Elucidating the Epigenetic Mechanisms that Modulate Biological Age

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Abstract

In recent years, changes in the epigenome have emerged as a key component in the aging process. DNA methylation, histone acetylation and methylation, and chromatin landscape remodeling are the most well researched of the types of epigenetic modifications. To best elucidate their functions, they should be understood as interacting parts in the context of an integrated epigenetic approach and not as exclusive mechanisms. Each type of modification undergoes some predictable changes with aging but may display variation depending on the individual, cell type, or organism. These epigenetic modifications correlate to changes in phenotypes common with aging and to increased susceptibility to age-related diseases, such as cancer or Alzheimer's Disease. While age may cause these changes, epigenetic modifications also contribute to aging by altering gene expression and interacting with other agents of aging to further the progression. Therefore, the scope of this review looks at epigenetic modifications as an effect and driver of aging.

Keywords: aging, epigenetics, DNA methylation, histone modification, chromatin, chromatin remodeling, chromatin landscape

1. Introduction

Cellular and molecular damage to cells over time accumulates in a phenomenon called aging, which is partly dependent on the passage of time, or chronological age. Biological age differs from chronological age, as it is the age of a person demonstrated by cellular health and is a more accurate predictor of lifespan and healthspan, the amount of time an individual lives healthily. The rate of aging was previously believed to be predetermined by the genes inherited at birth, but recent research has shown that the environment and the resulting epigenetic changes to the chromosomal landscape determine a significant portion of longevity and health (Kirkwood, 2005). Aging is also accompanied by modification of an organism's phenotype, which is due to the epigenetic modulation of transcription. (Kirkwood, 2005). Various hypotheses and hallmarks of aging have been proposed thereafter, including genomic instability, telomere attrition, cell senescence, and epigenetic modifications (López-Otín, et al., 2013). These hallmarks of aging should not be seen as mutually exclusive, but as an interconnected network of processes influencing each other and contributing to aging as a whole. However, this review will concentrate mainly on epigenetic modifications as the most crucial factor in aging because it controls gene regulation and phenotypic alterations, thus making it an underlying factor that many of the other hallmarks of aging possess.

Epigenetics refers to changes made to the chromosomal landscape without altering the genetic code directly and includes DNA methylation, histone modification, and chromatin remodeling. While there are other factors that influence epigenetic modification, such as modification of non-coding RNAs, which has also been established as an epigenetic factor more recently, this review will focus on the more established canonical methods of epigenetic modification. The most well-studied epigenetic modification being DNA methylation, which refers to the addition of methyl groups to the cytosine in CpG dinucleotides, where a cytosine precedes a guanine in the 5' to 3' direction, regulates the tightness of chromatin and therefore the rate of transcription of the genes at the methylated loci. Hypomethylation leads to reduced genomic stability and excessive gene expression, increasing the rate of mutations and the probability of age-related health issues, including cancer. Conversely, hypermethylation represses gene expression and may occur at protein-coding genes that carry out basic cell functions, henceforth depleting the cell of said protein which could have catastrophic consequences (Ashapkin, et al., 2017). Histone modification, defined as a post-translational modification (PTM) of histone proteins bound to chromatin, while less researched than DNA methylation, has also emerged as an important epigenetic factor in the remodeling of the genomic landscape. Histone acetylation and histone methylation in particular are amongst the various types of histone modifications that have

shown consistent correlations with aging. As DNA methylation and histone modification influence chromatin folding and thus the level of gene expression, this process of DNA and histone methylation tightening the landscape and DNA and histone acetylation loosening the landscape, chromatin remodeling.

As the average lifespan increases in developed countries, health in old age becomes an increasingly important concern. While it is not clear the order in which aging hallmarks arise, epigenetic research may be a crucial piece in improving healthspan in older age. The epigenome is alterable unlike the genetic code; therefore, it is theoretically possible to reverse some age-related epigenetic changes to revert a cell to a biologically younger age. Pinpointing the exact epigenetic modifications that affect biological age may aid in the development of pharmaceuticals that target age-related epigenetic modifications.

