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P53 and DNA Methylation in the Aging Process

Yuyu Wang, Jingshan Shi*

Department of Pharmacology and the Key Laboratory of Basic Pharmacology of Ministry of Education, Zunyi Medical University, Zunyi, China
Email: *shijs@zmu.edu.cn

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Abstract

Healthy aging is the ultimate goal of all life science research and the most ideal state of a human being. There are many factors that affect aging, including genetic background, the environment, mental state and living habits and so on, which affect the body's internal environment and its steady state. The ultimate starting point of the body's aging all comes down to cellular aging. At the cellular level, aging is an irreversible block in the cell cycle, and the P53 gene plays a pivotal role in regulating the cell cycle. Aging is not only regulated by genes but also influenced by epigenetics affecting gene expression. DNA methylation, a novel biomarker of aging, plays a major role in epigenetics. This paper's mini-review briefly summarizes P53 and DNA methylation in aging.

Keywords

Aging, P53, DNA Methylation

1. Introduction

The process of human aging is complicated and individualized, taking place in the biological, psychological and social fields. Under normal conditions, aging usually refers to the progressive changes in cell metabolism and physical functions with the increase of age after adulthood. Aging results in impaired self-regulation and regeneration, and leads to structural changes. This is a natural and irreversible process [1]. Diseases or other abnormal factors can cause pathological aging, making the above phenomena appear in advance [2]. Aging is characterized by the gradual loss of some physiological functions, and it drives the development of chronic diseases, including metabolic, cardiovascular, and neoplastic diseases [3]. It is therefore a risk factor for many diseases, such as cardiovascular disease [4], cancer [5], and dementia [6].

One of the main signs of aging is the modification of gene expression [7], and gradually up- or down-regulated with age. Gene expression changes are mainly achieved by epigenetic modification, including several DNA modifications and histone modifications [8]. Among them, DNA methylation occupies the dominant position [9]. The correlation between the methylation level of CpG sites and the chronological age is one of the best signs of aging, and is even considered as the "apparent clock" [10]. By detecting the methylation status of CPGs in human tissue samples, the researchers found that with age, hypermethylation occurs in the promoter region, and hypomethylation occurs outside the promoter [11].

The aging of life begins with the senescence of cells. The reason for cell senescence is that the cell cycle is out of control [12]. Among the cell cycle regulation mechanism, the P53 pathway plays a crucial role [13]. The P53 is the most common mutant tumor suppressor [14]. The activation of this protein can regulate and control the aging process and senescence of cells. It has been observed that increased P53 expression in senescent cells [15].

2. P53 Pathway and DNA Methylation

2.1. P53 Pathway

The P53 signaling pathway plays an important role in many aspects, such as cell cycle regulation, development, reproduction, metabolism, senescence, tumor inhibition, etc. [16] [17]. P53 also plays a regulatory role in Alzheimer's disease, Parkinson's disease and other age-related diseases [18] [19]. As a "guardian of the genome", it is particularly important during cell growth. When cells respond to, for example, genotoxic damage, proto-oncogene activation, hypoxia, or severe stress, normal wild-type P53 is activated and induces a variety of biological response, such as regulating the cell cycle, participating in DNA repair, inducing apoptosis and senescence as well as regulating cell differentiation. It can even interfere with the formation and regulation of blood vessels [20] [21]. In the normal physiological systems, the expression level of the P53 is mainly controlled by the negative feedback loop of intracellular ubiquitinated with the Mouse double minute 2 (MDM2)-P53. On the one hand, the P53 can activate the MDM2 expression [22] [23] [24] [25]; on the other hand, the P53 binds to MDM2 to form an oligomer, which inhibits the transcriptional function activated by P53. The function of mouse double minute 4 (MDM4) is similar to that of MDM2 [26], which can reduce the inhibitory effect of P53 on cell proliferation and induce cell apoptosis [27].

P53 would initiate its survival mechanism under some certain conditions, for example, when the nutrition in the cell is limited, autophagy occurs to remove unnecessary or dysfunctional intracellular components, accompanied by autophagy the cell would downregulate P53 function to prevent cell damage and tissue denaturation [28]. Due to drastic changes in metabolism, the P53 maintains its survival by reshaping the metabolic pathway [28]. For example, the P53 can

activate genes such as AMPKβ, TSC2, and PTEN, which suppress the mammalian target of rapamycin (mTOR, sensor of nutrient supply) signaling and the TP53-induced glycolysis and apoptotic regulator (TIGAR), regulate aerobic glycolysis and promote oxidative phosphorylation. Finally, P53 activates MDM2, indicate of its ability to destroy (called the feedback loop) the cell cycle to make it return to normal. This ability to regulate pathways can sense the availability of nutrients for cell survival and is an important P53 response to ensure homeostasis [29].

2.2. DNA Methylation

DNA methylation is catalyzed by DNA methyltransferases (DNMTs). The unmethylated cytosine on DNA is catalyzed by DNMT so that the fifth carbon atom is covalently linked with methyl to from 5-methylcytosine, which is provided by SAM, and SAM is converted to S-adenosine homocysteine (SAH) (**Figure 1**) [30]. In mammals, DNA methylation does not occur on non-CPG dinucleotide cytosine [31]. In many cases, the clusters of CpG sequence called "CpG island" are formed in the gene promoter region. Some studies have found that the overall DNA shows a low methylation status, while the DNA hypermethylation status appears locally, by copying mammalian senescent cells [32]. After the fifth carbon atom of cytosine on the CpG island of DNA is methylated, the gene expression is usually inhibited. On the contrary, demethylation can induce or reactivate gene expression. Demethylation is the conversion of methylated cytosine to unmethylated cytosine mediated by demethylase [33].

