome are the primary targets for structural changes to the epigenome.[19] The addition of a methyl or acetyl group at a specific amino acid residue upon the histone results in a conformational modification to the structure of the histone chromatin complex.[19] More recently, it has been demonstrated that histone tail residues can accept a variety of additional marks, including; phosphorylation, ubiquitination, sumoylation, citrullination, and glycosylation.[19] Histone tail modifications are identified by the histone number, the amino acid residue and the type of modification. For example; Histone 3/ Lysine 4/ dimethylation (H3K4me2).[19]

Nutritional Epigenetics

Human biochemistry is constantly evolving, with pathways frequently adapting to a changing environment.[20] Overtime; adaptations to changed environments result in subtle shifts in allele frequencies providing the foundations for evolution.[21,22]

Changes to epigenetic mechanisms as a result of environmental and dietary choices contribute to human physiology and biochemistry by altering gene expression.[23]

In medicine, manipulation of the epigenome is attractive as it can be rapid, reversible, specific, and capable of modulation beyond the blood-brain barrier (BBB).[24]

Vitamin, mineral, and phytochemical constituents derived from culinary foods have shown experimentally to have epigenomic modulatory capabilities with profound disease treatment potential.[25] The enzymatic activity for a variety of primary epigenetic histone modifiers are dependent on nutritionally derived cofactors such as nicotinamide adenonucleotide (NAD),[26] Zn2+,[26] ascorbate,[27,28] Fe2+,[29] endogenous tricarboxylic acid metabolites[30] and oxygen,[31] enabling specificity to nutritional modulation of the epigenome. Culinary, botanical herb and spice phytochemical constituents have demonstrated modulation of the epigenome in the laboratory also demonstrating disease treatment potential. However, transportation of a substance to a specific tissue is challenging.[32] In contrast; environmental contaminants have also demonstrated interaction at the epigenomic level influencing evolution and contributing to disease.[33] Collectively, research pertaining to alterations in gene expression and the resulting alterations to human biochemistry following short or long term exposure to exogenous dietary or environmental substance and the influence upon the structure and function of the epigenome can be defined as nutritional and environmental epigenetics.[1]

Rapid advances in technology have enabled a greater understanding of continual biological evolution and the bio-uniqueness of the human genome. Consequently, this requires unlearning and relearning of metabolic processes to keep up with the evolving biology, for the majority of metabolic pathways including research pertaining to nutrigenomics was originally undertaken without thorough consideration of the workings of the epigenome.

Old ideas and one carbon metabolism

Methylation has been a focus for nutritional epigenetic researchers since the mid-nineties when a group of leading researchers discovered associations between folate deficiency, 5,10 methylenetetrahydrofolate deficiency, methylenetetrahydrofolate reductase (MTHFR) inefficiency, neural tube defects, and cardiovascular disease.[34-41] Today, a PubMed search of the MTHFR enzyme at the National Library of Medicine generates over 7000 entries and has been associated with hundreds of conditions.[42] Single nucleotide variations (SNV) of MTHFR, the primary regulator of one-carbon metabolism and methyl group synthesis, was said to be the leading cause of active folate deficiencies leading to neural tube defects.[35]

B vitamins, including folate (B9) molecules derived from the diet, are considered cofactors for one-carbon metabolism, function as carriers of one-carbon methyl units[2] and are well known for the epigenetic modulation of methylation.[43] Analysis of metabolites and cofactors of one-carbon metabolism in erythrocytes, serum, and plasma, provide insight into vitamin deficiency, one-carbon metabolism activity and methylation.[2]

Fortification of grains, including wheat flour and corn maze for the prevention of folate deficiency, had been discussed since 1974,[44] but was not implemented in the United States until 1997 following repeated associations with folic acid depletion and the incidence of neural tube defects.[44]

DNA methylation

Methylation is a primary epigenetic mark essential for the regulation of gene expression and is generally associated with gene

repression.[45] Methyl groups for endogenous methylation of histones, nucleotides, and proteins are said to be derived from one-carbon metabolism.[2] DNA methylation is a widely studied but not very understood epigenetic modification. DNA methyltransferase enzymes (DNMT) initiate DNA methylation through the binding of a methyl group to position 5′ of cytosine bases neighboring guanosine (CpG), generating 5-methyl-cytosine (5-mC). 5-mC is then oxidized to 5-hydroxymethylcytosine (5-hmC), which is said to promote de-methylation.[45] The Ten-Eleven Translocation di-oxygenase (TET) family of enzymes are shown to contribute to the removal of the methyl group from cytosine, forming 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC).[45]

CpG's are found in clusters which are referred to as islands, spanning approximately 1000 bp at the promoter region of a gene and are generally found unmethylated.[46] Methylated CpG's at the promoter region of a gene are associated with gene repression.[45] Differential methylation regions (DMR) are regions upstream of gene promoters that also influence a gene's expression.[47]

5′ methylcytosines contribute to the rate of evolution as they are shown to deaminate at a much greater rate than unmethylated cytosines and are considered mutational hot spots within the genome.[48,49] Thymine DNA glycosylate (TDG) is essential for base excision repair throughout the DNA methylation and remethylating process.[45]

Promoter DNA methylation of any gene which results in a loss of gene function can have profound effects on phenotype and has the potential to cause disease.[50] Inhibition and gene knock out studies which result in loss of function can often predict phenotypical outcomes of DMR or promoter region hypermethylation.[47] For example; both promoter region CpG hypermethylation and DMR of the estrogen receptor1 (ESR1) which encodes the estrogen receptor alpha protein results in reduced ESR1 expression.[47,51] DMR of ESR1 has been implicated in a range of hormone-related diseases including ovarian endometriosis and resistance to hormone therapy.[47,51]

Tryptophan hydroxylase 2 (TPH2) is essential for the first rate-limiting step in the synthesis of the 5-hydroxytryptophan (5-HTP), the immediate precursor to serotonin.[52] In animal models, reduced TPH2 activity is associated with depression-related behavior, aggression and altered sexual preference.[53,54] DNA methylation and reduced expression of TPH2 in humans has also been associated with suicide attempts in major depression.[52]

Histone methylation

The genome is comprised of hundreds of genes encoding writer histone methyltransferase enzymes capable of writing methylation to the histone.[19] Methylation of a repressive histone residue such as histone-3 lysine-9 results in a heterochromatin structural change to the epigenome and the exclusion of RNA polymerase from the genome, preventing gene expression.[19]

Methyl groups derived from one-carbon metabolism are donated by a S-adenosylmethionine (SAM) dependant histone methyltransferase to a specific histone residue.[19] Dietary consumption of high folate foods, fortification or supplementation with cofactors of one-carbon metabolism such as folate and B12 contribute directly to histone methylation.[2,55]

Histone demethylation

Histone demethylase enzymes erase the previously written methylation of the histone residue induced by the histone methyltransferase.[19]

The enzymatic demethylase reaction by Jumonji C (JmjC)-domain-containing demethylases produce succinate and carbon dioxide, and is dependent on the presence of oxygen (O2), Fe(II) and the endogenous tricarboxylic acid (TCA) metabolite alpha ketoglutarate (aKG).[56] Dietary derived ascorbate was also revealed to be an essential cofactor for JmjC-domain-containing histone demethylases[57] which is supported in stem cell culture, with vitamin C treatment reducing global H3K9me2.[58] Demethylase reactions are showing to be necessary for natural killer cell activation and expression of interferon-y (INF-y) in the anti-viral response.[59] Inhibition of demethylase results in upregulation of glycolytic genes and downregulation of proinflammatory cytokines.[59] Hypoxia and environmental substances such as nitric oxide (NO) have also demonstrated down-regulation of histone demethylase activity.[29]

Lessons Learned and Limitations

Laboratory techniques

Seventeen years following experimentation of MTHFR within cultured cells,[60,61] the same researchers were the first to develop a polymorphism detection technique using the restriction enzymes produced by Bird[62] and self-synthesized primers derived from the RNA of a 90 bp porcine liver MTHFR gene.[63] Restriction enzymes are still used today for detection of mutation and commercial microarray genotyping.[64] However, the use of restriction enzymes for SNV detection have their limitations as they are incapable of differentiating between a deletion, non-methylated base, or unrepaired deaminated nucleotide making next-generation sequencing a preferable option for accurate genotyping.[65,66]

It is now understood that cell culture storage and preparation conditions such as the use of formaldehyde interfere directly with the epigenome and contribute to DNA damage and mutation, making many early studies of enzyme activity unreliable.[67] Moreover, cell culture media is enriched with a variety of vitamins, minerals, and amino acids, including folic acid,[68] which is known to interact directly with the dynamic epigenetic activities of a cell.[2]

Epigenetic patterns and gene expression are unique to each cell.[69] However, studies of MTHFR deficiency are frequently performed on non-specific, unsynchronised cultured dermal fibroblasts,[60,70] despite Rosenblatt's early discovery which

demonstrated MTHFR activity of fibroblasts performed differently to lymphoblasts when exposed to SAM.[60]

Cell specificity and feedback inhibition of one-carbon metabolism

Stokstad and Kutzbach first described feedback inhibition of MTHFR activity in cell culture with the addition of SAM, the primary methyl donor required for methylation reactions.[71,72] This finding was later supported by Rosenblatt who found the addition of SAM resulted in the inhibition of the one-carbon metabolic enzymatic reactions in some cells but not others, also demonstrating cell-specific regulation of methyl group production.[60] Collectively; this makes diagnosis and treatment of neurological disease, based on the MTHFR enzymatic activity of the skin or oral mucosa, unreliable.

