

Review

The Ability of Nutrition to Mitigate Epigenetic Drift: A Novel Look at Regulating Gene Expression

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Summary Epigenetic drift causes modification in gene expression during aging and a myriad of physiological changes that are mostly undesirable, remove youthful phenotype and are related to biological decay and disease onset. The epigenome is considered a stable regulator of genetic expression. Moreover, evidence is now accumulating that commonly available compounds found in foods can influence the epigenome to embrace a more youthful and therefore, more disease resistant state. Here we explore the correlation between nutriment and the epigenetic regulation through various types of alimentation. The aim is not to discuss specific chemicals involved in disease onset. Instead, we offer a brief glance at pathogens and offer a practical pathway into epigenetic regulation, hypothesizing that epigenetic drift might be attenuated by several foods able to drive a more youthful and disease resistant phenotype.

Key Words epigenetic drift, epigenetic diet, nutraceutical, gene expression, nutrigenomics, genomic diet, histones, methylation

Senescence is the inability of organisms to rejuvenate tissues or mitigate damage, and as intracellular damage accumulates, exerting its deleterious effect, impaired cell function ensues and ultimately contributes to age-related disease and aging itself. The control of gene expression and signaling processes involving the cell, its cellular matrix, and its surrounding environment appear to be crucial in understanding the aging process and biological dysfunction that is associated with organismal senescence (1).

According to Issa (2), epigenetics can be defined as a stable mechanism of the regulation of gene expression that occurs regardless of the levels of the transcription factors that regulate the target genes in the cell. Therefore, it is crucial to distinguish between epigenetic silencing and regulation of gene expression during aging, and differentiation from transient or dynamic changes that might involve chromatin remodeling or even deoxyribonucleic acid (DNA) demethylation, which maybe

induced in response to transcription factors or other signaling molecules. Many types of epigenetic processes have been identified such as modifications of chromatin structure or the regulation of transcription inside the promoter regions via methylation, acetylation, and phosphorylation (3). These regulation patterns mainly involve changes in DNA methylation and chromatin remodeling which are encoded via the epigenome—a collection of methyl groups on DNA or on histone tails that are established during embryogenesis (4).

During DNA methylation, DNA methyltransferase 1 (DNMT1) adds a methyl group (CH₃) covalently bonded to the symmetrical dinucleotide CG also known as CpG islands (areas of extreme methylation) that are embedded in the promoter region. Eventually, the 5-methylcytosine base can be further modified through sequential carboxylation by the ten-eleven translocation (TET) family of enzymes (5).

Apart from telomere shortening, modification of histone tails provides another example of epigenetic regulation of gene expression (6). Until recently and for a long time, histones were thought to be structural proteins without any other cellular function apart from being the beads on a string around which DNA is wrapped. Enzymes known as “writers” were recently found to be able to add label groups such as acetyl or methyl groups to amino acids, ultimately protruding from the nucleosome leading to modifications of the histone structure. These labels serve as signaling molecules for downstream “reader” proteins which in turn repress or activate gene expression through chromatin

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Abbreviations: AGEs, advanced glycation end products; *C. elegans*, *Caenorhabditis elegans*; CpG, 5'-C-phosphate-G-3'; DNMT, DNA methyltransferases; EGCG, epigallocatechin-3-gallate; H3K9me3, histone 3 lysine 9 trimethylation; HAT, histone acetyl transferase; HDAC, histone deacetylase; HP1, heterochromatin protein 1; hTERT, human telomerase reverse transcriptase; miRNA, micro RNA; mtUPR, mitochondrial unfolded protein response; nDNA, nuclear DNA; NF-kappaB, nuclear factor kappaB; p53, tumor protein p53; PAHs, polycyclic aromatic hydrocarbons; SASP, senescence-associated secretory phenotype.

remodeling. Therefore, histones play a key role in regulating both transient and long-term gene expression (7).

