Review

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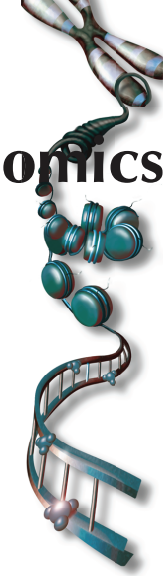
Epigenomics



DNA methylation changes and inflammaging in aging-associated diseases

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Aging as an inevitable phenomenon is associated with pervasive changes in physiological functions. There is a relationship between aging and the increase of several chronic diseases. Most age-related disorders are accompanied by an underlying chronic inflammatory state, as demonstrated by local infiltration of inflammatory cells and greater levels of proinflammatory cytokines in the bloodstream. Within inflammaging, many epigenetic events, especially DNA methylation, change. During the aging process, due to aberrations of DNA methylation, biological processes are disrupted, leading to the emergence or progression of a variety of human diseases, including cancer, neurodegenerative disorders, cardiovascular disease and diabetes. The focus of this review is on DNA methylation, which is involved in inflammaging-related activities, and how its dysregulation leads to human disorders.

Plain language summary: Aging as a natural process is associated with variation in physiological functions. One of the hallmarks of aging is epigenetic changes, which are directly involved in the aging process and aging-related diseases. DNA methylation is one of the epigenetic changes during aging. Consequently, changes in DNA methylation affect various cellular processes and cause age-related diseases. This review discusses the role of DNA methylation in aging processes and age-related diseases.

Graphical abstract:

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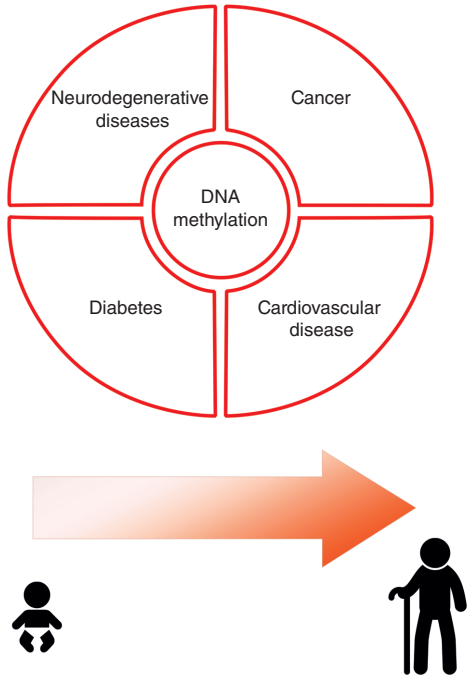

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Aging is a complicated biological mechanism characterized by decreasing physiological processes and increasing risk of aging-related chronic disorders, including cancer, cardiovascular malfunction, and musculoskeletal and neurological diseases [1]. Much research has concentrated on identifying the hallmarks of aging to find possible therapeutic targets for reversing the effects of aging in chronic diseases. Some of these hallmarks are directly associated with the pathogenesis of aging-associated diseases, such as exhaustion of stem cells, senescence, inflammation and epigenetic deregulation [2].

Understanding why the mechanism of aging results in progressively increasing vulnerability to chronic illnesses, disability and frailty has become a public health priority because of the extension of life span and the growing number of older adults in the general population [3]. Strong evidence suggests that inflammation is a risk factor for many age-related chronic diseases and undesirable health consequences. Many potential processes are involved in the mechanism of inflammaging, including genetic susceptibility, cellular degradation, immune dysfunction and chronic infection [4]. The term 'epigenetics' refers to inherited reversible genetic mechanisms like histone acetylation, methylation, phosphorylation and ubiquitination that can alter gene expression without causing fundamental changes in DNA sequences [5,6]. Separate from histone methylation, DNA methylation occurs through the addition of a methyl group to the cytosine ring's fifth position, forming a compound called 5-methylcytosine (5mC). This alteration is common in DNA areas with high CpG dinucleotides [7]. While DNA methylation has been recognized to regulate gene expression profiles for many years, the biological implications are still not thoroughly explored. Biologically and chemically, DNA methylation is a stable epigenetic modification that locks the patterns of long-term gene expression. CpGs in promoter regions are often hypermethylated during aging, but other CpGs undergo hypomethylation [8]. Recent reports have demonstrated the contribution of methylation patterns at specific DNA sites to aging and age-associated diseases and these pattern, therefore, can act as a reliable predictor in age-related processes [9,10]. In this review we highlight the contributions of inflammation and DNA methylation to the process of aging and focus on their implications in aging-associated diseases. Nowadays, due to the increasing global elderly population and the increasing rate of chronic diseases with advancing age, understanding the basic aging pathways can be useful in expanding health span and increasing life quality in older people [11]. Chronic inflammation, epigenetic changes and cellular senescence are considered fundamental aging characteristics [12,13].

Evidence suggests that specific features of aging, such as age-related diseases, can be delayed or reversed through interventions in laboratory models [13]. This important area of study provides distinctive perceptions of the aging process and the opportunity for screening and focused therapies.

In this context, we designed the current review based on a comprehensive literature search through the electronic databases PubMed, Web of Science and Google Scholar up to January 2022. The relative studies were selected using the following search terms: 'inflammation' OR 'inflammatory factor' AND 'aging' OR 'inflammaging' AND 'age-related disease' or 'Alzheimer' or 'Parkinson' or 'cancer' or 'CVD' or 'diabetes'.

Aging & inflammation

Aging is correlated with immunological dysregulation, with the most apparent manifestations including elevated blood levels of proinflammatory mediators in the absence of obvious triggers and a diminished ability to develop an efficient inflammatory response through appropriate immunogenic stimuli [14,15]. Despite issues with the use of high-sensitivity identification proteomics in serum and plasma, recent studies have revealed a list of proinflammatory markers associated with aging [16]. Even though there are no specific risk factors or clinically functional disorders, most older adults have increased levels of age-associated proinflammatory markers [14,17–19]. Despite the crucial physiological function of inflammation as a defensive mechanism against pathogens or foreign substances, chronic and persistent inflammation is harmful to health [20]. Increased proinflammatory status with age is the main characteristic of inflammaging. Inflammaging consists of five stages: low-grade, controlled, asymptomatic, chronic and systemic [21].

Nevertheless, inflammation caused by aging does not represent a controlled inflammatory state; rather, inflammation that occurs in the inflammaging process is considered non-resolving inflammation. In normal conditions, when proinflammatory elements are eliminated in infections and tissue damage, inflammatory responses are stopped and transform into resolving inflammation, a highly active and well-regulated balanced state. However, some factors that are still unclear, such as steady, low-severity stimulation and a long-term, uncontrolled response in target tissues, inhibit the common process of resolution and cause persistent (non-resolving) inflammation. Inflammation during the aging process falls under this category of persistent inflammation [21].

