

# Progress on the role of DNA methylation in aging and longevity

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## Abstract

Aging is a major risk factor for individuals' health problems. Moreover, environmental signals have a widespread influence on the aging process. Epigenetic modification, e.g. DNA methylation, represents a link between genetic and environmental signals via the regulation of gene transcription. An abundance of literature indicates that aberrant epigenetic change occurs throughout the aging process at both the cellular and the organismal level. In particular, DNA methylation presents globally decreasing and site-specific increasing in aging. In this review, we focus on the crucial roles of DNA methylation in aging and age-related disease and highlight the great potential of DNA methylation as a therapeutic target in preventing age-related diseases and promoting healthy longevity.

**Key words:** DNA methylation; epigenetics; aging; longevity; age-related disease

## Introduction

Aging is a natural phenomenon characterized by organ dysfunction and increased risk of age-related diseases, such as cardiovascular disease, neurodegenerative disease and cancer [1, 2]. It is predicted that by 2050, the number of people aged 65 years and older will reach about 15 billion, representing approximately 16% of the global population [3]. Consequently, understanding the cause and mechanisms of aging is imperative in assisting to suppress age-related diseases and promote healthy longevity. It is well-known that aging is influenced by a combination of genetic and environmental factors. Previous twin studies have shown that the genetic contribution to general human longevity is about 20–30% [4, 5], whereas environmental factors in human aging and longevity still account for the largest effect.

Epigenetic factors influence the regulation of gene expression without altering the DNA sequence and act as the bridge that links the intrinsic and extrinsic signals [6]. The most common epigenetic modifications include DNA methylation,

histone modification and RNA-based mechanisms [7]. In particular, DNA methylation is one of the best-studied epigenetic modifications in recent decades, and plays a crucial role in many biological processes, such as development, differentiation, genomic imprinting and X chromosome inactivation (XCI) [8, 9]. With the accumulation of findings, the biological importance of DNA methylation attracts more and more attention. For example, several studies suggest that DNA methylation participates in the process involving the formation and stabilization of memories [10, 11]. A recent study demonstrated that demethylation by silencing DNA methyltransferase enzymes (DNMTs) affects cell survival [12]. More interestingly, growing evidence is revealing that variations in DNA methylation caused by environmental stimuli can be transmitted from parents to offspring [13].

Intriguingly, abundant evidence has demonstrated that DNA methylation has a close association with aging, age-related diseases and longevity [14, 15]. In this review, we summarize the general DNA methylation change pattern in the aging process,

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and highlight the crucial roles of aberrant DNA methylation in the pathogenic process of age-related diseases. More importantly, accumulated studies suggest that age-dependent DNA methylation changes could be inverted by certain interventions, such as dietary control and chemicals [16–18], presenting the great potential of DNA methylation as a therapeutic target in preventing age-related diseases and promoting healthy aging.

### Function of DNA methylation in regulating gene transcription

DNA methylation involves the addition of a methyl group to DNA nucleotides, which was first described by Hotchkiss *et al.* in 1948 [19]. In mammals, most of the methyl groups were added to the 5' cytosine of CpG dinucleotides. Moreover, 60–90% of the CpGs across the genome were methylated. The unmethylated CpGs located on CpG-rich genomic sequences, termed CpG islands, are considered to be a marker of genes and to be functional in the regulation of transcription [20–22]. In humans, approximately 50% of the genes are associated with CpG islands in their promoter regions [23]. The methyl transfer reaction mainly involves three DNMTs, including DNMT1, DNMT3A and DNMT3B. The methyltransferase enzymes use S-adenosylmethionine as the methyl donor [24]. DNMT1 acts primarily in maintaining the genome methylation pattern during cell replication by methylating the hemimethylated CpG sites on one strand of double-stranded DNA, whereas DNMT3A and DNMT3B play roles in the *de novo* methylation of previously unmethylated DNA [25, 26]. However, recent studies show that DNMT1 also plays important roles in the process of *de novo* methylation, whereas DNMT3A and DNMT3B are also involved in maintaining the methylation pattern [27].