It is now well established that the genomic landscape of DNA methylation (DNAm) gets altered as a function of age, a process known as 'epigenetic drift.' As a consequence of aging the normal structure and patterns of DNA gradually become deregulated, leading to deviations from a normal epigenetic state. Furthermore, it is important to highlight that epigenetic drift is not directional or uniform across the genome, and is quite variable between individuals of the same age. It is also tissue specific (2) and influenced by trans-acting factors such as methylases (DNMTs), demethylases (TETs), and modifiers. Consequently, as DNA methylation is tissue specific, the epigenetic diet may not influence various parts of human physiology equally, resulting in different organs and systems expressing independent DNA methylation activity, which may result in more youthful or aged tissues. Even though the epigenetic diet aims to slow epigenetic drift due to the specificity of each tissue, system wide restoration of a youthful epigenetic configuration has not yet been observed. Moreover, in the last forty years, several studies have been published focusing on epigenetics and its correlation with aging and the onset of serious disease (8). It is clear that a wide variety of illnesses, including cancers, cognitive dysfunction, and cardiovascular diseases are linked with epigenetic mechanisms (9, 10).

The epigenetic mechanism holds definitive power over our health and mortality by being the ultimate decider on which genes are up-regulated or down-regulated and how those genes instruct cells to function.

Therefore, assessing what dietary sources may slow the epigenetic clock might be a valuable tool in preventing age-related diseases. Over the last few decades, several studies investigating the role and the effect of various species and food groups on the epigenome have been published. Results of these studies are varied whereas others are easily shown to have a robust positive effect on longevity genes via the epigenetic pathway (8, 11).

As our understanding of epigenetics and genetics increase, so does our manipulation of this machinery which will form the basis of this discussion. Understanding what molecules can enter the nuclear environment and the resultant effect on epigenetic expression is now becoming a little clearer as more research is conducted in this specific field.

Epigenetic Mechanisms

A major part of the epigenome is the methylome, and to induce changes in the methylome, nuclear DNA (nDNA) must first become loosely bound as euchromatin by unravelling from the protein molecules known as histones (12). This nDNA and histone combination is referred to as the nucleosome. To initiate changes to the nucleosome, a negatively charged acetyl group must first approach a histone to cause the unraveling of the tightly woven nDNA. The acetyl group achieves this by carrying an electron toward the positively charged

nucleosome.

Afterwards, histone acetylation leads to the partial unwrap of the DNA structure so that genes are accessible and epigenetic changes can be performed via the deposition of a methyl group. Methylation can either turn genes on for protein synthesis or the methyl group can turn genes off by activating suppressor genes that silence other genes (9). Methylation of DNA prevents transcription and this process may also be influenced by diet (13). When the epigenomic machinery induces gene expression, it must unravel the heterochromatin first. Histone acetyl transferase (HAT) is the enzyme used to activate histone acetylation (14). Only part of the histone may actually become unwrapped, as only specific terminals need to be manipulated by acetylation at any one time. However once transcription of that gene is complete, deacetylation can then take place which rewraps the nDNA tightly back over the histones. This is achieved by another enzyme called histone deacetylase (HDAC) (15).

The nuclear environment contains on average around 3 billion base pairs across the entire genome and remains tightly wrapped around the histones. Furthermore, this nuclear environment is only ~8% of the actual cell's volume (16). The nucleus remains approximately 6 micrometers in diameter even with billions of base pairs. The nucleus is incredibly complex and small (17); however, this is where the epigenetic diet begins. Certain foods can modify the inner workings of this intricate machinery and affect what genes are turned on and what genes are silenced (18). Therefore, the aim of this study is to offer a glance at the effect of food intake on epigenetic regulation of gene expression.

Windows into the Epigenome

Over the years, several studies investigated the connection of lifespan and epigenetics with promising results that epigenetic drift is highly correlated as a cause of biological aging and not a result of aging.

When mitochondrial electron transport genes are suppressed it results in the activation of the mitochondrial unfolded protein response (mtUPR) which is shown to increase lifespan in *C. elegans* (19). This study by Durieux et al. is just to cite one of many tantalizing studies that appear to indicate the gravity of epigenetics as a possible cause of aging as opposed to an outcome of aging.