Rosenblatt also showed how the enzymatic activity was dependant on the mitotic stage of cell cycle replication, demonstrating epigenetic fluctuation of gene expression in cell culture.[73] S-adenosyl-homocysteine has shown to be a potent inhibitor of SAM, establishing multiple mechanisms that regulate one-carbon metabolism and methyl group availability.[74]

In 1977, Rosenblatt showed inhibition of MTHFR enzymatic activity in cell culture with the addition of 5-methyltetrahydrofolate (5-MTHF) to the media, again demonstrating feedback inhibition of one-carbon metabolism.[60]

Spontaneous deamination

In DNA methylation identification via sequencing; sodium bisulfite is used to induce spontaneous mutation of 5′ methylcytosine. Methylated cytosines remain as C, where as unmethylated cytosines are deaminated to uracil and then thymine.[75] Compared to normal aging cells, immortalized cultured human fibroblasts are well known to deaminate resulting in a loss of their methylation marks due to epigenetic changes induced by constituents within the media.[76] A consequence of the cyclic process of DNA methylation, demethylation and remethylation is the deamination of cytosine and the underrepresentation of CpG's during the transition and prior to base excision repair by TDG.[49] A loss of CpG's in the genome is thought to be due to unrepaired deaminated bases,[77] and therefore a deficiency in TDG results in a super-mutator phenotype and the progressive loss of CpG in the genome.[78] These unrepaired transition mutations are said to account for half of the pathogenic mutations that occur within the human body,[77] and are a significant contributor to evolution in viruses,[79] eukaryotes and prokaryotes,[80] and may also play a role in antibiotic resistance[81] increased viral pathogenicity or cross-species infection.[79]

Rosenblatt also noticed that MTHFR was thermolabile, and its enzymatic activity was strongly inhibited following a 30-minute incubation at 55°.[61] Heat and alkaline conditions have also demonstrated to increase the rate of deamination of 5 mC dramatically[82] suggesting that heat-induced deamination may have contributed to the inhibition of this enzyme, and folates in cell-culture media and DNA preparation techniques, including heat-inducing polymerase chain reaction (PCR), may influence the rate of deamination resulting in a change in gene expression and the under-representation of cytosine in sequencing. Overall this makes accurate detection of MTHFR single nucleotide variants with microarray genotyping difficult.

Evolution and the MTHFR polymorphism

Populations that have swayed radically from traditional diets do the worst when it comes to health and longevity.[83] Using a model of hypertension, Laing explains how epigenetic modification as a result of a rapidly changing diet and environment explains this phenomenon.[84] Studies of positive selection and great ape diversification found the most significant acceleration of evolution in processes essential for environmental adaptation.[85] For example, the loss of human L-gulono-γ-lactone oxidase (GLO), the gene responsible for the final step in vitamin C synthesis, is said to be the result of changing oxidative conditions and increasing dietary consumption of fruit and vegetables.[86] Similarly; the well documented LCT gene encoding the enzyme lactase-phlorizin hydrolase has shown strong positive selection and is associated with lactase persistence after weaning.[87] However, shifts in allele frequencies alone do not explain this phenomenon, and DNA methylation of the LCT gene has also shown to contribute to lactose metabolism and its phenotype, indicating the epigenetic influence on alleles and evolution.[88]

DNA methylation patterns differentiate between ethnicity in population studies where a more significant percentage of methylation is seen in African-American, and Han-Chinese Americans compared to Caucasian-Americans.[89] Researchers have confirmed methylation regulatory genes, MTHFR and betaine homocysteine s-methyltransferase (BHMT), are also subject to positive selection as a result of increased DNA methylation from environmental pressures including fortification and ultraviolet radiation exposure.[90-94] A genetic drift model of positive selection of the reduced function MTHFR single nucleotide variant, rs1801133 was demonstrated at high altitude in Tibet, which was suspected to be associated with increased exposure to ultraviolet radiation.[95]

It was previously thought that methane was not utilised by humans and was therefore passed unmetabolized through faeces or breath, however it has been recently documented that methane-rich saline, can alter immune function via changes in gene expression.[96] Therefore, increasing levels of greenhouse gases, such as methane and carbon could drive the increased incidence of MTHFR single nucleotide variations through positive selection, resulting in the downregulation of endogenous methyl group production in some populations. It is therefore also likely that cells exposed directly to methane may require less endogenous methyl group synthesis and prolonged fortification may result in greater persistence of the reduced function MTHFR alleles such as the C677T genotype. In support of this

hypothesis, environmental methyl radicals have shown to directly induce the formation of 5-methylcytosine of DNA and are therefore capable of influencing both epigenetics and evolution.[97] This is proposing that activation of one-carbon metabolism may only be required when methyl groups are reduced, making dietary and supplemental enhancement of systemic methylation unnecessary. Additionally, the consequent increase in atmospheric temperature due to methane induced climate change, may also contribute to increased rates of evolution through increasing the rate of spontaneous deamination.

Methylation and Disease

Neural tube defects

The mechanism by which folic acid supplementation prevents neural tube defects is still unclear, however, knock out and hypermethylation studies of various genes, including Paired Box 3 (PAX3) and repressive H3K27 histone methyltransferase Enhancer of Zeste Homolog (EZH2) impair neural tube closure.[98,99] Folic acid supplementation acts by reactivating these genes, which would be possible through either histone methylation of an activating residue, such as H3K4 or the inhibition of one-carbon metabolism.[100] Mandatory fortification has shown to be effective as the incidence of neural tube defects has declined by 47%.[44] However, the biological effects of fortification upon other cell types throughout embryonic differentiation have been less explored. Research suggests that prenatal folic acid may have increased the incidence of ankyloglossia,[101] which is also associated with infantile developmental delay.[102] As a result of fortification and supplementation, circulating levels of unmetabolized folates has largely increased over the past two decades.[103] Elevated maternal serum folate in the 3rd trimester has been associated with reduced foetal growth[104] and a murine model of high dietary folate supplementation exhibited impaired gestational development and protein utilization, proposing exogenous folates to be xenobiotic as opposed to nutritional.[105]

Methylation and autism spectrum disorder (ASD)

Studies demonstrating the pathogenic effects of folate supplementation during neural development and their association with neurological disorders such as ASD are growing in number.[106] Researchers have proposed that the offspring of Mothers who have supplemented with folic acid have an increased risk of ASD.[103]

Polymorphic variations in the previously mentioned methyltransferase and chromatin remodeling gene EZH2 are also associated with the incidence of ASD, suggesting environmental and epigenetic adaptation of this gene may have played a role in the etiology.[107]

Methylation and development

It is well understood that histone modifications fluctuate during development. For example; fluctuation in the degree of expression of EZH2 is required for appropriate neural stem cell differentiation.[108] High expression of EZH2 and consequent upregulation of H3K27me3 results in oligodendrocyte differentiation.[108] In contrast, reduced expression results in the reduction H3K27me3 allowing gene expression for astrocyte or neuron differentiation.[109,110] It is understood that DNA methylation of CpG of promoter regions during development remain stable.[110] However, it has been demonstrated that deposition of the demethylation metabolite 5-hydroxymethylcytosine (5 hmC) in gene bodies during neurogenesis is associated with upregulation of EZH2 expression and the persistence of the repressive H3K27me3 mark,[111] collectively representing the dynamic nature of DNA methylation and epigenetic regulation of gene expression very different to that of embryonic stem cells during differentiation.[111] This implies that the use of folic acid during pregnancy may increase the expression of EZH2 during neurulation and prevent neural tube defects, but may also increase EZH2 expression persistently during neural cell development, with unknown neurological developmental consequences.