Excessive cytokines are released due to the subsequent inflammatory response, tissue damage and generation of reactive oxygen species that result in oxidative damage. These additional cytokines are primarily produced by cells of the innate immune system but can also be produced by the acquired immune response. In the case of the innate immune response, it is thought that more than any other cell type, monocytes and macrophages are responsible for inflammaging, resulting from the functional shift toward a proinflammatory phenotype and the diminished function of monocytes with aging [22]. Interestingly, in the cellular arm of the adaptive immune system, with aging, both the quantity and variety of peripherally circulating and tumor-infiltrating $CD8^+$ cytotoxic T cells decrease. A recent study identified that inflammaging was linked to a clonal population of $CD8^+$ T lymphocytes that have an exhausted phenotype and aggregate in tissues, promoting nearby cells to adopt a senescence-associated secretory phenotype (SASP) via secreting granzyme K, a process known as immunosenescence [23]. T-cell immunosenescence can cause the maintenance of inflammaging, which includes increased release of proinflammatory cytokines, increased memory and effector cells and reduced Treg cells (possible increased inflammation and autoreactivity). [22]. In particular, highly activated macrophages, natural killer cells, T cells and plasma cells as senescent cells (SCs) are released in the synovial tissue of rheumatoid arthritis patients, leading to increasing chronic inflammation, which determines the outcome of the local inflammatory process [24].

SCs have innate immune system components to detect molecular destruction and produce the SASP. Cytosolic RNA and DNA are major signals that induce the SASP and are modulated by cytoplasmic sensors in the senescence-related inflammatory condition [25,26]. The inflammasome is another important mediator of SASP formation [27]. Inflammasomes are collections of pattern recognition receptors that recognize different damage-associated molecular patterns (DAMP) and either activate the IL-1 inflammatory cascade or induce pyroptosis. SCs may be induced to stimulate tissue regeneration during growth and injury [28]. The SASP promotes the recruitment of immune cells capable of clearing SCs. However, regardless of its primary function, the SASP can provide both benefits and disadvantages. The SASP mediates the tumor suppressor mechanisms of senescence; for instance, SASP ingredients, including IL-8, IL-6, PAI-1 and IGFBP7, promote SC cycle arrest *in vitro* [29]. Furthermore, in a model of fibrosis-related liver cancer, the SASP can serve to develop an antitumor environment by polarization to the M1 tumor suppressor subset [30]. TGF family members, VEGF and chemokines such as CCL2 and CCL20 could further

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extend senescence to healthy neighboring cells, a process called paracrine senescence [27]. SC removal diminishes inflammatory mediator levels in old mice [31], implying that the SASP may also play a role in inflammaging.

Despite mounting evidence on the role of SCs in the aging phenotype, the mechanisms by which SCs stimulate aging and their contribution to age-related diseases in humans remain unknown. The lack of general biosensors for aging *in vivo* may have helped prevent the diagnosis of SCs in humans. However, previous research indicates that SCs have a negative impact on the tissue microenvironment, acting as pathological moderators or aggravators. As a result, it has been proposed that SCs are linked to the aging process and the emergence of age-related diseases via the SASP, which involves the release of a slew of cytokines and growth factors, as well as matrix-degrading compounds, all of which help maintain inflammaging [32]. Age-related SC accumulation, in turn, activates the immune system, and the chronic presence of immune system activation results in decreased SC clearance. Aside from immune cells, mesenchymal stem cells in elderly people are also influenced; studies indicate that a senescent environment may also minimize stemness or differentiation potential through both SASP systemic spread and SASP-related bystander consequences in different tissues [33,34]. Notably, SCs are abundant in all types of age-related diseases, including cancers and neurodegenerative diseases, implying that they may play a role in pathologies by inducing chronic inflammation [33,34]. Furthermore, senescence of endothelial cells (ECs) has been shown to lead to endothelial dysfunction, which is involved as a major stimulus not only in the incidence and development of cardiovascular diseases (CVDs) but also in other age-related diseases, including osteoporosis [35,36].

Epidemiological research has discovered that inflammaging is a risk factor for CVD, malignancy, chronic kidney disease, Alzheimer's disease (AD) and depression; in other words, several disabilities affecting activities of daily living among older adults [37–39]. According to these results, several researchers have claimed that inflammaging is a sign of accelerated aging that should be regarded as a fundamental process in aging biology [40]. Inflammaging is a multifactorial process in which many mechanisms are involved in developing inflammation associated with aging-associated diseases. Gene susceptibility has a crucial role in the process of inflammaging. Large-scale population studies have indicated several genetic variations that influence blood levels of inflammatory markers [41]. Several variants in the region of the *IL-1RN* gene, which codes for an IL-1 receptor antagonist, represent the high number of proinflammatory cytokines (e.g., IL-1β and IFN-γ) which affect the pathophysiology of various diseases, such as insulin resistance and osteoarthritis, through aging [41–44]. A particular mutation in the promoter of *IL-6* resulted in higher expression of *IL-6* in response to inflammatory stimulation, which is associated with significant diseases, including AD and CVD [45,46]. Visceral obesity is another reason for inflammation in aging; in particular, central obesity is associated with a proinflammatory situation [47–49]. Adipocytes can express proinflammatory cytokines (e.g., IL-1β, IL-6 and TNF-α) and also chemoattract monocytes and macrophages via the production of CCL-2 [49]. Infiltration of macrophages in various tissues is associated with chronic inflammation, which can promote many aging-related disorders. In AD and Parkinson's disease (PD), activated macrophages can infiltrate the senile plaques and other specific tissues in the brain [50,51]. Cellular senescence has been characterized as another hallmark of the biological aging process, accounting for inflammaging. In this regard, SCs can accumulate in multiple tissues and induce inflammation, representing various age-related diseases such as cancer, CVD and type 2 diabetes (T2D) [52,53]. These findings demonstrate the contribution of several inflammatory mechanisms involved in the process of aging with age-associated diseases.

Inflammatory mediator genes & genetic predisposition in aging

The inflammaging phenomenon provides novel molecular insights into the interactions between age-related changes and low-grade inflammation during aging, opening up new ways to explore the relationships between inflammation and age-related pathological processes [54–56]. Individuals' inflammatory mediators play a prominent role in aging and the molecular mechanisms that cause age-related inflammatory disorders [56]. Several studies suggest that aging is characterized by low-grade systemic inflammation and that inflammatory indicators are significant predictors of mortality in older adults [11]. Conceptually, genetic inflammation and inflammatory biomarkers are considered one of the main culprits in the progression of aging [12]. For example, variations in immune–inflammatory genes (e.g., *COX*) and cytokines have been thought to play a key role in the risk for AD [11]. Studies have indicated that in rodent models of severe brain injury, COX-2 inhibitors and *COX-2* genetic ablation also reduce neuronal loss [56]. In addition, CVD is a late result of an evolutionary proinflammatory response programmed to oppose infections in earlier life; thus genetic predispositions to inflammation may have opposing effects on CVD and life span. Therefore, genes involved in the regulation of inflammation should be involved in human aging, and high

levels of cytokines and C-reactive protein (CRP) in the serum seem to be a sign of unsuccessful aging [11]. In this section we focus on the inflammaging phenomenon by evaluating the role of genetic inflammation in aging.

Aging is a complex process influenced by several elements, including the environment, genetics and epigenetics. The presence of a chronic proinflammatory state is a common aspect of aging. In the absence of overt infection, the inflammaging phenomenon is a significant risk factor for morbidity and mortality in older adults [12]. Intriguingly, the remarkable trait of inflammaging is characterized by the upregulation of proinflammatory mediators such as IL-1, IL-6 and TNF-α as proinflammatory cytokines, as well as COX and inducible nitric oxide synthases (iNOSs) [4,21,57,58]. In line with this, accumulating evidence shows that continuous up-regulation of these proinflammatory mediators is induced during the aging process owing to an age-related redox imbalance that stimulates many proinflammatory signaling pathways, along with the NF-κB signaling pathway [56,58,59]. Notably, proinflammatory cytokines are important in immune system regeneration with age through downstream NF-κB signaling [59]. Inflammaging occurs when proinflammatory and anti-inflammatory networks become imbalanced as a result of aging. Polymorphisms in the genes encoding these cytokines can adjust their production and affect inflammatory processes [60]. Long-lived adults, particularly centenarians, appear to cope with persistent subclinical inflammation by triggering an anti-inflammatory response, dubbed 'anti-inflammaging' [61].