Moreover, several other groups obtained similar results: decreased levels of heterochromatin protein 1 (HP1) and lowered levels of histone acetylation with an accrual of histone 3 lysine 9 trimethylation (H3K9me3) (20, 21) which again was shown in *C. elegans*, (22) and also human fibroblast cells showing a senescent phenotype (23). Hu et al. in 2014 demonstrated that yeast loses almost half of its cardinal histone proteins genome-wide during age (24). These changes in nucleosome positioning and tenancy clearly indicate a loss of epigenetic control over genes that may add to a more disease resistant and youthful phenotype. Similar studies targeting other epigenomic factors also find clues

that are quite striking. A well-researched histone variant is macroH2A which has been shown to increase during aging across primates and human fibroblast cells which have become senescent (25). MacroH2A is a silencing histone variant and has been strongly linked to higher levels of senescent cells. When macroH2A is knocked out a strong reduction in the phenomenon of senescence-associated secretory phenotype (SASP) is observed (26). This evidence appears to indicate that epigenetic drift via macroH2A may induce SASP.

Furthermore, observations continue to show epigenetic machinery such as nucleosome remodeling complex lsw2, which when knocked down increases lifespan in yeast (27) and also the nucleosome remodeling complex SWI/SNF were also shown to lengthen lifespan by acting as a cofactor with DAF-16 (28).

When epigenetic machinery is prevented from drifting throughout age, a reversal of the age-related phenotype and a return to a more youthful epigenetic function is observed. When histones H3 and H4 are not lost during aging as a result of overexpression of their genes, again the elongation of lifespan is seen (24) and furthermore when genes are removed that downregulate H3 and H4 the same results of life span extension are seen (29). These clues all appear to indicate the epigenome as a regulating switch in the aging process, and the challenge for science is, how to influence epigenetic expression through diet (or at least supplementation with novel compounds).

DNA methylation clearly changes throughout aging and holds great promise for future research in human longevity. Methylation is seen to rise in mouse hematopoietic stem cells (30) which may lead to genomic instability and lead to a myriad of age-related pathophysiology. On the evidence it would be easy to draw the conclusion that the resetting of these methylation markers back into their unmethylated state would also remove the genomic instability that seems to ensue when these markers become methylated. Additionally, when the methylation patterns are examined in stem cells across the mouse model, global hypomethylation at the methyl sites and hypermethylation at the CpG islands is witnessed (31). These changes in the methylome could easily implicate the epigenetic framework in causing genomic instability, age related pathophysiology and decline.

Moreover, it was shown that there was a connection between the methylome undergoing severe entropy and a sharp increase in aging (when based upon the apparent methylomic aging rate $R=0.49$, $p\sim 0$) (32). Hannum et al. also found that methylation markers in young humans were very similar and that a divergence in those similarities became evident as epigenetic changes built up over time. This evidence points strongly to the importance of decelerating epigenetic drift for increased lifespan, as it becomes clear in Hannum's paper that these methylation changes induce an age-related phenotype. Ultimately it appears that the epigenome will become a major target for disease prevention, therapeutic medicines, and longevity technologies.

Epigenetic Diet

Ek and coworkers of the University of Uppsala, Sweden has shown that tea has been shown to upregulate genes. The influence of tea-drinking in epigenetic regulation is sex-dependent being higher in women more than men (33).

Although this might be explained by women drinking more tea than men, the epigenetic modification involved the expression of genes related to cancer and estrogen. Furthermore, in the same study, Ek et al. demonstrated that coffee does not appear to influence epigenetics. The primary polyphenol in green tea is epigallocatechin-3-gallate (EGCG) which is found to have a correlation with lower rates of cancer development (34, 35).

Moreover, the importance of curcumin and its role in epigenetic drift has been well established (36). Curcumin suppresses the expression of pro-inflammatory genes and upregulates pro-apoptotic genes via methylome regulation demonstrating that curcumin is an important factor in maintaining epigenetic regulation (37–39).

Other foods believed to influence epigenetics are broccoli, kale, cabbage, and watercress because of their isothiocyanates or sulforaphane content (40, 41). Folic acid or folate which is found in beans, grains and other green vegetables is also known to be a powerful epigenetic modifier (42). Folate seems to be extremely important in embryonic development and folate-free diets during pregnancy seems to promote major lifelong genomic instability (43, 44). Moreover, Wang et al. (44) also demonstrated that early fetal folate deficiency also renders the offspring to higher susceptibility of obesity-induced child metabolic risk, with the effect being inversely proportional when folate levels were sufficient. This is interesting due to the strong connection between folate and the one-carbon metabolism pathway, which is the basis for DNA and activated methyl groups that are essential for methylation of DNA and chromatin structures (45). Often referred as the folate-mediated 1-carbon metabolism, this pathway is paramount in preserving meticulous genomic function. Other nutrients such as vitamin B12, B6, methionine, choline and betaine are also implicated in the 1-carbon metabolism (46).