Folate and excitotoxicity

Natural sources of folate are conjugated to a mono, di or poly L-glutamate tail.[112] Early studies of folic acid activity demonstrated folic acid administration to have excitatory activity in neurons which is likely due to L-glutamate being the major excitatory neurotransmitter in the brain.[113,114] High dose folate supplementation during pregnancy has shown to alter synaptic transmission, lowering the threshold and increasing the susceptibility for seizure in offspring.[115] Glutamic acid decarboxylase (GAD) enzyme is responsible for the conversion of excitatory neurotransmitter L-glutamate to the inhibitory neurotransmitter gamma-aminobutryric acid (GABA).[116] Due to reduced GABA synthesis, anti-GAD antibodies in the brain are associated with a range of neurological disorders,[117] including stiff person syndrome, cerebellar ataxia, Miller Fisher syndrome, eye movement disorders and epilepsy.[118] In pancreatic islet beta cells, GAD is the target for autoantibodies and autoreactive T cells in insulin and non-insulin-dependent diabetes mellitus.[116,118] In a laboratory; monoclonal antibodies against GAD are produced by injecting mice with 70 to 210 mcg dose of GAD.[119] Similarly, L-glutamate induces overexpression of GAD, potentially contributing to the production of GAD antibodies.[116] Moreover; folate polyglutamate tails have also demonstrated antagonism of glutamate receptor binding sites, potentially interfering directly with neural development or GAD antibody production.[120]

Methylation and affective disorders

In a murine model; increasing maternal or post-weaning folic acid alters gene expression resulting in anxiety-like behavior and hyperactivity.[121] Folic acid toxicity showed both down and upregulation of genes, including the upregulation of

X-inactive specific transcript (XIST).[121] Overexpression of XIST is often present in affective disorders including bipolar and psychosis.[122]

Macrocytic anemia

Since 1931, folate and B12 deficiencies have been associated with macrocytic anemia, a condition of anemia due to a defect in erythrocyte differentiation resulting in abnormally large red blood cells.[44,123] During erythropoiesis, dynamic fluctuations in the expression of epigenetic modifiers and transcription factors are essential for effective differentiation of the erythroblast into a reticulocyte.[124] The mature erythrocyte is anucleate and therefore no longer requires nuclear methylation, implying folate depletion throughout differentiation may be a natural consequence,[125] and reduced erythrocyte folate may be the outcome for the appropriate use of substrate as opposed to a deficiency or a requirement for folate repletion.

Therefore, over-supplementation of folate may lead to epigenomic inflexibility and inadequate differentiation. Moreover, a PubMed search of macrocytic anemia shows significant evidence of mitochondrial dysfunction and reduced expression of cell cycle transcription factors playing a significant role in the development of macrocytic erythropoiesis.[126-128] This suggests that folate supplementation may contribute to disease in genetically susceptible individuals through enhanced methylation and its effectiveness for those who are treated successfully may have been due to feedback inhibition of one-carbon metabolism.

Homocysteine

Early studies have demonstrated elevated plasma or urinary homocysteine to be associated with inadequate methylation and are hallmark risk factors for cardiovascular disease.[129,130] Homocysteine is said to be converted to methionine by the vitamin B12 dependant methionine synthase (MS) in a reaction that also transforms 5-methyltetrahydrofolate into tetrahydrofolate, and by the zinc dependant BHMT enzyme.[131,132] From here, the ATP dependant methionine adenosyltransferase (MAT) converts methionine to SAM for epigenetic histone and DNA methylation reactions.[133] Excess homocysteine is catalyzed to cystathionine by the enzyme cystathionine beta-synthase (CBS).[134]

Folic acid is shown to reduce plasma homocysteine levels; however, its efficacy is irregular and dependent on the cell-specific DNA methylation status of either BHMT or CBS[134] further supporting self-regulated feedback mechanisms of one-carbon metabolism and demonstrates the phenotypical effects of biological manipulation to be highly individualized.

It is understood that s-adenosylhomocysteine is a primary by-product of DNA methylation and a potent inhibitor of DNMT enzymes. However, Ponnaluri and colleagues demonstrated overexpression of s-adenosylhomocysteine hydrolase (AHCY), the key enzyme responsible for the last step in the synthesis of homocysteine to bind DNMT1 increasing its expression and participating in global DNA methylation inheritance and global hypermethylation.[135] Together, this suggests that elevated homocysteine can also increase DNA methylation as opposed to being a hallmark feature of reduced methylation as demonstrated by earlier studies.

A Fresh Perspective

Nutritional modification of the epigenome goes far beyond methylation.[26] Due to early inaccessibility of epigenetic sequencing technology, we are only beginning to understand the epigenetic mechanisms for many herbs, vitamins, and pharmaceuticals.[25,136,137]

Histone acetylation

Acetylation is a primary writer of epigenetic modification and is essential for both gene accessibility and activation.[19] In contrast to methylation, acetylation of the same histone residue can result in open euchromatin allowing genes to be accessible to RNA polymerase for transcription (Figure 1).[19]

Despite the early discovery,[18] the importance of acetylation has not been recognized until recently. Histone acetyltransferases (HAT) or histone lysine acetyltransferases (KAT) are the primary enzymes responsible for acetylation.[19] Acetyl groups are generated through oxidation of pyruvate, fatty acid beta-oxidation, and direct synthesis using acetate as a substrate.[138,139] Like one-carbon metabolism, acetyl-CoA synthesis is epigenetically self-regulated,[140] and a cellular reduction in acetyl-CoA production or a loss of mitochondrial DNA copy numbers results in genome-wide hypoacetylation.[141,142]

When a HAT binds an acetyl group to a histone residue, it neutralizes the positive charge maintained by methylation (Figure 1), weakening the chromatins tightly wound hydrogen bonds, making histone acetylation directly antagonistic to histone methylation.[143]

Evidence indicates that HATs are essential for the expression of the vast majority of genes, including the epigenetic modulating demethylases,[144] and one-carbon metabolic genes, including MTHFR,[145] making acetylation essential for all biological processes.

Histone deacetylase

Like histone demethylases reverse methylation reactions, histone deacetylases reverse the action of acetylation with the removal of the acetyl group from the histone residue.[146]

Nicotinamide adenosine dinucleotide (NAD) and Zn2+ are the primary cofactors for class 1 and 2 and sirtuin (class 3) histone deacetylase enzymes.[146]

Classically, the sirtuin reaction removes the acetyl group specifically from a lysine residue.[147] The first step is the release of nicotinamide (B3) from NAD+, followed by the release of

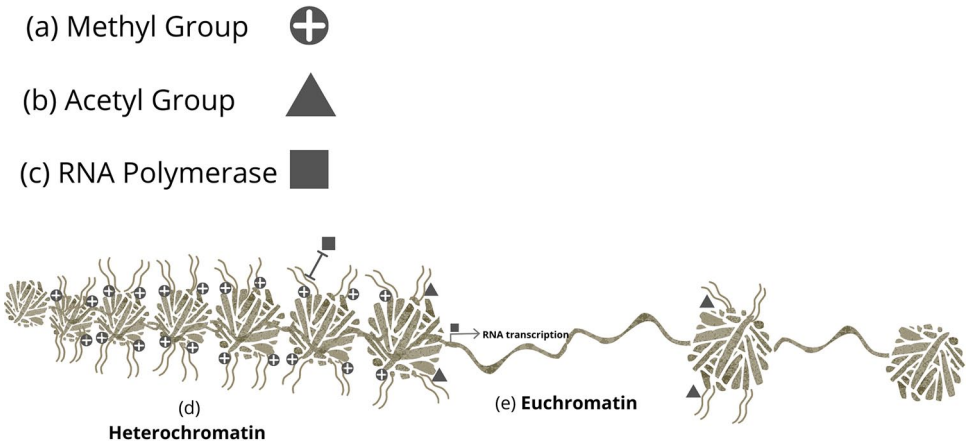

Figure 1. Heterochromatin and euchromatin. The methyl group (a) bound to the histone tail maintains a positive charge resulting in tight hydrogen bonds and compact genomic structure; heterochromatin (b) Heterochromatin excludes ribonucleic acid polymerase (RNA) (c) from binding the gene and initiating gene transcription. The acetyl group (d) neutralises the methylating positive charge maintaining heterochromatin, loosening the hydrogen bonds and enabling open structural conformation; euchromatin (e) and RNA polymerase to access the genome and initiate gene transcription.

a peptidyl ADP-ribose intermediate attached the to the acetyl group.[147]

B3 is an essential component of the NAD molecule and has demonstrated to increase NAD levels in the blood, increasing the activity of SIRT3 and improving mitochondrial function.[148] However; in cell culture, B3 has been widely studied for its non-selective inhibition of sirtuin histone deacetylase activity.[148] Again, this demonstrates time-specific substrate-level feedback regulation of epigenetic mechanisms. Moreover; fluctuation of SIRT3 acetylation is showing to regulate additional epigenetic modifiers through controlling the available source of succinate-CoA[149] and acetyl CoA[150] for histone acetylation and succinylation.