In general, DNA methylation participates in regulating gene transcription by influencing the binding of transcription factors or changing the status of chromatin [28]. Early studies mainly focused on the function of DNA methylation in promoter regions and revealed a negative relationship between methylation and gene expression. However, accumulated evidence shows that the functional consequences of DNA methylation in promoter regions depend closely on the CpG content [29, 30]. Besides, several studies have shown that the methylation in the gene body region was correlated with enhanced gene transcription [31–33]. Additionally, there is sound evidence for the complex relationship between DNA methylation and gene expression. For example, the expression of the *Igf2r* gene is repressed by the methylation of the CpG island in its second intron [34]. Furthermore, hypermethylation of the *hTERT* promoter is reported to be positively correlated with its expression in some tumor cells and normal human tissues [35].

### Changes in DNA methylation throughout the aging process

In recent years, a large number of studies have revealed the close link between DNA methylation and the aging process. Previous studies revealed that a globally decreased methylation occurs as individuals age. At the cellular level, Wilson and Jones [36] found that the 5mC content of the genome was decreased markedly in cultured normal diploid fibroblasts from mice, hamsters and humans. This observation was also verified in other cells types, including lymphocytes [37] and T cells [38]. At the organismal level, Singhal *et al.* [39] revealed that the total DNA methylation level decreased with age in mice, and

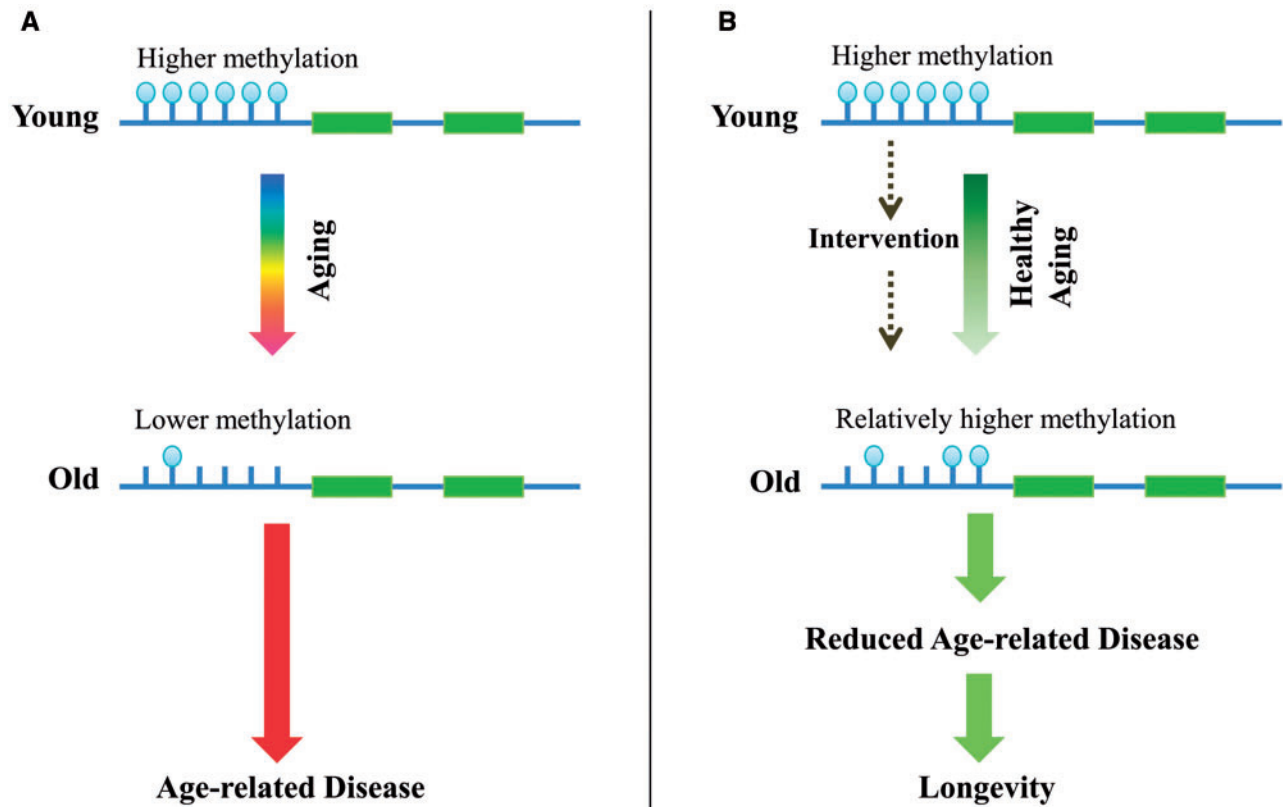
furthermore, that the rate of demethylation was inversely associated with life span. Moreover, it has been shown that the 5mC content in DNA decreases in the brain, heart and spleen, but does not change in the liver or lungs [40]. Similarly, decreased genome methylation is also observed in the blood of humans [41, 42]. Locus-specific hypermethylation has also been observed in the aging process [43, 44]. In addition, research has shown that the global loss of methylation generally occurs on repetitive genomic sequences [41], whereas the aging-associated DNA hypermethylation occurs preferentially at CpG islands and bivalent chromatin domain promoters [43, 45].