IL-1α and IL-1β are vital mediators of the stress-induced inflammatory response. Caspase-1 cleaves IL-1β into the active form, while calpain protease activates IL-1α. IL-1R antagonists inhibit IL-1-mediated signaling by downregulating both *IL-1α* and *IL-1β*, which are activated by binding to the IL-1R [62]. In this context, accumulation studies suggest that in old individuals, including centenarians, IL-1R antagonist (IL-1Rα) exhibits an age-related increase, while there is no discernible age-related trend in IL-1β [59]. Interestingly, some *IL-1* haplotype carriers generate increased *IL-1* gene polymorphisms that correlate with earlier onset or severity of AD advancement but not with osteoporosis [63–65]. No specific variation in the *IL-1* gene confers an advantage in centenarian survival; however, a polymorphism in the *IL-1* gene was associated with reduced life span in elderly Swedish males [66,67]. Hence, because *IL-1* gene variants would seem to increase the chance of age-related disorders, IL-1R blockers as a recombinant medicine could be a clinical solution for inflammation control [59].

IL-6 is one of the critical cytokines involved in aging and age-related disease, and is thus termed the 'gerontologist cytokine' [61,68]. In normal conditions, the level of IL-6 is low in the blood, but it rises with age and in people who have vulnerability indicators and chronic conditions, with a high risk of death [59]. For instance, a study conducted on patients with stage 2–5 chronic kidney disease indicated that high serum IL-6 is related to a history of CVD and predicts cardiovascular events. This study reported that patients who were homozygous for the risk allele (C) of the –174 G/C polymorphism had higher levels of IL-6 compared with those who had other genotypes [69]. Therefore, evidence-based IL-6 blockers can improve age-related diseases [70,71].

TNF-α is another key player in inflammaging and acts as a proinflammatory cytokine [72]. TNF-α starts the inflammatory cascade and is associated with several age-related diseases [73]. In a study by Bruunsgaard *et al.*, TNF-α was found to be an independent prognostic marker for mortality in people over the age of 100 years, implying that it has unique biological effects and serves as a marker of frailty in the elderly [74]. Another study investigated the associations of the *TNF-α* –308 G/A and *IL-10* –1082 G/A (promoter region) polymorphisms with aging and survival selection in the Jordanian population. The results showed no significant differences in the genotype and allele frequencies of *TNF-α* gene variants between the two groups, whereas the *IL-10* genotype and allele frequencies were significantly associated with longevity in men but not in women [75]. However, the study results indicated that TNF-α was linked to death in men but not in women, but low-grade IL-6 increases were strongly linked to mortality in both genders. These data suggest that persistently increased levels of TNF-α and IL-6, at least in older populations, have different biological activities that induce age-related disease and cause mortality [73]. Based on this research, further evaluations are needed to elucidate the relationship between TNF-α and the risk of age-related disease, but it is possible that the use of TNF-α blockers may ameliorate the progression of some age-related disorders [76].

As mentioned above, the COX and iNOS enzymes are upregulated during aging, which contributes to proinflammatory status via NF-κB activation during the aging process [58]. COX is a critical enzyme in the production of the inflammatory mediators prostaglandins. An inducible variant of COX, known as COX-2, has been associated with age-related diseases such as cancer; based on epidemiological findings, the use of NSAIDs as prototypical COX inhibitors is related to a lower incidence of numerous cancers, including colorectal cancer [77]. Additionally, NSAID-mediated COX inhibition may decrease inflammation and amyloid-β buildup *in vivo* by interfering with a potential feed-forward inflammatory mechanism [56].

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On the other hand, the use of NSAIDs is associated with some adverse effects, including gastrointestinal problems. In the absence of sufficient randomized studies, it is too soon to offer clinical advice, but there are several promising future study directions [78]. In this context, NF-κB is considered the main transcription factor with a critical role in the inflammatory processes that, after phosphorylation of IκB, translocates to the nucleus, promoting proinflammatory genes [79].

In summary, inflammation is required to deal with harmful agents and is essential for survival. However, the low chronic inflammatory status in aging may be one of the mechanisms that result in age-related disease. Overall, in the case of centenarians, they have a higher frequency of genetic markers linked to better inflammatory control [77].

Epigenetic modifications

Over the last two decades, there has been a surge in interest in studying the biological foundation of human longevity to better understand the complex biological and environmental factors that influence the quality and rate of human aging, along with stochastic elements [80]. Epigenetics influences gene expression status without altering the DNA structure [81]. Most nuclear functions are affected by changes in epigenetic mechanisms, including gene transcription and silencing, DNA replication and repair, cell cycle progression, and telomere and centromere structure and function [82]. and compelling evidence suggests that epigenetic changes are one of the pillars of the multifaceted aging process [82,83]. These modifications can be stratified into three general categories of epigenetic alterations: DNA methylation or hydroxymethylation, histone modifications and chromatin remodeling. Moreover, recent investigations have recognized histone variants, miRNAs and lncRNAs as further epigenetic indicators [81].

Ultimately, a set of epigenetic changes that occur during aging result in improper gene expression, reactivation of transposable components, and genomic instability by changing local accessibility to the genetic material [83]. Currently, DNA methylation and histone methylation, acetylation, ubiquitination and phosphorylation as post-transcriptional modifications are the best-studied epigenetic modifications [84]. Chemical modifications in the nucleosomes, which are essential for chromatin-dependent gene regulation, are carried by histone proteins as well as DNA molecules [85]. Histone modifications have the potential to be both repressive and activating because they have an impact on how tightly chromatin folds together and recruits different effector proteins [86]. DNA methylation contributes to the silencing of gene expression: the main mechanism consists of the methylation of DNA and the following recruitment and attachment of proteins that recognize methylated DNA. Then, condensed chromatin becomes stabilized as a result of these proteins' interactions with chromatin remodeling and histone deacetylase complexes (Figure 1) [87].

Inhibiting epigenetic changes in the aging process is one of the most interesting therapeutic approaches to reducing aging and age-related disease; it is not surprising that there are currently new hopes for the use of so-called 'epigenetic drugs' that can control the activity of enzymes that can alter the epigenetic state in the treatment of diseases [88]. Studies have implicated the importance of epigenetic modification in CVDs, cancer and neurodegenerative disorders (e.g., AD, PD and Huntington's disease) and have described novel therapeutic interventions for these diseases [23,81,89]. In animal models of neurodegenerative disorders, small drugs such as histone deacetylase inhibitors can pass the blood–brain barrier, delaying the onset and progression of symptoms, and some epigenetic medications have been shown to be effective in boosting cognitive performance and preventing neurodegeneration in AD models [89]. Given the importance of epigenetic changes in cancer, drugs that target epigenetic modifying proteins and pathways have been created and are currently being used in the clinic; the histone deacetylase inhibitors and DNA methyltransferase inhibitors are two notable examples [23]. The results of the current studies may assist in identifying new epigenetic treatments and strategies for managing inflammaging and delaying aging and age-related disease.