Histone deacetylase inhibition

In 1978, Davie, Candido, and Reeves revealed epigenetic inhibitory modulation of histone deacetylase with the bacterially produced fatty-acid butyrate[151] and supplemental butyrate prevented insulin resistance and obesity in mice fed a high-fat diet.[152] Histone deacetylases are pH sensitive, and a variety of acids, including endogenous lactate, have shown histone deacetylase inhibitory (HDACi) activity.[153] The valerian derived valeric acid and analog Valproic acid was developed as a solvent in 1881 and used medically as an anticonvulsant by the late sixties.[154] Its action as an HDACi was not discovered until 2001,[155] and its ability to alter chromatin structure was not identified until 2005.[137] Today; the mood-stabilizing medicine is used for the treatment of bipolar disorder, migraine, depression, epilepsy and some cancers.[154,155]

Ketones and ketogenic diets

In 2014, an endogenous ketone beta-hydroxybutyrate was found to have HDACi activity.[156] Ketogenic diets are highly beneficial for the management of epilepsy, depression, reducing mortality and improving memory.[157-159] Together this demonstrates the importance for open chromatin and active gene expression in the management of neurological disease.

Mitochondria and Epigenetics

Beyond acetyl group synthesis, a cell's energy production relies on acetyl-CoA entering the tricarboxylic acid cycle (TCA) for the production of adenosine triphosphate (ATP) and a variety of metabolites that also regulate epigenetic mechanisms, such as succinate and akG.[139] Mitochondrial oxidative phosphorylation is the primary source of cellular ATP.[160] Many epigenetic post-translational modifications depend on ATP, including chromatin remodeling complexes,[161] ubiquitination,[162] and phosphorylation.[163-166]

MAT is a well-documented ATP dependant enzyme.[164] This enzyme catalyzes the last step in the formation of SAM from methionine for both histone and DNA methylation epigenetic modifications.[164] Figure 2 depicts some of the many cross-talk combinations that exist between primary epigenetic modifiers.[165-170]

For example, unmethylated histone-3 lysine-4 (H3K4) acts as an allosteric activator of DNA methyltransferase (DNMT3a) activating DNA methylation making H3K4 an auto-regulator of de novo methylation, and similarly, DNA methylation or histone methylation of genes within the one-carbon metabolic pathway reduces methyl group transfer and ultimately DNA or histone methylation.

Energy metabolism

Energy metabolism can also be used to demonstrate epigenetic cross-talk (Figure 3). For example; respiratory quotients (VCO2/VO2) have been used to estimate the use of energy substrate during rest and exercise.[171] RQ > 0.7 is said to represent mix substrate utilization, 0.8-0.9 protein utilization, 1 glycolysis only and >1.2 anaerobic metabolism.[172] Making RQ below 1 desired for high energy output and sustainability.

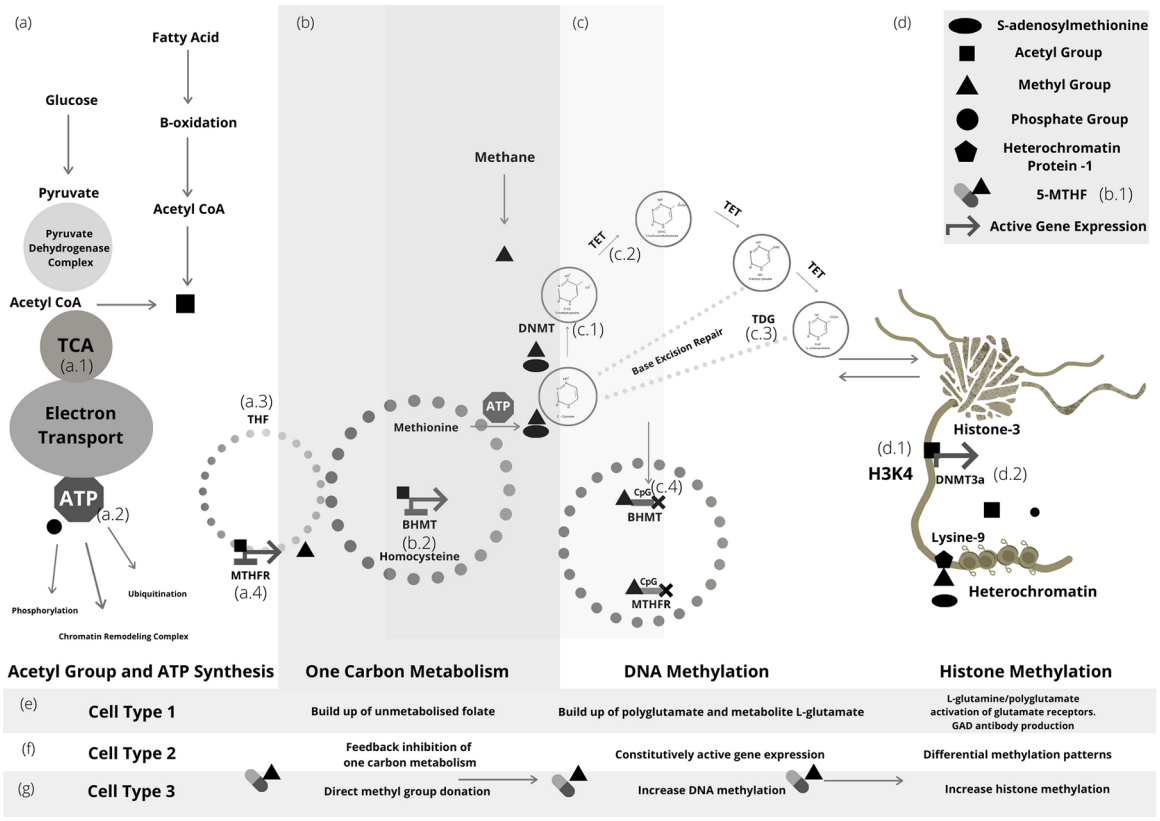

Figure 2. A cell-specific hypothesis—metabolic effects of bypassing a reduced function MTHFR polymorphism with exogenous methyl. (a) Acetyl-CoA enters the tricarboxylic acid (TCA) (a.1) cycle for adenosine triphosphate (ATP) (a.2) synthesis or is catabolized to an acetyl group for histone and protein acetylation. ATP is used for additional post translational modifications. Acetyl group binds the promoter region of the methylenetetrahydrofolate reductase (MTHFR) (a.4) and initiates transcription for enzymatic reduction of 5,10 methylenetetrahydrofolate from tetrahydrofolate (THF) (a.3). (b) During active one-carbon metabolism, MTHFR carries the one-carbon methyl unit to methionine for ATP dependant methionine adenosyltransferase (MAT) synthesis of S-adenosylmethionine for DNA and histone methylation reactions. Exogenous methane or supplemental methyl groups (5-MTHF) (b.1), supply methyl directly without the need for one-carbon metabolism. Betaine-homocysteine-S-methyltransferase (BMHT) (b.2) has a bound acetyl group and is transcribed to catalyze the conversion of betaine and homocysteine to dimethylglycine and methionine respectively. (c) Deoxyribonucleic acid (DNA) methyltransferase (DNMT) (c.1) utilises exogenous methyl groups, or one-carbon derived S-adenosylmethionine for DNA methylation. A Methyl group is bound to the promoter region cytosine-phosphate guanosine (CpG c.4) of BHMT and MTHFR to depict feedback inhibition of one-carbon metabolism. DNMT initiates DNA methylation through the binding of a methyl group to position 5′ of cytosine bases neighboring guanosine (CpG), generating 5-methyl-cytosine (5-mC). 5-mC is then oxidized to 5-hydroxymethylcytosine (5-hmC). Ten-Eleven translocation di-oxygenase (TET) (c.2) contribute to the removal of the methyl group from cytosine, forming 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC). Thymine DNA glycosylate (TDG) (c.3) initiates base excision repair of deaminated bases. (d) Unmethylated histone 3-lysine 4 (H3K4) (d.1) allosterically activates DNA methyltransferase 3a (DNMT3a) (d.2) depicting epigenetic cross-talk between DNA and histone methylation. Lysine 9 residue of histone 3 is unphosphorylated and has been methylated by a s-adenosylmethionine dependant histone methyltransferase with bound heterochromatin protein 1, resulting in heterochromatin and the exclusion of RNA polymerase for active gene expression. The metabolic effects of supplemental or environmental methyl group donation are cell-specific. Cell type 1 (e) A build-up of unmetabolized folate, resulting in excess extracellular L-glutamate or polyglutamate activation of glutamate receptors. Cell type 2 (f) Feedback inhibition of one-carbon metabolism, constitutively active gene expression and differential methylation patterns. Cell type 3 (g) Direct methyl group donation, increased DNA or histone methylation.