Many of the studies on epigenetics and its role in aging have been done in model organisms such as budding yeast (*Saccharomyces cerevisiae*) and the fruit fly (*Drosophila melanogaster*), which have short generations and high reproduction rates, providing ideal conditions for researchers to track target genes and phenotypes related to aging over many generations. These organisms also possess some highly conserved cellular processes, allowing us to make inferences about aging in humans (Kaeberlein, et al., 2007). To elucidate the importance of epigenetic mechanisms in the process of aging, this review highlights patterns of DNA methylation, histone modification, and chromatin remodeling as it relates to other hallmarks of aging, providing a holistic perspective on epigenetic modifications and their roles in aging. This holistic review will bring to light the extensive interplay between different epigenetic modifications, allowing scientists to easily identify the key questions in the field and investigate them experimentally.

2. DNA Methylation

As aging progresses, a global pattern of hypomethylation occurs, but specific CpG sites show hypermethylation (Day, et al., 2013). Aging is accompanied by an increase in heterogeneity between methylomes and amongst cells of the same tissue over time due to random errors in DNA methylation that may arise during replication (Winnefeld and Lyko., 2012; Bormann, et al., 2016; Ashapkin, et al., 2017). The increasing differences between individual methylomes accounts for many of the phenotypic differences that arise in identical twins as they progress through life, and the heterogeneity within the same tissue increases risk of organ failure (Tan, et al., 2016; Ashapkin, et al., 2017). Studies in *D. melanogaster* and *C. elegans* have contributed to our understanding of the correlation of DNA methylation and aging, however, the type of DNA methylation that occurs in *D. melanogaster* and *C. elegans* differs from the traditional mammalian (5mC) methylation (Booth and Brunet, 2016; Greer and Shi, 2012). Therefore, while model organisms can provide insight into the global correlations of DNA methylation and aging, DNA methylation must be studied as well in mammalian cells to provide insight into the cellular mechanisms behind epigenetic patterns and aging.

Tracking DNA methylation at CpG sites has been established as a reliable way to measure chronological age, more so than other well studied age predictors such as telomere length (Hannum, et al., 2012; Horvath, 2013; Jylhävä, et al., 2017). Although there are individual variations in patterns of DNA methylation, some methylation sites are highly conserved among mammalian species, demonstrating a clear link between aging and specific genes (Li, et al., 2022). The ELOVL2 gene, which is associated with the synthesis of long fatty acid chains primarily in the liver, has been identified as the strongest correlative gene between methylation and age (Li, et al., 2022; Spólnicka, et al., 2018; Garagnani, et al., 2012). As chronological age increases, the ELOVL2 gene becomes hypermethylated, decreasing gene expression (Garagnani, et al., 2012). Other CpG sites with methylation patterns highly correlated to age have been found to play a role in cancer and Alzheimer's Disease. Interestingly, some CpG sites have shown strong positive correlation between DNA methylation and age in certain regions of the brain, among other common symptoms of aging and decline in health (Spólnicka, et al., 2018; Hernandez, et al., 2011).

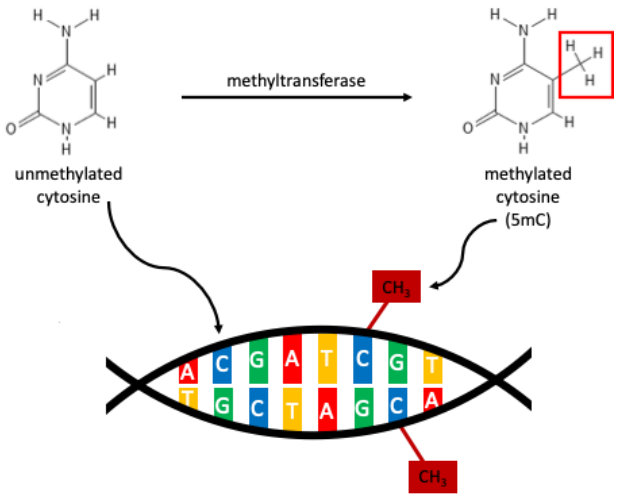

Figure 1. Structure and Mechanism of DNA Methylation. DNA is methylated by methyltransferases which adds a methyl group onto the 5' carbon at CpG sites.