Emerging studies suggest that the change in DNA methylation of some specific CpG sites presents good linear regression with age and can be used to predict the age of individuals, offering great potential value to forensic science owing to widely available materials, such as saliva and blood [46–49]. For instance, one study identified three CpG sites with age-related DNA methylation in saliva and used just two of them to predict the age of an individual with a mean accuracy of 5.2 years [47]. Another study identified three CpG sites that can track the aging of blood with a mean absolute deviation of less than 5 years [50]. Recently, yet another study identified two CpG sites in the *ELOVL2* gene that can be used to predict age with a prediction error of 6.85 years using a linear regression model, and showed that the DNA methylation status remained stable even when the blood was stored at room temperature for 4 weeks [51]. Furthermore, a new study demonstrated that the support vector regression model might be more robust than other statistical models, including multivariate linear/nonlinear regression and back propagation neural network in predicting age by using DNA methylation as a marker, and the average predictive accuracy was estimated to be 4.7 years when using six identified CpG loci with this model [52].

### Relationship between DNA methylation and age-related diseases

The aging-dependent DNA methylation changes are closely associated with age-related traits and diseases (Figure 1A). The most striking evidence comes from the study of monozygotic twins, who share the same genotype while exhibiting many phenotypic differences [53–55]. Epigenetic differences caused by a diverse living environment are considered to be one explanation for this phenomenon. Using a simple method known as amplification of intermethylated sites, it has been shown that the methylation differences between monozygotic twins increase with age [53]. A recent longitudinal Danish twin study also revealed that DNA methylation in aging has a relationship with mortality [55]. Significantly decreased global methylation has also been reported to be associated with increased frailty [56]. The hypomethylation of Alu and LINE1 in the aging process was observed to be linked to rapid lung function decline in elderly individuals [57]. In addition, studies indicate that the age-related methylation change might be one potential cause of impaired T-cell function [58]. Moreover, research has identified several CpG sites with an association between age-related methylation changes and aging-related phenotypes, such as cg03001305 in *STAT5A* with levels of serum low-density cholesterol, cg16463460 in *WT1* with lung function and cg09259772 and cg13870866 in *ARL4A* and *TBX20* with maternal longevity, respectively [59].