JmjC histone demethylase enzymes have demonstrated direct cellular O2 sensing.[173] A reduction in histone de-methylase substrates akG or O2[56] results in potent inhibition of histone demethylation and upregulation of repressive H3K9me2,[174] and activating H3K4me[174] resulting in reduced expression of DHFR[174] and upregulation of hypoxia-inducible factor a (HIF1a) respectively.[174] Upregulation of HIF1a is well known for its oxygen conservation via feedback inhibition of pyruvate dehydrogenase (PDH) activity, through upregulation of pyruvate dehydrogenase kinase (PDK) resulting in increased lactate synthesis and a reduction in the available acetyl CoA to enter the TCA and mitochondria for sustainable ATP synthesis,[175] subsequently increasing RQ to 1 or >1.2, resulting in excessive muscle lactate. Moreover; upregulation of HIF1a has been associated with the upregulation of eNOS,[176,177] resulting in increased NO and greater downregulation of demethylase activity[29] and O2 conservation.

B vitamins have long been associated with energy production; however, the mechanisms have remained undefined. The above describes how a reduction in sustainable ATP synthesis, through hypoxia, or hypermethylation at the level of acetyl CoA synthesis could result in downregulation of all primary

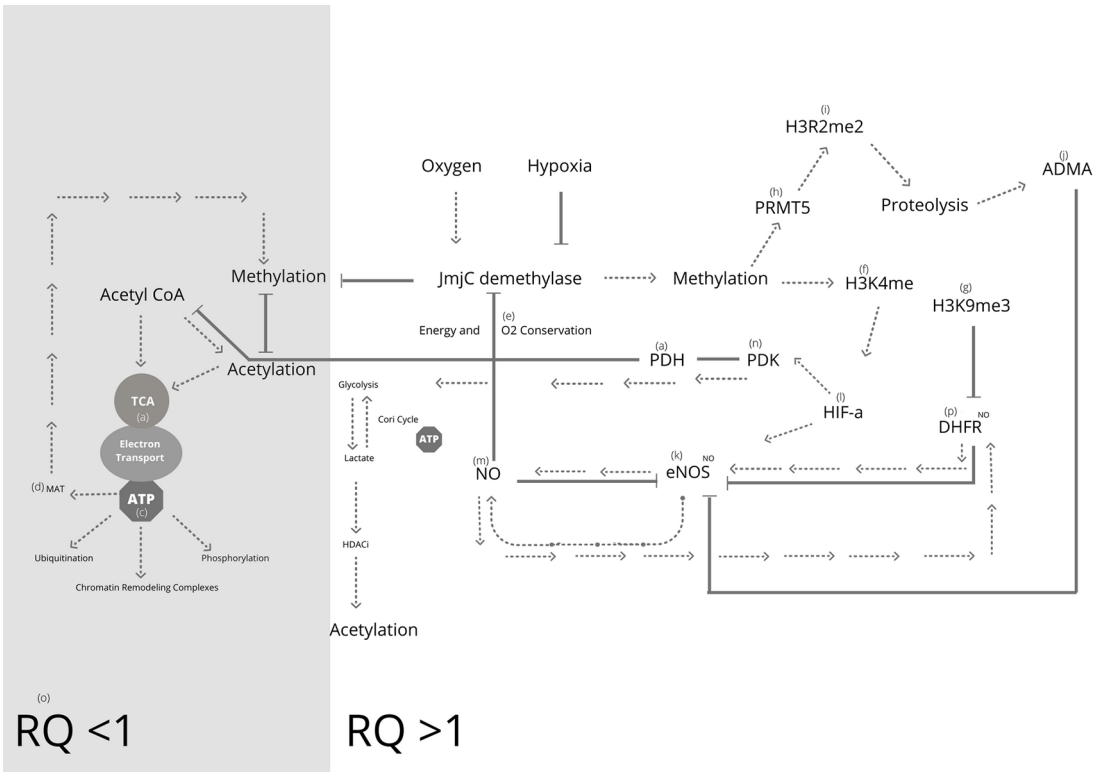

Figure 3. Epigenetic cross talk in energy conservation. Pyruvate dehydrogenase (PDH) (a) synthesized acetyl-CoA enters the tricarboxylic acid (TCA) (b) cycle for mixed energy substrate and sustainable adenosine triphosphate (ATP) (c) synthesis. ATP is used for epigenetic methionine synthase, (MAT) (d) ubiquitination, phosphorylation, and chromatin remodeling complexes. Acetyl-CoA catabolized acetyl groups participate in protein and histone acetylation. Oxygen (O_2) (e) intake increases activity jmjC demethylation, reducing methylation and enabling active gene expression and high energy output. Hypoxic reduction of O_2 dependant demethylase and consequent upregulation of methylation marks including activating histone-3-lysine-4 methylation (H3K4me) (f), repressive histone-3-lysine-9 trimethylation (H3K9me) (g) and protein arginine methyltransferase activity (PRMT) (h) and histone-3-arginine-2 dimethylation (H3R2me) (i). Upregulation of PRMT5 and consequent proteolysis of methylated arginine increases asymmetric dimethylarginine (ADMA) (j) resulting in downregulation of endothelial nitric oxide (eNOS) (k). Upregulation of H3K4me results in the induction of hypoxia-inducible factor (HIF) (l) and subsequent upregulation of eNOS, increasing nitric oxide (NO) (m). Upregulated HIF, upregulates pyruvate kinase (PDK) (n), inhibiting PDH, reducing acetyl CoA production and acetylation. Increased HIF induced NO inhibits demethylation, causing persistent methylation, conserving energy and oxygen through switching to glycolysis only metabolism and advancing to respiratory quotient (RQ) (o) = >1. Dihydrofolate reductase (DHFR) (p) promotes eNOS coupling and NO synthesis. Nitrosylation of DHFR stabilises the protein and prevents uncoupling. eNOS is self-regulated by eNOS protein nitrosylation, inhibiting its expression. Hypoxic upregulation of inhibiting H3K9me3 reduces expression of DHFR, resulting in eNOS uncoupling, reduced NO synthesis, superoxide generation, and upregulation of demethylase activity signifying precise epigenetic regulation of energy and O2 conservation in hypoxic conditions.

ATP dependant epigenetic modifiers, relying on environmental or supplemental direct methyl group donation to drive methylation and glycolysis only metabolism (RQ > 1) which is unlikely to be sustainable for exercise such as endurance. Moreover; TCA cycle or mitochondrial ATP synthesis defects have been repeatedly implicated in diseases of cancer and dysfunctional immunity.[178] As a result the excessive lactate production and consequent HDACi[153] may result in acetylation of undesired genes as seen in the metabolic switch known as the Warburg effect, which is commonly detected in cancer cells.[179]

Chronic fatigue syndrome (CFS)/Myalgic encephalomyelitis (ME)

A reduced ability to produce ATP, which is often characterized as deregulation of glycolysis at the pyruvate dehydrogenase complex is seen in patients with post-exertional malaise, or CFS.[16] McGregor and colleagues revealed changes in glycolysis and concentrations of metabolic pyruvate, acetate and lactate influencing both acetylation and deacetylation which were indicative of hypoacetylation in patients with CFS/ME.[16]

Epigenetic fluctuation

Immune cells fluctuate levels of epigenetic modifiers.[180] Specifically, T cells require coordinated suppression of methylation for memory T cell differentiation and appropriate response to antigen.[180] Similarly, the menstrual cycle[181] and circadian rhythms[182] require coordinated and a timely fluctuation of epigenetic modifications for regularity and synchronicity. Therefore, uncoordinated supplementation of methyl donors or modern epigenetic modifiers may result in dysregulated menstrual cycles, abnormal sleep patterns or immune dysfunction.

Succinylation

Succinate is another TCA cycle metabolite and mitochondrial substrate involved in epigenetic histone modification capable of altering gene expression.[183] Succinate groups are derived from TCA succinyl-CoA, in an enzymatic reaction that produces ATP or GTP.[183]

Succinate groups used for histone succinylation rely on the enzymatic activity of succinylate dehydrogenase (SDH) within the mitochondria.[184] Increased SDH enzymatic activity within the mitochondria reduces the succinate available for succinylation of histones.[184] SDH enzymatic activity is dependent on the riboflavin containing flavin adenosine deoxyribunucleaic acid (FAD), and ubiquinone (CoQ10) for redox reduction to ubiquinol and it's multiple subunits are dependent on both iron and haem.[184] Similar to acetyl group availability for acetylation; multiple upstream feedback mechanisms determine substrate availability for histone epigenetic succinylation.