In the next section, we will discuss in detail how DNA methylation arising during the life span can influence aging and aging-associated disease.

Mechanisms of DNA methylation in aging cells & tissues

DNA methylation is one of the heritable epigenetic hallmarks during aging [2]. This epigenetic modification induces biological changes by affecting the gene expression (either induction or suppressing) through influencing DNA-binding proteins and chromatin structure [90,91]. The mechanism underlying DNA methylation is characterized by the transition of a methyl (CH_3) group from S-adenosyl methionine to the fifth position of cytosine nucleotides, creating 5mC [92,93]. These processes involve three DNA methyltransferases: DNMT1, DNMT3A and DNMT3B [90]. DNMT1 acts as a maintenance DNA methyltransferase that primarily replicates methylation

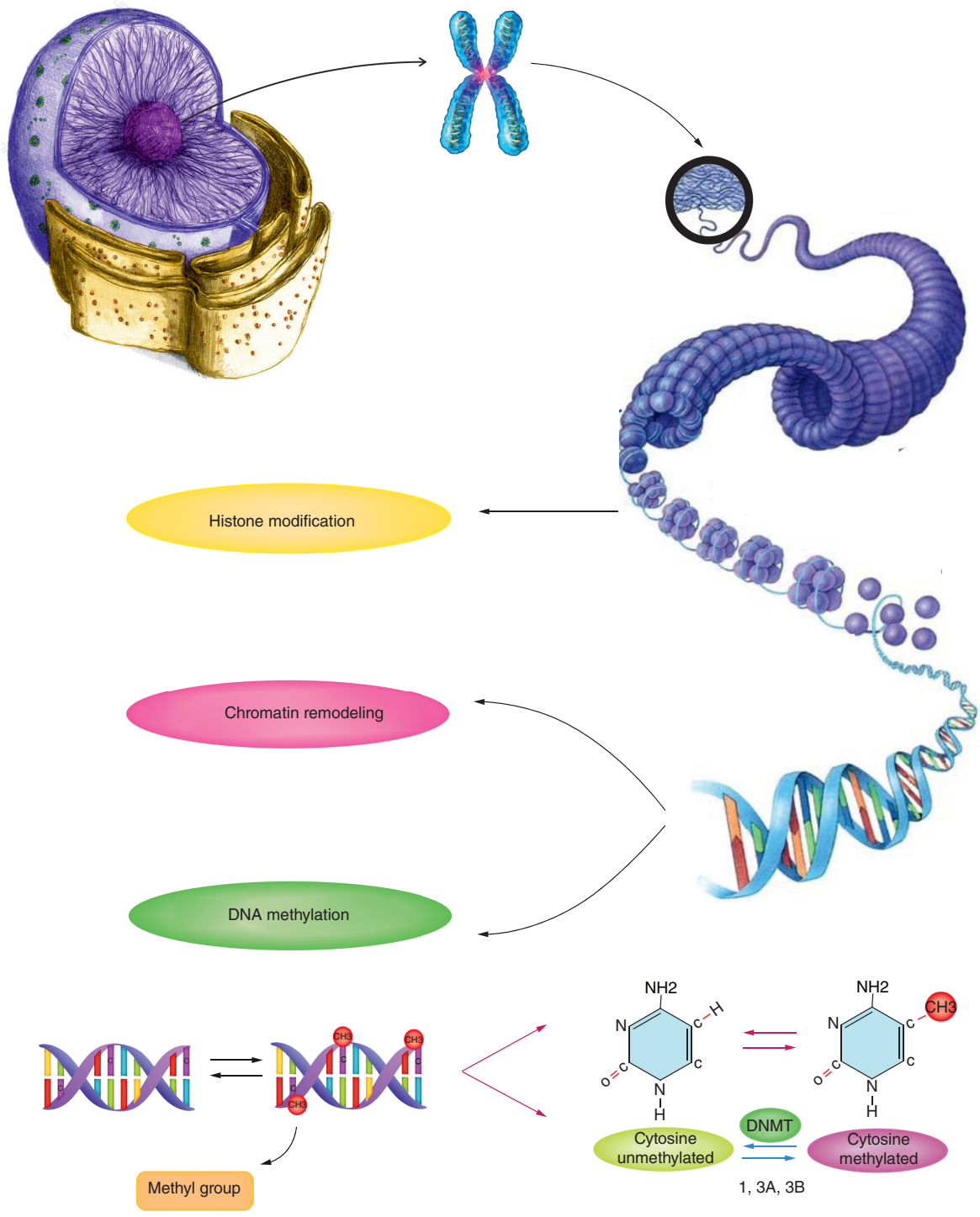

Figure 1. Epigenetic changes during aging. Aging-related epigenetic alterations include histone modifications, chromatin remodeling and DNA methylation that cause the general changes in chromatin structure in senescent cells. In the DNA methylation process, DNA can be methylated directly by covalently attaching a methyl group to the fifth position of the cytosine ring, resulting in the formation of 5-methylcytosine. The transfer of a methyl group to the unmethylated cytosine is mediated by DNMT1, DNMT3A and DNMT3B.

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patterns, whereas DNMT3A and DNMT3B can methylate previously unmethylated DNA, a process known as *de novo* methylation [90,92]. The activity of DNMT1, DNMT3A and DNMT3B primarily forms methylated cytosines on CpG dinucleotides in mammals. CG sites are under-represented in mammalian species, and they tend to cluster in areas where gene promoters are found and where CG frequency is unusually high ('CpG islands'). In contrast to the hypermethylated portion of the genome, 20–40% of the approximately 28 million CG sites in the human genome are usually unmethylated [94,95]. DNA methylation is vital in preventing aberrant X-chromosome activation, repressing 'parasitic' DNA and averting aberrant centromere recombination. These functions of the methylation process are vital for maintaining whole-genome integrity at various levels [90,94].

In the case of aging, a large body of evidence affirms the alteration of DNA methylation patterns during a lifetime [93,96]. When people get older, their DNA methylation pattern changes in two ways: global 5mC decreases, and specific loci become hypermethylated (primarily CpG island promoters) [97]. DNMT1 gradual loss or incorrect enzyme targeting by other cofactors (or both) is most likely the primary cause of aging-related global DNA methylation loss, although this hypothesis requires confirmation [84,98]. Upregulation of the *de novo* DNA methylase DNMT3B, which was previously discovered in cultured fibroblasts, could also be a normal cell response to DNA methylation loss at repetitive DNA sequences [84,98]. For the time being, evidence indicates that the gradual loss of methylation or global hypomethylation occurs in bronchial epithelial cells and leukocytes with age [97].