When methyl groups bind to CpG dinucleotides, the new methylated form of cytosine called 5-methylcytosine (5mC) (Figure 1) inhibits binding of transcriptional activators or promotes binding of transcriptional repressors to

repress gene expression (Watt and Molloy, 1988; Booth and Brunet, 2016). Repression of expression has also been attributed to the recruitment of proteins that contribute to chromatin tightening and silencing (Fuks, 2005). CpG sites are often located at the promoter regions of genes, therefore regulating gene expression (Day, et al., 2013). However, methylation correlated with aging most dramatically occurs not in the promoter regions, but in the enhancer regions which are sites important to regulating gene expression (Johansson, et al., 2013). Furthermore, methylation at noncoding sites has emerged in recent years as a significant factor in genome stability (Winnefeld and Lyko, 2012). It is thought that hypomethylation of noncoding DNA sites decreases chromatin density and allows insertion of transposable elements, thereby increasing genomic instability and mutations prevalence (Pal and Tyler, 2016). Therefore, breast and other types of tissue that typically indicate a higher biological age according to DNA methylation patterns also demonstrate a higher incidence of cancer and tumors (Horvath, 2013).

DNA methylation data shows modest correlation with other hallmarks of aging and lifespan: cell senescence, the cessation of cellular division, and telomere attrition, the shortening of the DNA sequence at the ends of chromosomes (López-Otín, et al., 2013). Cellular senescence significantly correlates with changes in DNA methylation at certain loci, which may indicate some degree of interplay between the molecular mechanisms, although the nature of the relationship is not clear (Koch, et al., 2013). However, some hallmarks of aging demonstrate a degree of exclusivity from DNA methylation, such as telomere attrition, as epigenetic age increases even in the presence of telomerase, an enzyme that lengthens telomeres (Kabacik, et al., 2018). The greatest correlation between a hallmark of aging and DNA methylation is seen between stem cell exhaustion and patterns of DNA methylation, as DNA methylation is crucial in maintaining the undifferentiated state of stem cells (Li, et al., 2022). These correlations between DNA methylation patterns and hallmarks of aging need to be further investigated experimentally to determine the exact degree of molecular mechanism interplay between the two hallmarks of aging.

3. Histone Modification

Histone proteins can undergo many types of PTMs, such as phosphorylation, acetylation, and methylation. However, histone acetylation and methylation are by far the most researched. (Sterner and Burger, 2000). It is more challenging to track histone modification patterns, as it shows less of an overall pattern as it does specific alterations at certain gene loci. In general, histone modifications are fluid and dynamic in nature which makes it difficult to track (Sen, et al., 2016). Research in *D. melanogaster*, *C. elegans*, and *S. cerevisiae* has demonstrated that sites of modification on histones, or histone marks, are associated with regulation of gene expression, either by promoting or inhibiting transcription (Booth and Brunet, 2016). Like DNA methylation, histone modification regulates gene expression by affecting the density of chromatin packaging (Kouzarides, 2007). However, not all mechanisms of histone modification are conserved between model organisms and mammals, increasing the demand for thorough histone modification research in mammalian cell cultures (Dang, et al., 2009). This section of the review covers known histone acetylation and methylation events that are correlated to aging.