Ubiquitination

Ubiquitination is an ATP dependant modification and is considered the kiss of death modification, for it is responsible for the degradation of proteins.[167] Dysregulation of ubiquitination is implicated in various diseases, including cancer and autoimmunity, and modulation of the ubiquitin system has shown promise in the management of these conditions.[167] Nutritional modulation of ubiquitin systems is promising. Ubiquitin molecules are found in dairy milk[185] and both up, and down-modulation of ubiquitination have been described.[185,186]

Nitrosylation

Tetrahydrobiopterin (BH4) content is maintained by the salvage and regeneration pathways.[186] Salvage of BH4 by dihydrofolate reductase (DHFR) promotes endothelial-derived nitric oxide synthase (eNOS) coupling and ultimately NO synthesis.[187] Like methylation; histone nitrosylation is demonstrating to be self-regulating, where the nitrosylation of eNOS inhibits its own expression,[188] and the nitrosylation of DHFR stabilises the protein and prevents it from ubiquitination and degradation increasing NO synthesis (Figure 3).[189] BH4 deficiency or inhibition of DHFR by the methylated folate analog methotrexate (MTX) has demonstrated eNOS uncoupling resulting in the production of superoxide anions.[189] Similar to the hypoxic lack of O2 substrate and downregulation of demethylation; NO has also demonstrated direct inhibition of jmjC demethylase activity.[29] Interestingly; this may explain the downregulation of DHFR during hypoxia by providing direct superoxide production which has also demonstrated epigenetic modulation[190] and indicates a highly precise epigenetic regulation of gene expression, protein availability and energy conservation in the hypoxic state (Figure 3).

Like the environmental methyl radicals, exogenous nitrogenous radicals may also influence epigenetic nitrosylation and ultimately the evolution of NOS.[191]

Animal-derived peptides such as those extracted from whey protein also play a role in epigenetic nitrosylation due to the up-regulation of nitric oxide synthase.[192]

Endothelial damage

Two primary mechanisms that have been implicated in the development of endothelial dysfunction are elevated levels of asymmetric dimethylarginine (ADMA) and a lack of dimethylarginine dimethylaminohydrolase (DDAH) enzyme activity.[193] ADMA is an endogenous eNOS inhibitor derived from the proteolysis of methylated arginine residues following arginine methylation by a group of epigenetic modifying enzymes referred to as protein-arginine methyltransferases (PRMT).[193] PRMTs are dependent on the one-carbon metabolite SAM for methyl group donation.[194] Increased ADMA has demonstrated superoxide release, uncoupling and inhibition of eNOS activity by up to 40%, and its presence predicts cardiovascular mortality, endothelial dysfunction in hypertension, hyperlipidemia, diabetes, and coronary artery disease.[193]

In contrast to MTX, in vivo vascular infusion of 5-MTHF has demonstrated improved endothelial function, eNOS coupling and decreased superoxide production.[195] However; in other cells, overexpression of eNOS has exhibited both male and female infertility.[196,197] Validating the unpredictable nature of orally administered 5-MTHF.

Glycosylation

Glycosylation is another nutritionally moderated post-translational modification; its substrate, the nutrient-sensing molecule UDP-N-acetylglucosamine is derived from the hexosamine biosynthetic pathway from extracellular glucose and has been associated with cancer, metabolic and neuronal disease.[198] Overconsumption of dietary glucose, resulting in increased production of advanced glycation end products, has demonstrated to manipulate histone glycation resulting in disease.[199]

Phosphorylation

Inorganic phosphate for histone and protein phosphorylation are stored in the form of phosphocreatine, and ATP.[163] Phosphorylation histone -3 tyrosine-41 (H3Y41) by members of the Janus Kinase (JAK) family introduces a negative charge at the histone, excluding heterochromatin protein 1a (HP1a) from binding to H3K9me3[200] resulting in euchromatin and gene accessibility. Janus Kinase-2 (JAK2) gene expression is essential for hematopoietic differentiation[201] and many biological processes, most notably within the immune system.[202] Hypermethylation or loss of function of various genes within the JAK/STAT pathway have been implicated in a range of immunological conditions including recurrent staphylococci infection and allergic disease.[203-205] For example; DNA hypermethylation of JAK2 is responsible for dampened host immune responses in patients with tuberculosis.[205]

Protein phosphorylation of the primary immune transcription factor T box protein (T-bet) is essential for T helper cell 1 (TH1) differentiation.[206] Knockdown of phosphorylation or T-bet results in impaired T helper cell 2 (TH2) suppression resulting in the allergic phenotype.[207,208] T-bet induction of interferon-gamma (IFN-y) for viral immune response is dependant on H3K9 acetylation at the IFN-y locus,[208,209] which would not be possible without JAK2 exclusion of HP1a and prevention of H3K9me3.[200] Moreover; it has been determined that the histone lysine N methyltransferase (SUV39H1) dependant H3K9me3 incorporation of HP1a and the subsequent heterochromatin transcriptional silencing maintains the stability of TH2 cells and the induction of T-bet results in a resolution of allergic inflammatory lung pathology.[209]

Myeloproliferative neoplasms

An acquired JAK2 V617f mutation is present in the majority of patients with myeloproliferative neoplasms.[209] Several phenotypical manifestations result from the same mutation, and the presence of this mutation does not always present with disease.[210] The phenotypical presentation is dependent on the JAK2 burden, which is the accumulative JAK2 RNA from JAK2 copy numbers and the ratio of JAK2 RNA between wild type and its mutants.[211] However, the neoplastic cell line UKE-1 shows the accumulation of JAK2 copy numbers in culture, suggesting epigenetic adaptation to the media.[209]

As previously described, human fibroblast cell lines frequently deaminate, losing methylation marks resulting in longevity or immortalization of cells.[76] Negative regulation of JAK2 is demonstrated by the upregulation of G9a methyltransferase at H3K9me2, and comparably the inhibition of G9a H3K9 methyltransferase increases JAK2 expression and ultimately H3Y41 phosphorylation, demonstrating direct epigenetic modulation by methylation.[201] Similarly, in cell culture, folic acid and the folate analog methotrexate also inhibit JAK2 phosphorylation.[212,213]

Like other cell lines, nutritional media for the UKE-1 cell line is also abundant in epigenetic modulators such as folic acid capable of supporting epigenetic inhibition of JAK2 and potentially a feedback loss of JAK2 methylation.[210,214] This suggests that chronic epigenetic modification may influence genomic adaptation, resulting in increased gene copy number or lead to constitutive activation of oncogenes such as JAK2 (Figure 4).