Conversely, promoter-specific hypermethylation is also observed in various aged tissues such as colon and gastric mucosa [97,99]. For instance, a cohort study conducted on 784 men at approximately 3- to 5-year intervals showed that aging was associated with a reduction in methylation of *GCR*, *iNOS* and *TLR2* and also with an increase in methylation of *IFN-γ*, *F3*, *CRAT* and *OGG*; the decreased *iNOS* DNA methylation may be related to increases in the inflammatory response in aging by enhancing *iNOS* gene expression [100]. Moreover, Bollati *et al.* showed that DNA methylation in 718 elderly individuals (55–92 years) studied at 8-year intervals was associated with a progressive loss in repetitive elements dispersed throughout the genome [101]. In addition to the mentioned factors involved in inflammation, CRP is considered a sensitive marker in low-grade inflammation. A meta-analysis of epigenome-wide association studies conducted on serum CRP determined 58 DNA methylation sites that are remarkably associated with levels of CRP in individuals of European or African-American ancestry [102]. Another study indicated that increased blood CRP levels, according to Mendelian randomization analysis, should lead to altered CpG methylation [103]. Moreover, the results of another study highlight the potential of DNA methylation proxies for measuring chronic inflammatory status, which supports the idea that chronic inflammation may contribute to neurodegenerative brain alterations that underlie disparities in cognitive capacity in later life [104].

Collectively, depending on the tissue and gene, both increases and reductions in methylation occur as people age. These alterations can have pathological repercussions, contributing to the development of cancers and autoimmunity as people age and the development of other illnesses. As a result, while aging can affect DNA methylation, alterations can also affect aging [90]. Although current research has suggested new avenues toward understanding the causal association between DNA methylation and aging, further investigations are needed in this context.

The following sections discuss the associations between age-related DNA methylation and age-related disease.

Crosstalk between DNA methylation changes, inflammaging & aging-related diseases

DNA methylation mechanisms in the management of aging and the development of pathologies associated with inflammaging have aided the groundwork for a better understanding of a variety of diseases. In aging organisms, some epigenetic mechanisms, such as DNA methylation accumulation, contribute to age-related changes in gene transcription resulting in gene transcription and translation and the destruction or stabilization of molecular components [105]. Here we summarize the essential roles of DNA methylation modifications associated with inflammaging and a selected range of medical conditions, focusing on certain cancers, neurodegenerative diseases, CVDs and diabetes.

Cancer

Chronic inflammation is a vital contributor to carcinogenesis; according to recent estimates, inflammatory pathways are directly involved in approximately 25% of all malignancies [106]. Large-scale investigations have discovered a strong link between circulatory inflammatory variables and the risk of several cancers [107–109]. Carcinogenesis may be facilitated by dysregulation of the inflammatory immune response through various mechanisms. Inflammatory environments – including elevated cytokines, chemokines and active oxygen and nitrogen species – lead to DNA

mutations, epigenetic alterations and genomic instability, contributing to tumor onset [110–112]. Tumor propagation involves the proliferation of genetically changed cells; increased tumor size, additional genetic changes and the tumor's spread from its original site to several sites are affected by chronic inflammation and oncogene-induced senescence [113].

Other research has suggested that inflammaging potentially can generate epigenetic changes in blood leukocytes which are crucial in the development of carcinogenesis caused by inflammation [111,114,115]. Blood leukocyte methylation is a vital part of the body's immune response and inflammatory processes [115–118], both of which are associated with various cancers [116,117].

Evidence has shown that promoter landscapes are reorganized during oncogene-induced senescence by the recruitment of enhancer components such as BRD4 near SASP genes [118]. Other chromosomal modifications include downregulated histone H3K9 dimethylation at promoter sequences of important SASP elements, and upregulated histone variant macroH2A1 is influenced in the SASP regulatory system [119,120]. H2AJ, a histone variant found in SCs, is also involved in SASP formation [121]. DNA methylation is commonly seen in non-neoplastic tissues in inflammatory-related cancers; unlike genetic mutations, minimal amounts of methylated DNA can be detected in tissues [122]. Due to DNA hypermethylation or epigenetic modulator dysregulation, the injured cell may multiply endlessly during tumor development. Methylation of promoter regions related to tumor suppressor genes (e.g., *VHL*) is corrected with angiogenesis and increased blood supply to the tumor environment [123]. Epigenetic modification of apoptosis-related genes or critical actors in the cell cycle, such as *CDKN2A*, hypermethylation of which is seen in many malignancies and results in loss of function, can disturb cell death [124–126]. More aberrant proteins, such as RUNX1, RAR and CBFB, have been linked to leukemogenesis [127].

Based on the above, aberrant methylation can be used as a marker to estimate the risk of cancer. One of the landmark studies showed that DNA methylation is observed in both cancerous tissues and non-neoplastic digestive tissues and that the rate of methylation is accelerated with inflammaging [122]. Although the source of the aberrant methylation or stimulus that initiates this process is yet to be verified, one possibility is that it is a function of SCs [128]. *De novo* methylation of select CpG islands occurs in tumor tissues and increases with age [129,130]. The pattern of overall DNA methylation has been shown to be highly conserved with increasing age between human and mouse species, and CpG islands that are hypermethylated in an age-dependent manner are associated with EZH2 [131]. However, initial observations showed that *de novo* methylation of select CpG islands occurs primarily in promoters of tumor suppressor genes and is the result of growth selection; it now appears to be an extensively planned process that may relate to polycomb complex targeting [132,133]. Despite the presence of more than 13,000 unmethylated islets of CpG in the human genome, about 2000 of them are labeled with polycomb, a protein complex that acts as an inhibitor by inducing local heterochromatinization [134]. In tumors, this complex is probably responsible for applying the *de novo* methylases DNMT3A and DNMT3B [135,136], which appear to cause the abnormal modification observed at these sites.

Inflammaging stimulates markedly aberrant methylation patterns which are correlated to colorectal cancer [111,114,115]. A broad pattern of increased DNA methylation has been found not only in inflammatory but also in malignant epithelial tissue [137,138]. Higher levels of DNMT1 are related to increased methylation. Guo *et al.* identified an increase in *DNMT1* expression along with DNA hypermethylation in some human colon cancer cell lines (HT29, LoVo and SW480) and publicly accessible data from colorectal adenocarcinoma patients [139]; because the incidence of colorectal cancer rises with age, a thorough examination of the effects of DNMT1 depletion appears to be required. By monitoring SssI methylase in numerous tissues and lowering the indications of aging in heterozygous *DNMT* null mice, Yung *et al.* discovered a pattern of enhanced DNA methylation [140]. As a significant risk factor for colorectal cancer, colorectal inflammation is strongly associated with *p16INK4a* methylation [141]. In colonoscopic biopsies of inflammatory rectal mucosa, Yang *et al.* [142] discovered hypermethylation in the *p16* promoter region, while Foran *et al.* [143] proposed that hypermethylation of the *p16INK4a* promoter region takes place in the neoplastic advancement of ulcerative colitis related to colectomy samples. In addition, proinflammatory cytokines have been reported to be prone to altered expression in SCs through aberrant DNA hypermethylation in prostate cancer and, as a result, to change the regulation of additional cancer-related genes [144,145]. Prostate cancer is thought to be caused by genetic and epigenetic alterations influencing genes that regulate inflammaging [146]. Environmental carcinogens can cause aberrant DNA methylation in inflammatory genes [147–149], and this aberrant methylation is a strong determinant of cancer rates [150,151]. Environmental factors such as oxygen stress can directly affect the production of aberrant DNA methylation patterns by recruiting DNA methyltransferases into polycomb target genes, thereby elevating a profile of tumor-like methylation [152]. The conversion of chronic inflammation

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to cancer through DNA methylation can be linked to the major pathways of NF-κB and STAT3 [153]. One of the downstream targets of this pathway is IL-6 signaling, which is suppressed by treatment with the DNMT1 inhibitor 5-azadeoxycytidine [142]. These cases suggest the direction of research into DNA methylation as a plausible option for particular pathways through which environmental toxins and inflammaging can lead to the development of cancer risk.