Other considerations involve the crucial roles of abnormal DNA methylation in the occurrence of age-related diseases. For



**Figure 1.** Aberrant changes in age-related DNA methylation in the aging process. (A) DNA methylation level decreases in the aging process and is associated with age-related disease occurrence. (B) In the aging process, internal or external interventions could modify the methylation status, which may reduce the prevalence of age-related diseases and promote longevity. (A colour version of this figure is available online at: <https://academic.oup.com/bfg>)

type-2 diabetes mellitus (T2DM), a study found a CpG site in the first intron of fat mass and obesity-associated gene with a lower methylation level in the peripheral blood of patients [60]. Insulin gene promoter is also hypermethylated in pancreatic islets from patients with type-2 diabetes compared with controls, which is negatively associated with insulin mRNA expression [61]. In addition, by analyzing the methylation profiles of freshly isolated islets from 5 diabetic and 11 nondiabetic Caucasian donors, 276 CpGs with significantly differential methylation levels were identified in diabetic islets that related to genes involved in beta-cell survival and function [62]. For Alzheimer's disease, a globally decreased methylation level was observed in the hippocampus of diseased patients [63]. Increased expression of the APP gene contributes to the occurrence of Alzheimer's disease, and it has been shown that there is a lower methylation level on the promoter region of APP in Alzheimer's disease patients [64]. Sanchez-Mut et al. [65] revealed that neurodegenerative diseases, including Alzheimer's disease, dementia and Parkinson's disease, present a similar pattern of aberrant changes in DNA methylation. For cardiovascular disease, it has been shown that subjects with ischemic heart disease and stroke exhibit lower LINE-1 methylation [66]. Furthermore, the association between age-dependent changes in DNA methylation and cancer development is well-characterized. In 1994, the 5' CpG island of the estrogen receptor gene was found to be hypermethylated in both aged colon tissue and colon tumor, which first linked the age-associated change in DNA methylation with the occurrence of tumor [67]. Moreover, it has been shown that the promoter methylation levels of *RARβ2*, *RASSF1A*, *GSTP1*, *NKX2-5* and *ESR1* are increased with

age in normal prostate tissue, and, meanwhile, hypermethylated in prostate cancer tissue [68]. Similarly, 24 CpGs with age-related hypermethylation in normal breast tissue have been identified, also showing hypermethylation in breast tumor tissues [69]. These findings suggest the importance of DNA methylation in the occurrence of age-related diseases.

### Potential therapeutic strategy of DNA methylation in preventing age-related diseases and promoting longevity

An abundance of evidence indicates that reversal of aberrant DNA methylation could be an effective strategy to suppress disease and promote longevity (Figure 1B). Calorie restriction (CR) is a well-proven and effective intervention strategy to prevent/delay age-related diseases and extend life span [70–73]. Recent studies suggest that changing aberrant methylation status is one underlying mechanism involved in CR response. For example, it has been shown that glucose restriction could increase promoter methylation level of *p16<sup>INK4x</sup>* (a tumor suppressor and aging-related gene) in normal WI-38 cells that blocks the binding of an active transcription factor (E2F-1) and further suppresses the expression of *p16<sup>INK4x</sup>* [73]. In addition, studies show that CR has the ability to attenuate the age-associated change in expression of *Dnmt3a* in mouse hippocampus, which may delay the age-related decrease in brain function [16]. Similarly, a diet rich in vitamins and antioxidants can increase the promoter methylation of DNA mismatch repair gene *MLH1* in T2DM subjects [17].

Other obvious evidence coming from cancer therapy involves DNA methylation. It is well-known that silencing the expression of tumor suppressor genes induced by age-dependent hypermethylation of their promoter regions is regarded as one cause of tumorigenesis. Previous studies have indicated that suppression of DNA hypermethylation through inhibition of DNMTs is one potential anti-cancer strategy [18]. Currently, two pyrimidine analogs, including 5-azacytidine (5-Aza-CR) and 5-aza-2'-deoxycytidine (5-Aza-CdR), are the best-studied DNA methylation inhibitors. Both chemicals can be incorporated into DNA and inhibit the binding of DNMTs to methylated DNA [74]. In addition, 5-Aza-CdR can promote the degradation of DNMT1 through a proteasomal pathway [75]. Taken together, all of the above researches indicate that DNA methylation holds great potential as a therapeutic target in disease treatment and life span extension.