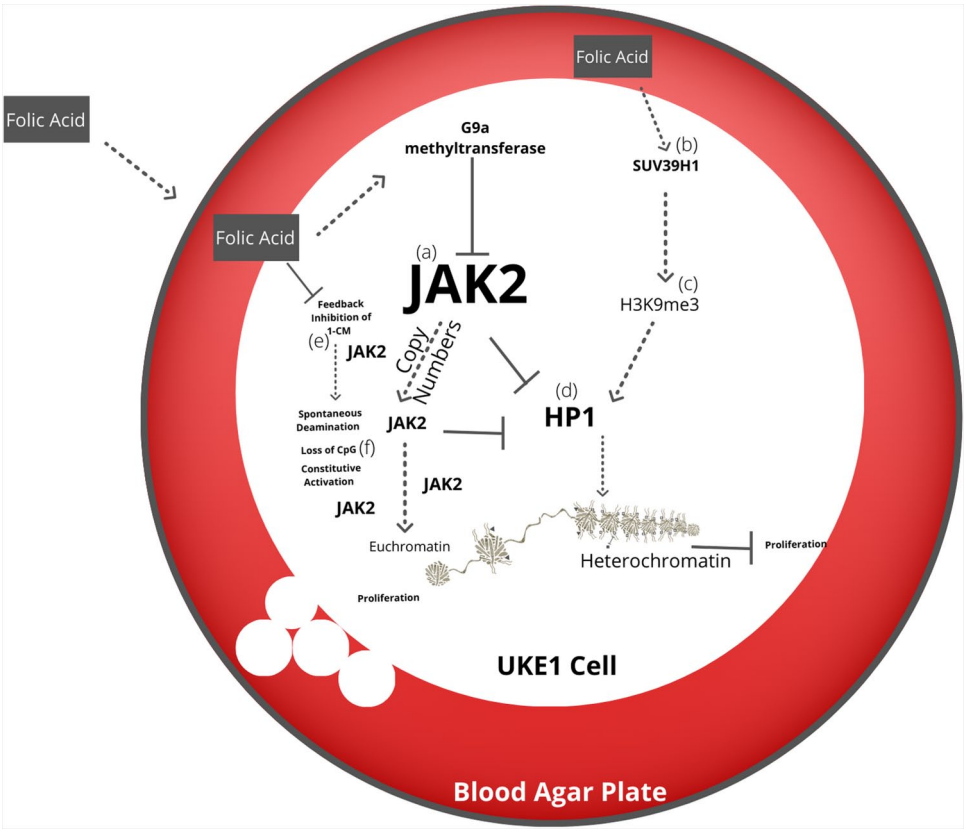

Figure 4. Hypothetical cell culture epigenetic manipulation of Janus kinase-2. Folic acid is added to the UKE1 cell line culture media, influencing epigenetic methylation. Upregulation of G9a methyltransferase inhibits Janus kinase-2 (JAK2) (a). Upregulation of histone-lysine N-methyltransferase (SUV39H1) (b) induces trimethylation of histone-3-lysine-9 (H3K9me3 (c) stimulating the binding of heterochromatin protein 1 (HP1) (d) and initiating heterochromatin. Reduced expression of JAK2 is incapable of excluding HP1 and maintaining euchromatin. Excess methylation inhibits one-carbon metabolism (1 Cm) (e) and induces spontaneous deamination of methylated cytosine-phosphate-guanosine (CpG) (f) and loss of CpG resulting in JAK2 constitutive activation or copy number accumulation.

Diet and Peptides

Many minerals, culinary, botanical herb, and spice phytochemical constituents, such as curcumin, resveratrol, quercetin, and sulforaphane have demonstrated powerful epigenetic modification in cell culture.[32,215] The fat-soluble vitamin retinol (vitamin A) has been described to drive epigenetic DNA methylation erasure through potentiation of TET demethylation enzyme activity.[216]

Vitamin D has also demonstrated DNA demethylation regulation through upregulation of jmjC domain-containing demethylase activity in cell culture; however, the cell-specific mechanisms are yet to be elucidated.[217,218]

Vitamin K has also demonstrated epigenetic modification in cell culture, producing histone deacetylase inhibition and hyperacetylation for treatment of cultured cancer cells.[219,220]

Animal-derived peptides have also demonstrated modulation of the epigenome with disease treatment potential in the laboratory.[221] Animals have very similar genomes to humans, and therefore animal-derived peptides from specific organs, sex-specific animals, animals with high metabolic rates or cuts of meat that produce specific proteins may increase the specificity of nutritional epigenetic modulation for treatment of disease.

Hormones

Dietary consumption of small endogenous biologically active molecules such as estrogen may play essential roles in health and evolutionary selection.

Estrogen

Animal organ and muscle estrogen can be stable up to 180 degrees and absorbed by the gastrointestinal tract of the consumer.[222] Estrogen is a potent epigenetic modulator influencing gene expression and has been associated with cognition, memory, mood, immunity, and bones.[223] Estrogen has shown to interact directly with the previously mentioned epigenetic transcription factor JAK2,[224,225] signifying that a sudden reduction in consumption of biologically active molecules like estrogen may result in epigenetic adaptation resulting in hundreds of molecular changes to gene expression required to ensure adequate endogenous synthesis of estrogen. Therefore, concomitant hypermethylation, SNV or deletion in the genome due to advanced evolution within the hormonal synthesis pathway, may result in pathological symptomology and/or disease.

Prolactin

Prolactin is a pituitary hormone essential for lactation and is found abundantly in breast milk.[226] Prolactin is commonly associated with reproduction; however, it is also produced by many immune cells and can be considered a cytokine for it has demonstrated production of a variety of cytokines.[227] Activation of the prolactin receptor also stimulates activation of JAK2 which may result in histone post-translational phosphorylation.[228,229]

In the gastrointestinal tract, prolactin has displayed stimulation of intestinal calcium absorption, increasing bone turnover and reducing renal calcium excretion.[230] Taken together, this suggests that a sudden withdrawal of dairy products from the diet may require epigenetic modification of the immune system, cytokine production and calcium absorption.

Amino Acids and Biogenic Amines

Serotonylation

Serotonin (*5-hydroxytryptamine—5-HT*) is a biogenic amine derived from the amino acid tryptophan and has demonstrated multiple functions within multiple organs.[227] Serotonin and its precursor 5-hydroxytryptophan (5-HTP) are found abundantly in animal tissues [231] including breast milk,[232,233] is orally bioavailable and has a half-life of over 17 hours.[234]

In the brain, both serotonin and its precursor are neurotransmitters, with serotonin having 3000 fold greater biological activity at some receptors but less affinity at others.[235] 5-HTP has also shown to be directly antagonistic to serotonin at the receptor.[236] It was once thought that serotonin was incapable of crossing the BBB, however more recent research demonstrates otherwise.[237-239] The results of earlier studies may have been due to a lack of active gene expression in cell culture.

Histone seronylation of the 5th glutamine residue of histone 3 (H3Q5) results in significant changes to gastrointestinal and brain gene expression profiles.[240] This demonstrates the potential for peripheral serotonylation to influence gene expression beyond the BBB. The Coeliac disease implicated transglutaminase 2 (TGM2)[241] is said to initiate the serotonylation adjacent to the activating H3K4me3, enhancing the binding of chromatin transcription factors for gene expression.[241]

Dopaminylation

Dopamine is a neurotransmitter derived from the amino acid tyrosine.[242] It plays a pivotal role in reward and movement regulation.[242] Unmodified H3Q5 can also be dopaminylated.[243] The lack of marked serotonylation at this residue allows for dopaminlylation to occur, which likely explains the antagonistic relationship frequently reported between dopamine and serotonin.[244,245] In a murine model of cocaine addiction, a fall in H3Q5 dopaminylation demonstrated decreases in the rate of spontaneous action potentials and an increase in cocaine-seeking behavior[243] demonstrating direct epigenetic influence by stimulants and their withdrawal.

Dopamine has a relatively short half-life and poor oral bioavailability, and therefore the epigenetic effects of dietary consumed dopamine is likely to be limited.[246]

Gamma-ammino butyric acid (GABA)

GABA is an inhibitory neurotransmitter found abundantly in almost all organisms and therefore can be obtained directly from many foods.[247] It is found in over 20 peripheral tissues[248,

and in all regions of the central nervous system.[249] GABA is renowned for its high potency.[249] As previously discussed, defective GABA function has been associated with a variety of neurological and non-neurological conditions including mood, cognition, motor function, flight response, sexual and reproductive behavior, anxiety, pain, violence, and aggression.[248]

Like serotonin, GABA was once thought to be incapable of crossing the BBB; however, a handful of studies have demonstrated otherwise yet the mechanisms remain unclear.[250] Unlike serotonin, GABA has a short half-life of approximately 17 minutes, and therefore direct modulation of the brain following dietary consumption seems unlikely.[249,250] However; Yamastu and colleagues displayed rapid gastrointestinal absorption and a peak in blood concentrations of GABA 30 minutes after consumption.[251] Moreover; GABA was revealed to successfully inhibit class I, II and III histone deacetylases, upregulate H3K9 acetylation and H4K12 acetylation in SH-SY5Y cells; therefore, we cannot rule out epigenetic modulation that may rapidly influence gene expression beyond the BBB.[247] In addition, neurosteroids are highly lipophilic and therefore, readily cross the BBB.[252] Plasma and brain synthesised neurosteroids have demonstrated rapid effects on neuro-excitability via modulation of the GABA-a receptor.[253] Therapeutic neurosteroids have established varied oral bioavailability, [254,255] the primary steroid cholesterol of which all neurosteroids are derived has an individualised oral bioavailability between 29 and 81%.[255] This suggests that a sudden reduction in dietary neurosteroids may negatively impact mood or cognition in a patient who has a genetic synthesis pathway defect.

Alcohol. Alcohol abuse causes extensive changes to gene expression in the human brain.[256] Some of these changes have been associated with alcohol dependence.[256] A 30% downregulation of DNMT1 was seen in 3 brain regions of alcoholics, accompanied by an increase in promoter H3K4me3 and the upregulation of GC rich genes including ubiquitination modifier ubiquitin-activating enzyme 1(UBE1)[256] again representing direct cross-talk between DNA and histone methylation.

Environment

Populational dietary choices also impact the environment, and with that impacts epigenetics. For example, sugar cane production produces 11% of carbon and methane greenhouse gas emissions,[257] contributing to increased methyl group donation and an increase in the prevalence of reduced function MTHFR alleles.