Neurodegenerative diseases

One of the newer expanding epigenetic applications is the association of methylation modifications with an increased incidence of several age-related neurological disorders or diseases, including AD and PD [154]. These disorders show the involvement of DNA methylation aberrations which can occur due to mutations causing partial loss of function in genes encoding methylation factors or mosaicism for X-linked recessive mutations, and are correlated to chronic inflammation which, although not causative, may significantly affect pathogenesis [155]. Experimental evidence in cell culture and animal models also shows that impaired expression and altered function of DNA methyltransferases modulates neurodegeneration. For instance, overexpression of *DNMT3A* has been shown to cause neurodegeneration and apoptosis, while depletion or mutation induces loss of function, and DNA methyltransferase inhibitors decrease the processes of apoptosis in motor neurons [156]. Methylation modifications could be relevant mechanisms mediating the onset and progression of AD, and specific loci such as *PSEN1* have been found to be demethylated in AD subjects [157]. The principal epigenetic alteration of DNA that inhibits gene expression is cytosine methylation at DNA CpG [158]. Hypomethylated DNA was identified in the temporal neocortical nuclei of the affected twin in a monozygotic pair discordant for AD, showing that aberrant methylation patterns enhance the likelihood of AD [159]. Late-onset AD (LOAD) is one of the most common neurological disorders and, unlike the hereditary (monogenic) forms of the disease, has been attributed to a number of risk factors. Different biochemical pathways implicated in LOAD have been associated with differential DNA methylation of certain gene promoters [155]. For example, in a case–control study in Colombia, 50 individuals with LOAD and 50 age- and sex-matched control individuals were evaluated to assess DNA methylation patterns in the region of *BIN1*. The results suggest that loss of DNA methylation in CpGs in *BIN1* may play a vital role in *BIN1* expression and may be a biomarker to identify individuals at high risk of developing LOAD [160]. Neuroinflammation is a common occurrence in LOAD [161,162]. Inflammation has been observed in the brains of patients with PD and AD for several years [163]. However, it is largely unknown whether the inflammation processes in these etiologically distinct syndromes account for specific components of the disease or are associated with pathology in response to abnormal protein accumulation or signals from damaged neurons.

Recently, mRNA expression of inflammatory cytokines and immune system mediators has been investigated in the human frontal cortex in middle-aged adults and LOAD patients in stages I–II/0(A), III–IV/A–B and V–VI/C. Step-dependent changes in the expression of various components have been found. In the early stages of I–II/0(A), for example, *IL-1B* and *IL-6* mRNA expression peaked, then declined in the late stages of V–VI/C disorder [164]. Research has highlighted the need to study the sites of complete methylation in DNA promoters and flanking regions and to demonstrate that the production of certain cytokine genes is related to imbalance or aberrant DNA methylation in the brains of LOAD patients [164]. Furthermore, while comparing blood levels of TNF-α in controls and AD patients, one study found considerable hypermethylated CpG in the brain [165]. Facilitated *TNF-α* expression, which leads to higher protein levels, may have a role in the pathogenesis of AD. TNF-α causes neural death through the TNF p55 receptor, which is structurally organized in a pan-neural pattern, and inflammation through the TNF p75 receptor [166,167]. As a result, prolonged activation of the TNF-α receptors p55 or p75 in the brain will have detrimental implications [168]. TNF-α upregulation is prevented by a high level of methylation, which protects neurons against TNF-α-related cell death and an unrestricted inflammaging condition in the brain. In one study, TNF-α levels in the hippocampus were considerably higher in patients with mild cognitive impairment and AD compared with age-matched controls [169].

Furthermore, reduced global methylation has been documented in the hippocampus of patients with AD [170]. Among the many cytokines involved in inflammaging processes in neurological disorders, TNF-α seems particularly important. TNF-α in the peripheral blood plays an important function in immune defense, has a vital role in the cytokine cascade and is a major protein that helps the body respond quickly to infections. Because of its minimal promoter methylation, TNF-α in the blood can be modulated quickly during infectious stages [165]. The methylation status of a promoter regulates the binding of transcription factors and expression levels [158]. One of the first studies based on the DNA methylation hypothesis in PD examined *TNF-α* promoter DNA in the cortex, striatum

and substantia nigra (SNpc). Methylated DNA was found significantly in the SNpc, indicating that the *TNF-α* promoter was more active in the SNpc, while less promoter activity was expected in the cortex or striatum [171]. The *TNF-α* promoter has several potential transcription factor binding sites for the activation of NF-κB, AP-1, AP-2 and Sp1, and binding of AP-2 and Sp1 has been found to be sensitive to methylation [172,173]. Methylation of specific CpGs in the *TNF-α* promoter reduced the binding of transcription factors AP-2 and Sp1, suppressing *TNF-α* promoter activity [171]. Although no obvious differences were found between PD patients and healthy individuals, these data are consistent with the hypothesis that the SNpc is particularly susceptible to inflammatory lesions, and provides clarity for early histopathological studies that detected increased microglia and *TNF-α* expression in the SNpc [174]. In addition, TNF-α and TNF-α1 receptor levels (TNFR1, p55) were increased in the SNpc of PD patients [175]. According to the hypothesis that elevated TNF-α is involved in the pathophysiology of PD, mice with TNF-α deficiency are slightly resistant to neurotoxic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the murine model of PD [175]. Deficiency in both TNFR receptors (*TNFR* double knockout) suppresses microglia activation in these mice and fully preserves against MPTP-induced dopaminergic neurotoxicity [176,177]. Therefore, less methylation of the *TNF-α* promoter can increase the sensitivity of dopaminergic neurons to TNF-α-mediated inflammaging reactions in neurodegenerative diseases. However, further studies on the role of methylation of genes involved in inflammaging are needed to determine their exact role in the development and progression of neurodegenerative diseases.

Cardiovascular diseases

Atherosclerosis is the major reason of coronary artery disease and stroke, and is classified as a chronic inflammatory disease among a wide range of CVDs [178]. Hypermethylation of multiple genes (e.g., *TFPI2* and *ERa*) has been detected in atherosclerotic plaques and is linked to inhibited gene expression [179,180]. Hypermethylation of global genomic DNA has also been linked to the incidence of CVD-related diseases [181]. Researchers discovered that high levels of genomic DNA methylation were the most substantial predisposing factor for CVD mortality after controlling for aging, CVD and diabetes [182]. One study found that hypermethylation was present in 13 individuals who died of CVD and in patients with inflammation (high CRP values); according to this study, increased inflammation and mortality in chronic kidney disease are also linked to hypermethylation of global DNA [181]. Hypermethylation was found to be the most significant risk factor for CVD-related death. A disease-related epigenetic component was also found in coronary artery disease: Sharma *et al.* verified angiographically that patients with coronary artery disease had enhanced amounts of genomic DNA methylation in peripheral blood cells compared with healthy individuals [181].