3.1 Histone Acetylation

Histone acetylation loosens chromatin folding due to the acetyl group neutralizing the charge of lysine residues in histone side chains, leading to transcriptional derepression (Kouzarides, 2007). Enzymes called histone deacetylases, such as sirtuins, deacetylate histones (Houtkooper, et al., 2012). A loss of sirtuins has been linked with aging in both *S. cerevisiae* and humans, due to the loss of telomere silencing as histone acetylation increases (Dang, et al., 2009; Michishita, et al., 2008). Global histone deacetylation correlates with increased longevity in *S. cerevisiae* through suppression of oxidative stress responses, as chronic oxidative stress is known to play a role in cell damage, death, and aging (Eisenberg, et al., 2009). Research in *S. cerevisiae* has also shown that acetylation of H3K56 at the promoters of histone genes decreases with age, corresponding to decreased gene expression and loss of core histones (Dang, et al., 2009). Conversely, acetylation at the H4K16 mark in telomeres increases with age, correlating to the loss of silencing in these regions and the arrest of the cell cycle, or cell senescence (Dang, et al., 2009). Premature cell senescence is associated with the loss of telomere silencing and telomere dysfunction (Michishita, et al., 2008), once again demonstrating interplay of histone acetylation and other hallmarks of aging.

3.2 Histone Methylation

Histone methylation is the addition of methyl groups to arginine, histidine, or lysine residues in histone side chains. Compared to DNA methylation or histone acetylation, histone methylation often results in more versatile effects. Furthermore, histone methylation can occur simultaneously with other histone PTMs on the same histone, or

histone methylation may occur exclusively (Greer and Shi, 2012). Henceforth, when combination of histone PTMs occurs on a single histone, the effect on chromatin remodeling and gene expression may be different from when the modifications present exclusively or may differ depending on the amino acid methylated (Greer and Shi, 2012).

Lysine methylations, such as H3K4me3 and H3K27me3, are modifications that significantly influence transcription and gene expression as aging progresses (Sen, et al., 2016). H3K4me3 is traditionally associated with transcriptional activation, while H3K27me3 is associated with transcriptional repression (Rothbart and Strahl, 2014). However, these generalized functions of histone methylation can vary widely between cell types and organisms. For example, global H3K27me3 increases in murine quiescent cells, where quiescence is a hallmark of aging, yet H3K27me3 decreases in *C. elegans* with age (Liu, et al., 2013; Maures, et al., 2011). In murine hematopoietic stem cells, H3K27me3 levels mostly increase with age, but some loci show decreased levels of methylation (Sun, et al., 2014). Similarly, studies in *C. elegans* and *D. melanogaster* show varying patterns of H3K4me3 with age depending on cell type (Booth and Brunet, 2016). Nonetheless, the causal relationship of histone methylation and aging is clear. The ability to methylate and demethylate is a crucial process that decreases with age, where overexpression of enzymes that add methyl groups (methyltransferases) or strip them away (demethylases) have resulted in increased longevity in model organisms, and many of these pathways are highly conserved (Maures, et al., 2011; Greer and Shi, 2012).

It is hypothesized that DNA methylation compensates for defective histone methylation, indicating an interaction between these two types of epigenetic modifications to repress gene expression (Fuks, 2005). Some evidence also suggests that DNA methylation may promote histone methylation, and further research of this phenomenon may lead to a deeper understanding of the order and progress of aging (Bartke, et al., 2010).

4. Chromatin Landscape Remodeling

Chromatin is wrapped around proteins called histones, forming units of nucleosomes. These nucleosomes can be packaged tightly, called heterochromatin which represses transcription, or packaged loosely, called euchromatin which enables transcription. The genome may display various states of chromatin density depending on loci and the phase of the cell cycle. The heterochromatin loss model of aging presents the loss of tightly packed DNA as a cause of aging due to the increase in genome instability (López-Otín, et al., 2013). Research in *C. elegans* and *D. melanogaster* has demonstrated that maintaining heterochromatin is conserved process important for muscle strength and longevity (Larson, et al., 2012).

Changes in the chromatin landscape associated with aging can be attributed to patterns of DNA methylation and histone modification throughout the genome (Bannister and Kouzarides, 2011). For example, heterochromatin was seen to decrease in aging cells and in cells of individuals with Hutchinson-Gilford progeria syndrome (HGPS), a premature aging disease (Greer and Shi, 2012). The HGPS cells also displayed a decreased methylation at the H3K27me3 mark on the inactivated X chromosome (Shumaker, et al., 2006).