Heavy metals

It has been established that environmental heavy metals interact with the epigenome.[258] However, their epigenetic modulation is often contrasting and difficult to define due to their effects being cell and dose-specific. Metabolic transformation of inorganic arsenic produces metabolites monomethylarsinic, dimethylarsinic acid, and trimethylarsinic acid, and the one-carbon metabolic product SAM donates the methyl group in the reaction.[259] It was once thought that the methyl and glutathione conjugation of carcinogenic metals was essential for detoxification and elimination, however more recent research is demonstrating the conjugated metabolites to have greater toxicities.[259-261] Arsenic induced malignant cellular transformation is said to be due to global DNA hypomethylation and aberrant gene expression.[262] The highly toxic lead and methyl-mercury have both demonstrated non-consistent differential epigenetic modifications which are dependent on the specific tissue.[263,264]

Endocrine disrupting chemicals

Endocrine-disrupting chemicals found in pesticides and plastics such as bisphenol A (BPA) have also demonstrated interaction at the epigenetic level, potentially contributing to rapid evolution and disease.[34,265]

Fluoride

In the 1930s researchers found an excess of the natural mineral fluoride in drinking water supplies was associated with mottled teeth enamel yet interestingly, also prevented dental caries.[266] The concentrations of natural sources of fluoride in water wells across the globe and regionally are highly variable. In the early 1900s natural sources fluoride In the United States were measured to be as low as 0.1 parts per million (ppm).[266]

On January 5th 1945; Michigan state, United States were the first to introduce fluoride fortification of their town water, other states and Countries followed shortly after.[266] Today water concentrations of fluoride in water supplies can range anywhere between 0.7 ppm and 16 ppm. However; around the same time as fluoride fortification was implemented around the World, researchers in India were discovering that natural sources of fluoride in the water supply as low as 1ppm were associated with fluoride toxicity and skeletal fluorosis.[266] Fluoride toxicity and subsequent fluorosis have been assessed for its epigenetic influence upon genes associated with skeletal development.[267] Promoter region DNA hypermethylation and sequential down-regulation of expression were demonstrated for genes associated with extracellular matrix deposition and cartilage formation.[267] Together, this demonstrates classic epigenetics and evolutionary differences in populations, where one man's medicine may be the cause of another's ailment and exemplifies the damaging effects of nutritional conformity and a need for personalised nutritional management.

Future Directions

Nutritional deficiency

Reduced erythrocyte, serum or plasma nutrient levels comparable to the average cohort have long been used to identify a nutritional deficiency. However, it is rarely considered that a reduction in an epigenetic modifier such as folate may be due to excessive use of substrate and enzymatic activity—for

example; folate depletion due to excessive DNA methylation. The methylation requirement for each cell is different, making an evaluation of serum folate difficult to predict the level of folate within and required for glial cells compared to cardiomyocytes. Therefore; nutritional epigenetic research must look at nutrition from both angles keeping bio-individuality in mind, and practitioners should use caution in interpretation.

Rhythmicity and cell specificity

Outlining the cell-specific epigenetic regulation of rhythmic and coordinated biological processes will add to precision and patient management accuracy.

Laboratory techniques

Nutritional modulation of the epigenome in the laboratory is becoming increasingly popular and has profound disease modulatory potential. The cell, tissue and dose-specific epigenetic mechanisms for many environmental substances including bioactive peptides are yet to be determined; however, when it comes to experimentation, researchers should pay special attention to detail with the use of excipients, extraction, storage, reagents, media, and temperature as to avoid unintentional epigenetic modification and human error.

Accurate genotyping with the use of next-generation sequencing technologies is still an expensive operation, and the use of first-generation micro-array genotyping technologies is affordable and provides valuable insight into a patient's ancestry and origin for precise nutritional health management. However; the use of restriction enzymes in this technique makes it impossible to differentiate a deletion from a methylated base or unrepaired deaminated base. Moreover; this technology does not provide information pertaining to haplotype selective expression, cell-specificity or gene epigenetic regulation, all of which largely affect the pathogenic effect of an SNV. This makes nutritional epigenetic modulation of specific genes based on these results currently unreliable, and therefore standardization of accurate genotyping is of great importance. It is now understood that regardless of genotype, epigenetics is the foundation of a gene's regulation and therefore, RNA based sequencing techniques may provide a more reliable insight into cell-specific gene expression.

Novel epigenetic post translational modifications

A deeper understanding of the novel epigenetic post-translational modifications such as serotonylation and dopaminylation will aid suicide prevention and the natural management of mental health conditions and addiction.

Evolution

Understanding the impact of environments on gene evolution and epigenetics within populations or cultural subgroups should take priority as this opens up bio-individuality moving away from nutritional conformity and greater health for all populations.

Conclusion

With advances in genomic sequencing technology and the evolution into personalized medicine, nutritional epigenetics is showing to be an attractive non-invasive natural alternative to gene editing for the treatment of disease.

Insufficient MTHFR enzymatic activity is said to account for insufficient DNA and histone methylation, whereas an excess of DNA methylation and its etiology has been less explored. This is likely due to the difficulty of monitoring the regulation of the epigenome in cell culture.

Depending on the cell's bio-uniqueness, the addition of 5-MTHF to the media may result in feedback inhibition of one-carbon metabolism and a loss of methylation or progresses to DNA methylation resulting in repression of gene expression, two completely opposing results, making nutritional supplementation without a thorough understanding of the patient's bio-individuality unreliable.

The rapid epigenetic fluctuation that drive core human biological processes requires coordinated activation and deactivation of all potential modulators, suggesting prolonged supplementation with methyl group cofactors or other supplemental epigenetic modifiers may result in epigenetic inflexibility and metabolic blockages, providing symptom relief and disease concomitantly, which may or may not be immediately recognized. Knowledge of such paradoxical mechanisms emphasizes the importance and the need for highly specific personalized patient management.

This conceptional research has demonstrated that the underappreciated acetylation and downstream epigenetic post-translational modifications may be the pinnacle of the epigenomic hierarchy and that many variables are possible when it comes to epigenetic regulation of gene expression and DNA methylation of any primary epigenetic modifier has the potential to cause disease. Implications of excessive cellular methylation has been unclearly defined; however, this research demonstrates that an excess of methylation in cell culture results in epigenetic adaptation, immortality, differential methylation patterns, advanced evolution and a loss of CpG. Environmental factors have influenced the increasing incidence of reduced function MTHFR alleles through natural selection and bypassing evolution's natural slowing of methyl group synthesis with methylated B vitamins may result in dysregulated feedback inhibition of one-carbon metabolism with unpredictable consequences. Given that methylation is directly antagonistic to acetylation upon the histone suggests that an excess of cellular methylation can result in significant chromatin inaccessibility and chronic gene inactivation. Moreover; hypermethylation of acetyl CoA production or a reduction in mitochondrial ATP synthesis due to hypoxia may result in epigenetic inflexibility, leaving primarily supplemental or environmentally derived methyl groups to drive aerobic glycolysis resulting in inefficient ATP production, and an excess of lactate.

It has become overwhelmingly clear that our genomic complex adapts to environment and diet, and the evolutionary consequence of differences between populations shapes our genomic blueprints resulting in subtle differences in the human phenotype. Human populations that undergo rapid dietary or environmental change tend to suffer the most with disease compared to those who remain true to tradition, such as centenarians of blue zone communities. Current international nutritional guidelines do not consider ancestry, bio-individuality or epigenetics so we must consider whether nutritional conformity may be negatively influencing the rate of evolution and contributing to disease.

The fortification of milled grains with B vitamins may have been implemented premature as the consequence of such intervention on other cells throughout differentiation, and its impact on neurodevelopment has been previously undefined. This report demonstrates that excessive oral or intravenous supplementation with 5-MTHF is non-cell specific, unpredictable and therefore may result in differential methylation patterns in cells with already sufficient cofactors.

In conclusion; this paper has explored the potential for nutritional epigenetics and has found personalized nutritional epigenetic modulation of disease through dietary, environmental, and lifestyle intervention to be a safe and highly effective option and should be a first-line treatment approach for the management and prevention of disease. In contrast; this report has established a requirement for cautionary measures in the interpretation of genomic sequencing data and revealed the irregularity and unpredictability for prolonged supplementation with epigenetic modifiers and the dangers associated with it. Therefore, the administration of epigenetic modulators through fortification or supplementation should be administered with caution and consideration for cell specificity, epigenetics, ancestry, and environmental influence.

Author Contribution

All contribution is solely done by Lynda Sedley.

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