In addition, as atherosclerosis progresses, the proliferation of vascular smooth muscle cells and the increase of inflammatory cells in progressive fibromuscular lesions may either result in methyl group loss or upset the global genome methylation balance due to faulty maintenance of the DNA methylation pattern. Consequently, predominant DNA hypomethylation is achieved [183]. In the pathogenesis of atherosclerosis, inflammaging involves a complex relationship between inflammation and the status of DNA methylation. Several proinflammatory cytokines are regulated by methylation; lipopolysaccharides, for example, lower tumor *TNF-α* promoter methylation and consequently increase macrophage *TNF-α* expression. The stimulatory actions of lipopolysaccharides increase *TNF-α* expression when 5-azadeoxycytidine is administered [184].

Furthermore, 5-azadeoxycytidine has been found to have a stimulatory effect on *IL-6* expression in human breast cancer *in vitro* [185]. Inflammatory cytokines, in turn, cause aberrant methylation of the gene; this can affect the expression of vital regulatory genes and stimulate a proatherogenic cellular profile, all of which are crucial in the destruction of endothelial cells, the proliferation of abnormal vascular smooth muscle cells, formation of extracellular matrix and CVD inflammation [183]. By promoting *DNMT1* gene expression and hence metabolic activity, IL-6 influences DNA methylation [186]. In a human multiple myeloma cell line, IL-6 also elevated methylation of the *p53* promoter, a critical tumor suppressor gene, lowering *p53* expression via activating *DNMT1* expression [187]. In addition to its action on DNA methyltransferases, IL-6 can inhibit the 'suppressor of cytokine signaling' (SOCS) family proteins through hypermethylation. SOCS is a family of proteins that play a role in the downregulation and upregulation of transcription of inflammatory cytokine pathways such as IL-4, IL-6, interferons, growth hormone, thrombopoietin and prolactin. Silencing SOCS increases the cytokine signaling response, resulting in severe inflammation and the persistence of an aberrant cycle of inflammaging and gene hypermethylation [188].

Some evidence suggests that the atherosclerotic process is harmed by effector T cell-based responses [189]. T-cell activation plays a major role in plaque instability, which may lead to disruption of plaques and the onset of acute

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coronary syndromes [189]. On the other hand, Treg cells are specialized in suppressing immune responses associated with T-cell pathogenicity and maintaining T-cell homeostasis [190]. According to recent research, epigenetic modifications in a highly conserved region of the *FOXP3* gene (the Treg-specific demethylated region) can be useful for detecting a stable Treg phenotype [191,192]. $FOXP3^+$ Treg cells play a crucial role in suppressing the atherosclerotic inflammatory process by releasing cytokines like IL-10 and TGF-β and inhibiting other cellular targets such as dendritic cells, monocytes/macrophages, B cells and natural killer T cells [193]. Several investigations have revealed that the stability of *FOXP3* expression in Tregs is naturally regulated by DNA methylation [192,194,195]. Demethylation of the human *FOXP3* gene in the Treg-specific demethylated region is limited to Treg cells, enabling them to be differentiated from other kinds of peripheral blood cells and tissue cells in analysis [192,196]. In numerous clinical situations, demethylation analysis in the *FOXP3* Treg-specific demethylated region has been regarded as a potential rapid screening approach for Tregs [197,198]. Recently we reported diminished *TSDR* demethylation and reduced expression of *FOXP3* in patients with newly diagnosed rheumatoid arthritis [199].

Diabetes

It is hypothesized that DNA methylation regulates human metabolism and that epigenetic changes contribute to the pathogenesis of T2D [200,201]. Suppression or upregulation of several differentially methylated genes decreases β-cell proliferation and glucose-stimulated insulin release [202,203]. Bacos *et al.* discovered more than 50% similarity among genes linked to aging-related alterations in terms of epigenetic modifications in leukocytes and pancreatic cells and that a subset of methylated genes influenced insulin production in distinct ways [204]. Vanderjagt *et al.* identified that the blood of pre-T2D patients could include differential DNA methylation, indicating the possibility of earlier DNA methylation through T2D progression [205]. Eliminating pancreatic β-cells is associated with the progression of T2D with some hallmarks such as hyperglycemia and obesity-related insulin resistance, characterized by infiltration of inflammatory cells into various tissues [206]. Obesity-related inflammation switches from an anti-inflammatory to a proinflammatory condition, which is linked with the upregulation of proinflammatory cytokines [207]. Guenard *et al.* discovered that genes implicated in glucoregulatory and inflammatory disorders were differentially methylated among siblings born before and after maternal biliopancreatic diversion (denoted as BMS and AMS, respectively) [208]. They also discovered lower abnormalities in heart metabolic gene methylation and inflammatory regulation pathways in AMS compared with BMS siblings [209]. Maternal obesity surgery is related to increased methylation of various locations in genes implicated in proinflammatory signaling of cytokines [210]. Previous reports indicated the contribution of hypermethylated or hypomethylated genes involved in T1D and T2D among differentially methylated sites (DMS) of BMS and AMS siblings, including *HLA-DQB1*, *IGF-2*, *INSR*, *FTO* and *TNF* [210]. Gene methylation can mediate different gene expression levels through the 5′ and 3′ UTRs, associated with increased gene expression, while methylation in the promoter regions is related to gene regulation [211]. The reduction of confounding factors due to genetic modification and lifestyle from one pregnancy to the subsequent pregnancy and siblings born in different conditions of maternal obesity and intrauterine environment confirm the epigenetic regulatory role of genes related to inflammation and immunity in determining offspring phenotypes [210]. It is hypothesized that the recent significant increases in the prevalence of age-related T2D and obesity may be the result of fetal events contributing to lifetime vulnerability to these conditions [208]. Previous research has shown modifications in the function of methylated genes after bariatric surgery. Barres *et al.* [212] showed differences in the methylation of promoter *PGC-1a* and *PDK4*, while Kirchner *et al.* [213] illustrated enhanced methylation in *PDK4*, *IL-1B*, *IL-6* and *TNF* promoters in patients undergoing Roux-en-Y gastric bypass. Remarkably, a link has been established between acquired senescence caused by SASP regulation and main regulator alterations such as diabetes complications, biological aging, endoplasmic reticulum and oxidative stress [120,214]. These findings imply that the SASP may play a role in the endothelial dysfunction that characterizes aging and T2D. A recent hypothesis suggests that the accumulation of SASP-expressing cells may cause diabetes and its vascular consequences [215,216]. Diabetes cells and tissues have chronically stimulated SASP genes, including *IL-1α*, *IL-1β*, *IL-6* and *TNF-α* [217]. Moreover, the most inflammatory cytokines produced by ECs and leukocytes during the hyperglycemia implicated in the vascular diabetic complications are SASP-secreted particles, implying that the SASP plays a vital role in diabetes-related chronic systemic inflammation [218,219]. Besides, involved pathways in inflammation, T1D, T2D, and IGF1 signaling, as well as various methylated genes such as *HLA-DQB1* associated with T1D and *FTO* and *ATP10A* associated with T2D, were discovered when comparing sibling BMS and AMS [208]. The relationship between aging and DNA methylation is well known. An intensive epigenome association analysis revealed that *FTO* is more methylated in diabetic patients' peripheral blood lymphocytes than in nondiabetic individuals [220]. Subsequent