More interestingly, a recent study compared the genome DNA methylation profiles between centenarians, their offspring and controls born from both long-lived and non-long-lived parents. It was revealed that the offspring of long-lived subjects possess a better preservation of DNA methylation status, as well as a higher methylation level of genes involved in DNA/RNA synthesis, metabolism and cellular signaling [76]. Another study showed that the rate of age-related DNA methylation in semi-supercentenarians and their offspring was slower than their age-matched controls [77]. A previous study of Gentilini *et al.* revealed that age-related XCI skewing, which is linked with late-onset X-linked disorders, was significantly less severe and frequent in centenarians' offspring compared with controls with both long-lived and non-long-lived parents [78], and recently, they revealed a correlation between XCI skewing and increased stochastic epigenetic mutations (DNA methylation) in females [79]. All these observations indicate the potential roles DNA methylation in determining human healthy aging and longevity. In addition, our previous study also found that the centenarians exhibited different methylation status preferentially on genes involved in age-related diseases, suggesting the great potential to suppress the occurrence of age-related diseases via DNA methylation [80]. Furthermore, a recent study revealed that the methylation level in some sites in nonagenarians presented good linear regression with the life span of their father, suggesting the potential role of DNA methylation in the heritability of human life span [81]. However, all these studies lack corresponding transcriptome data and functional assays. Much more future work should be done to verify the function of DNA methylation in human longevity.

## Conclusion and future prospects

The changes in DNA methylation across a life span is a hallmark of aging. There is considerable research indicating that the abnormal age-dependent changes in DNA methylation are a potential cause for organ function decline and the occurrence of age-related diseases (Figure 1A). In addition, there is evidence to show that aberrant DNA methylation is caused by various external risk factors throughout life, such as smoking [82].

It is well-known that DNA methylation is a reversible epigenetic modification. Currently, emerging evidence suggests that certain interventions (e.g. CR, dietary supplementation and chemical drugs) can prevent age-related diseases and promote longevity, at least in part, through reversing the aberrant age-associated changes in DNA methylation, suggesting the great potential of DNA methylation in therapeutic strategies against age-related diseases (Figure 1B).

However, to further understand the roles of DNA methylation in human aging or longevity, much more future efforts should be taken in exploring (1) the functional CpG sites with aberrant DNA methylation changes in aging and their corresponding roles in the occurrence of age-related diseases; (2) the risk factors, such as smoking, that contribute to aberrant age-associated DNA methylation changes; and (3) strategies to modify DNA methylation status at target-specific levels.

Finally, it is worth stressing that epigenetic and genetic factors are interactive in regulating biological processes including aging and age-related diseases. Modulation of risk factors (genetic and epigenetic) related to aging and age-related disease is one effective strategy in improving human health and promoting longevity. In particular, the emergence of the CRISPR/Cas9 system in recent years, a RNA-guided genome editing technology, has led to a revolution in genetic engineering, which presents tremendous application value in disease therapy [83–86]. Moreover, emerging studies have revealed that this technology can also achieve epigenetic editing. For example, Hilton *et al.* [87] generated a new CRISPR/Cas9 system that could regulate the expression of target genes via catalyzing acetylation of histone H3 lysine 27 at their promoter or enhancer regions. Taken together, the improvement of the CRISPR system and invention of other new technologies will have great practical value in the future.

### Key Points

- DNA methylation plays crucial roles in regulating gene transcription.
- A globally decreased and site-specific increased DNA methylation occurs in aging.
- The age-associated change in DNA methylation is one cause for the increased risk of age-related diseases.
- Reversal of aberrant DNA methylation may be one potential strategy to suppress disease and promote longevity.

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