Selenium rich foods such as nuts, legumes, beans and some meats also have been shown to influence the epigenome (47). In a pivotal study, Speckmann and Grune demonstrated that selenium species have been shown to modify the methylome at various gene loci and even globally (48). The authors also point out that further studies are required to determine exactly how selenium species are able to inhibit DNA methyltransferases (DNMT) and histone deacetylases (HDAC). When DNMTs and HDAC fluctuate, genomic instability can be induced, so suppression of DNMTs and HDAC via selenium species may deliver beneficial effects against epigenetic drift.

Interestingly a natural isoflavone of the soybean known as genistein has been shown to inhibit the transcription of the gene hTERT, which is responsible for telomerase induction, and leads to lower cell prolifera-

tion. Levels of DNA methyltransferases 1, 3a and 3b were also shown to be inhibited by genistein (49). Suppressing telomerase activity via hTERT inhibition may not be an attractive epigenetic regulator for those wishing to maintain longer telomeres for longevity. Therefore, genistein may not be an attractive epigenetic regulator for attenuating epigenetic drift (50). Shorter telomeres have been associated with an increase in mortality due to factors such as oncogenic transformation of somatic cells, cellular senescence and apoptosis (51), and therefore epigenetic regulators that speed up telomere attrition such as genistein should be reconsidered when considering an epigenetic diet.

Cooked meat has been classed as a class one carcinogen (52). It in fact contains high levels of polycyclic aromatic hydrocarbons (PAHs) and advanced glycation end products (AGEs) (53, 54). Therefore, even though meat can influence the epigenome, the influence is considered detrimental to human health and not advised for effective mitigation of epigenetic control. Due to different breeding and feeding methods, steroids contained in meat may be present in high concentrations, as well as high levels of antibiotics which are also known to be detrimental epigenetic expression factors. However, the literature on meat containing steroids and/or antibiotics and the effect on epigenomic machinery is sparse and much more research is required in this specific field.

Temporary spikes of high glucose are also shown to influence the epigenome which can negatively influence gene expression (55). These transient levels of hyperglycemia induce extended variations of the epigenome in the promoter of the nuclear factor kappaB (NF-kappaB) subunit p65. The effect on the epigenome and gene expression was noted to last up to 6 d *in vitro* and in non-diabetic mice from transient high glucose spikes. This vivid effect demonstrates how a diet with regular sugar spikes can be harmful to human health. The evidence is also consistent with prolonged life from diets that are comprised of foods with a low-glycemic index, or from fasting (56).

Choline is another methyl donor that is shown to influence the epigenome and therefore gene expression. When pregnant mice are fed diets high in the methyl donor choline, the coat colour of mice is brown, however when dietary intake of methyl donors such as choline is restricted in pregnant mice the coat colour is yellow and the offspring tend to suffer from obesity and even tail kinking (57). This change in coat color can be observed in agouti viable yellow mice. Choline is also shown to contribute in the production of S-adenosylmethionine which is a major methyl donor used for DNA and histone methylation (58) which appears to establish choline as an important instrument against epigenetic drift. The authors note that much of these studies are associations and therefore more in-depth examinations should be performed.

On the Evidence

The studies discussed here are just a brief snapshot of the available evidence which implicates the epigenome

as a master regulator in aging. Even though many of the studies we have just discussed may not be directly the result of food ingestion causing the epigenetic changes, they do demonstrate that the epigenome is most likely involved in accelerating aging and disease processes and therefore warrants clever food choices to influence the nuclear environment. This level of influence over the epigenome remains an etiological argument that warrants further research on nutritional epigenetics.

Evidence clearly shows that our genes are at some level influenced by food intake via the resultant epigenetic expression, though how much of our genome can be influenced remains to be seen. This epigenetic expression is also mitotically heritable, therefore, minimizing exposure to molecules whether found in foods or elsewhere that can change the epigenetic framework is extremely important for disease prevention and positive outcomes. The epigenetic machinery can be affected through histone modifications, DNA methylation either with single points of methylation or regions such as CpG islands, micro RNA (miRNA) (59) and other nuclear machinery such as interference of chromatin remodeling or enzymes that regulate the structure of nucleosomes.