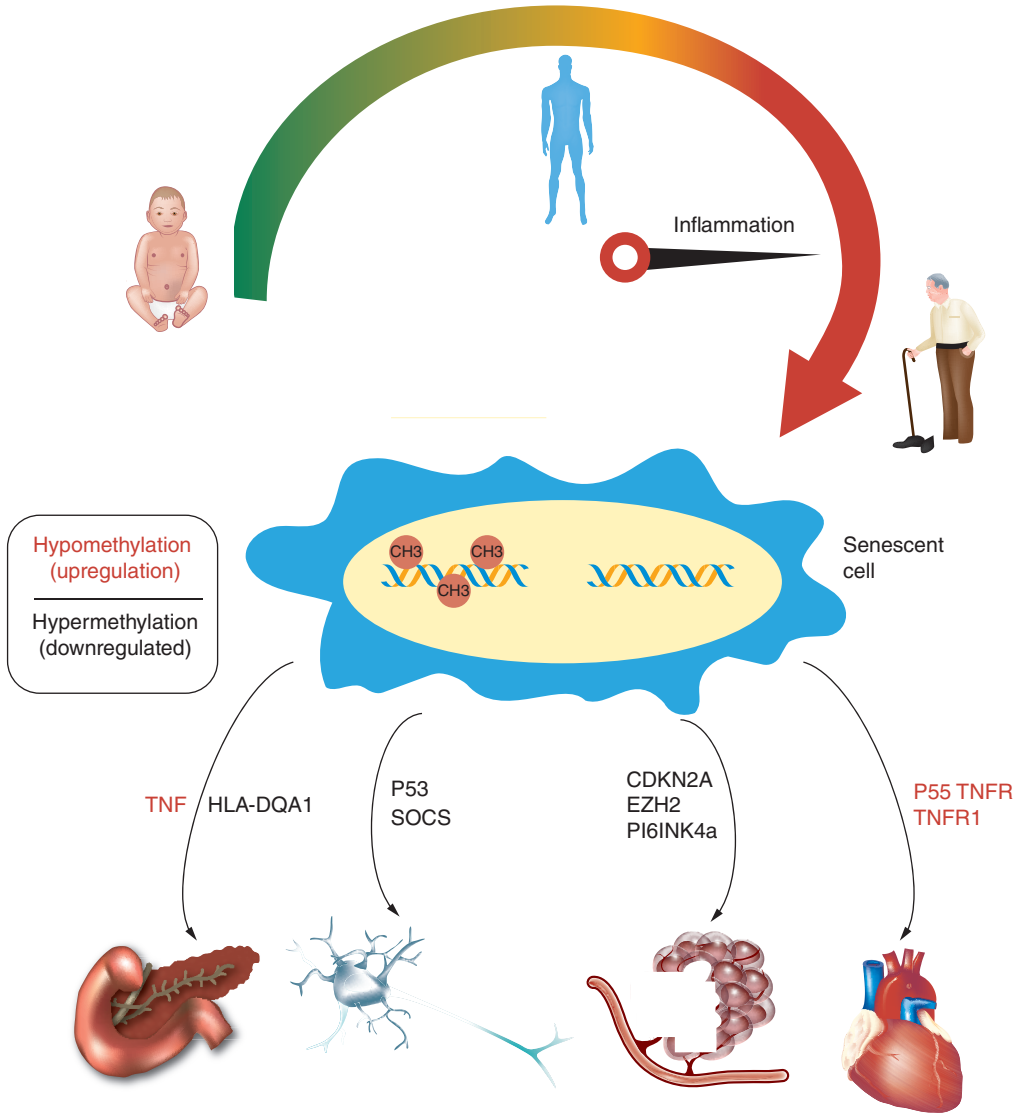

Figure 2. Schematic showing DNA methylation changes and aging-related diseases. Alterations in DNA methylation can occur with age. Accumulating these changes may affect the expression status of various genes; these dysregulations in gene expression contribute to multiple diseases, including neurodegenerative diseases, cancer, diabetes and cardiovascular diseases. Hypermethylation (downregulation) and hypomethylation (upregulation) of DNA in specific genes are shown in red and blue, respectively.

research found a methylation pattern for a CpG site within an intron of the *FTO* gene in diabetic and control young subjects but not in older subjects [221]. It was recently discovered that a different *FTO* site in a considerably older sample population seemed to show DNA methylation differences in patients with T2D and metabolic syndrome; taken together, these results suggest that *FTO* gene methylation in peripheral blood lymphocytes could be used as a biomarker for T2D or metabolic syndrome [222], although differences in DNA methylation between T2D and metabolic syndrome patients and controls may be more prominent in young adults. DNA methylation may be used as an indicator for other metabolic abnormalities like metabolic syndrome in subjects with higher insulin resistance or those previously diagnosed with T2D. More research is required, nevertheless, to determine the correlation between the intensity of these associations and inflammation.

Conclusion

The link between DNA methylation and inflammaging is examined in this review, which provides a body of experimental evidence of the key role of methylation modifications in the prevention of aging as well as aging-

related diseases. DNA methylation in specific sites of inflammatory markers can be used as an indicator of age-related disease progression. Previous studies did not establish the association between DNA methylation and inflammaging in similar diseases. Epigenetic deregulation has a role in aging-related alterations in the transcription of inflammatory genes and, as a result, translation and the stabilization and destruction of molecular components. Furthermore, alterations in DNA methylation in the same location may differ among age-related disorders (Figure 2). Epigenetic medicines, in particular, are of clinical interest because of their reversible and temporary effects. Using cutting-edge techniques to characterize molecular alterations in different species during aging can shed light on the importance of epigenetics in inflammaging and aging-related disorders.

Future perspective

Unfortunately, few studies on DNA methylation of inflammation-related genes have been conducted to date, and this could be one of the next paths in inflammatory research. While aging-related disease processes are still being unraveled, several investigations have found an epigenetic component. Indeed, epigenetic alterations have been demonstrated to play critical roles in diseases such as cancer, neurodegenerative diseases, CVDs and diabetes. Although the specific mechanisms and links between various epigenetic modifications and human illnesses are still unknown, a better understanding of epigenetic pathways influencing life expectancy will be an important starting point in identifying new potential treatment targets. Unlike DNA mutations, epigenetic alterations such as DNA methylation changes are reversible, making them interesting targets for developing therapeutic ways to reduce aging. As a result, DNA methylation in various inflammatory markers may be used as a biomarker to diagnose, stage and treat many aging-related diseases in the future. A better understanding of the epigenetic mechanism's effect on longevity will also identify novel potential therapeutic targets.

Executive summary

- Aging is related to increased inflammatory mediators without proper immune stimuli.
- Epigenetic alterations like methylation are involved in inflammaging-related diseases.
- Alterations in DNA methylation at one location may vary between age-related diseases.
- DNA methylation in inflammatory markers implies the progression of age-related disease.
- The impact of DNA methylation on different gene expression statuses and its association with age-related diseases such as cancer, neurodegenerative and cardiovascular diseases and diabetes are discussed.
- DNA hypermethylation, for example, has been identified in colorectal and prostate cancer, cardiovascular diseases and other conditions, whereas DNA hypomethylation has been found in conditions like Alzheimer's disease, atherosclerosis and diabetes.
- Cutting-edge technology now enables the characterization of the molecular alterations in different species during aging and sheds light on the importance of epigenetics in inflammaging and aging-related disorders.

Author contributions

Planning, writing and initial editing of the manuscript: M Alimohammadi, S Makaremi and A Rahimi. Conducting and designing the figures: V Asghariazar. Conceiving the presented idea, monitoring the project and revising the main text of the paper: M Taghadosi and E Safarzadeh. All authors discussed and contributed to the final manuscript.

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