Histone methylation may also indirectly contribute to the loss of heterochromatin through the loss of nucleosomes (Booth and Brunet, 2016). Methylation at H3K27me3 downregulates genes that code for histones and has been linked to the loss of core histones with age (Liu, et al., 2013). This may cause the loss of nucleosome occupancy and the loss of chromatin density, possibly resulting in genomic instability, insertions of transposable elements, and an increase in genomic mutations (Liu, et al., 2013). As mutations accumulate, the susceptibility to age-related diseases increases, leading to decreased health- and lifespan.

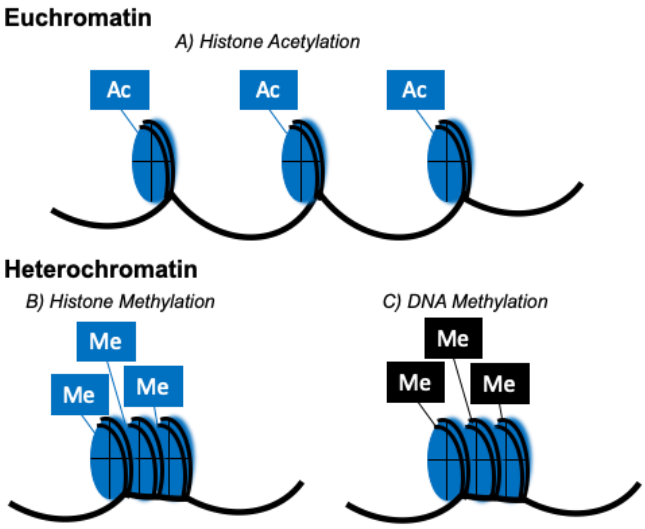

Figure 2. Chromatin Types and the Effects on the Chromatin Landscape. Euchromatin is when DNA is open due to histone acetylation and ready for use via transcription or replication. Heterochromatin is when DNA is tightly wound and inaccessible to proteins important for transcription and replication processes. This DNA tightening is due to histone and DNA methylation.

Similarly, as DNA becomes globally hypomethylated with aging, chromatin becomes less tightly packed (Figure 2), resulting in genomic instability and transposon activation. When heterochromatin unravels into euchromatin, enzymes have easier access to the DNA and gene expression becomes less regulated. Uncontrolled gene expression may lead to the conversion of healthy cells to cancer cells, which is indicative of accelerated age (Horvath, 2013).

Epigenetic alterations also result in the loss of heterochromatin at transposable elements, increasing the probability of transposition and consequently mutations that occur due to transposition and result DNA damage.

Increased histone acetylation also contributes to aging by increasing genomic instability and telomere dysfunction through loss of heterochromatin (Figure 2). Telomeres are ideally maintained permanently in the heterochromatin state outside of DNA replication (Bannister and Kouzarides, 2011). However, telomere damage results in the loss of chromatin density due to the loss of sirtuins (Michishita, et al., 2008). This in turn, may cause the loss of core histones, perpetuating this state of genomic instability (O'Sullivan, et al., 2010). This state of genomic instability can be further perpetuated through DNA methylation and histone modification (Sen, et al., 2016). Thus, we can see that epigenetic modifications and other hallmarks of aging may work synergistically to contribute to the molecular causes of aging.

5. Conclusion

Epigenetic modifications demonstrate correlation with various other hallmarks of aging and shape the chromatin landscape of the genome, regulating gene expression and altering phenotype. While large strides have been made in the field of DNA methylation and its implications on aging, information on histone modification is comparatively lacking. The fluidity of histone modification may offer an easier path toward chromatin remodeling and suppression of age-related ailments than may DNA methylation alone, and the coexistence or mutual exclusivity of some histone marks may be a property to take advantage of in the development of targeted pharmaceuticals. Further research is needed to understand the true relationship between each hallmark of aging and where they show correlation or causation to better comprehend the initiation of cellular decline and to extend healthspan as lifespan increases.

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