The addition of methyl groups on either allele can turn genes on or off. As we age these methyl groups may begin to change their methylation patterns and it is this epigenetic drifting that can become dangerous to human health. The epigenetic diet which may inhibit this drifting requires further research by scientists so that we can peer into what parts of the epigenome may continue to drift after the epigenetic diet is applied and also which foods are responsible for influencing exact parts of the epigenome. Additional research to develop novel compounds to assist with epigenomic maintenance and what other foods may attenuate drift is warranted.

Evidence also points to parents being able to preprogram the epigenome of their offspring, predisposing them to a life of obesity even though the genes remain the same (60). The epigenomic expression and not just the genomic expression is passed down through the generations which demonstrate the importance of avoiding harmful epigenetic factors, as an individual's lifestyle may echo into the health of future generations.

As a final note, epigenetics can also be used to determine biological age (61). Specific markers on the DNA which can tell us the state or age of various tissues are now used to deliver what is known as an epigenetic clock (32, 62). Research to see if the epigenetic diet can move or rearrange the hundreds of biomarkers in this epigenetic clock could further deliver clues about epigenetic drift and aging. The epigenetic clock can also deliver knowledge about what pathology an individual may die from, and therefore future research into whether there are connections between an epigenetic diet and biomarkers on an epigenetic clock would make sense for the field. Such knowledge may deliver firm data about biological age when combined with an epi-

genetic diet. Understanding the combined connection between the epigenetic diet, epigenetic drift and the epigenetic clock could be a useful weapon in the fight against aging.

Human tissues age at different rates naturally, epigenetics allows us to see into the future and know what organs are likely to fail first, years in advance (63). Two issues that have been found not to age at the same rate as other tissues is the heart tissue and female breast tissue which appears to age faster than surrounding tissues (64).

The epigenetic diet is a nutritional program based on foods able to influence gene expression. Moreover, epigenetics is affected by environmental factors (65) and it is these factors that should be examined in depth. Some foods and chemicals such as those found in cigarette smoke (66) can change epigenetic expression and ultimately cascade down into either wanted or unwanted gene expression. Some genes such as p53 have proapoptotic function which protects from disease onset whilst others have oncogenic function (67, 68).

Moreover, longevity genes which are also known as age suppressor genes such as the Klotho gene are studied by scientists to understand and extend health span (69). When overexpressed Klotho extends lifespan in mice, whilst Klotho deficient mice show a premature aging phenotype (70–72). The epigenetic diet is comprised of foods that are shown to activate age suppressor genes whilst at the same time downregulating genes that may cause disease or other biological insults (73). Possessing knowledge on what foods to select to influence the epigenome holds great promise for aging research (74). Epigenetic control over genes shifts during age allowing expression from unwanted or disease-causing genes. Chronological age is now believed to be less accurate than epigenetic clocks as a predictor for mortality, as the epigenetic clocks are able to reveal how far your epigenome has drifted, clearly pointing to impending genetic mutations which cause disease and death (75). This prediction is performed by observing methylation markers that establish themselves along the genome to either turn on or turn off various genes (76). The removal of methylation markers can restore youthful gene expression and prevent the onset of biological decay, and furthermore, methylation markers can activate some genes, to suppress or inhibit other genes, therefore DNA methylation is essential machinery that is able to deliver control over epigenetic drift and is paramount to maintain a disease-free phenotype (77, 78).

Conclusions

Ultimately, the evidence appears to indicate that the epigenetic expression during youth is the most disease resistant configuration and most effective at maintaining a youthful genomic phenotype. Epigenetic drift seems to be part of the aging process itself, however with new knowledge showing us what foods assist in maintaining a youthful epigenetic landscape, the epigenome should now become a primary target for longevity technologies and strategic dietary choices.

The importance of attenuating epigenetic drift through the epigenetic diet cannot be overestimated. It is clearly a case of nurture over nature; human lifespan is controlled by how well you take care of yourself; genes are not destiny, but genes will mirror their master's behaviour, so take care of them.

Authorship

RDP research and formulation, MV review, contributions, modified. VP further review and contributions.

Disclosure of state of COI

RDP is Chief Scientific Officer for Helium 3 Biotech, Author of the Anti-Aging Toolkit, Host of the Longevity Experts television series. VP and MV have no conflict to declare.

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