DNMTs are the managers of the conversion of unmethylated cytosines to 5-methylcytosines in mammals. They include DNMT1, DMMT3A, DNMT3b and DNMT3L, which work together to catalyze and maintain mammalian DNA methylation and methylation levels [34] [35]. In the process of aging, During DNA replication, the methylation of the hemi-methylation site is achieved by a

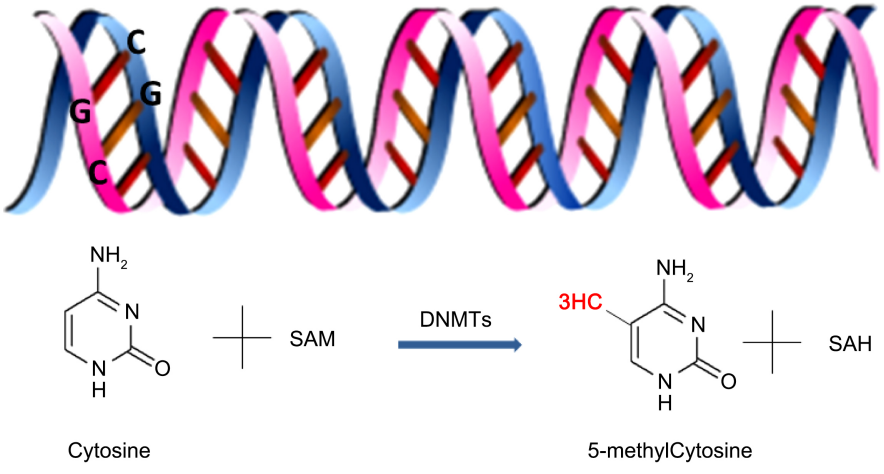

Figure 1. Under the action of methyl catalase, the methyl provided by S-adenosylmethionine (SAM) is covalently linked to the fifth carbon atom of cytosine on the double strand of DNA to form 5-methylcytosine, convert SAM into SAH.

conservative methylation pattern by DNMT1; in this way, the production of a methylated DNA double strand using hemi-methylated DNA as a substrate is intended to stabilize the inheritance of specific DNA methylation patterns in the body [36].

There are two mechanisms to achieve DNA demethylation, including active mechanism and passive mechanism: 1) In the active mechanism, the demethylation process is mainly mediated by DNA demethyltransferase; 2) In the passive mechanism, DNA methylation cannot be completely removed because nuclear factors attach to methylated DNA in this mechanism, which can only block the effect of DNMT1. One of the demethyltransferases is the methyl-CpG binding domain 2 (Mbd2) protein, a member of the conservative methyl-CpG binding domain (MBD) family. These proteins include MBD1, MBD2, MBD3, MBD4 and Methyl-CpG binding protein 2 (MeCP2). Methylated binding proteins specifically recognize methylated DNA and silence genes by recruiting co-repressors. MBD1 can inhibit transcription inhibition of genes, which can be partially reversed by histone acetylase inhibitors through DNA methylation, and MBD1 binds to symmetric methylated CpG sequences. It has also been found that chromatin-related factors (MCAF) containing MBD1 are considered to have a transcriptional regulatory role, through its binding to the transcriptional inhibitory domain (TRD) of MBD1 to form an inhibitory complex [37]. All MBDs may lead to silencing of regions showing DNA methylation. Therefore, there are mainly two ways to inhibit the expression of DNA methylation genes: 1) the promoter region of DNA methylation directly affects the binding of transcription activator and recognition sequence, and directly blocks the transcription of genes; 2) MBD identifies the methylated CpG dinucleotide sequence and recruits HDAC to the methylated site, indirectly inhibiting gene transcription under the synergistic effect of transcription factors and RNA polymerase II [38].

In addition, methylcytosine dioxygenase TET contributes to DNA demethylation. There are three members of the TET family protein, namely TET1, TET2 and TET3 [39]. The TET protein can even oxidize 5m C to 5f C and 5ca C in vitro [40]. Then thymine deoxyribonucleic acid glycosylase (TDG) converts it into unmethylated cytosine through a base excision repair mechanism [41] [42]. There is currently no consensus on how the expression of DMNTs or TETs changes with age; some studies have shown that with age, some decline, and some remain unchanged [43].

3. P53 and DNA Methylation in Aging

3.1. The P53

The most common phenotype of aging is cell senescence [44]. There are two classic pathways related to aging are P16INK4a/RB and P53/P21. P16INK4a can block the cell cycle process by inhibiting the cyclin dependent kinases4/6 (CDK4/6) complex, thereby activating Rb (retinoblastoma) pathway to inhibit E2F transcription [45].

P53 signaling pathway plays a key role in regulating cell senescence and body aging [46]. Moreover, studies have shown that an appropriate P53 expression level is very important in the process of controlling cell senescence [47]. The first identified P53 target is the cyclin dependent kinases1A (CDKN1A), commonly known as P21, which also plays an important role in cell cycle arrest and aging [48]. In normal cells, the expression of P53 is maintained at a low level through negative feedback regulation. When P53 is activated, cell proliferation is effectively inhibited by cell cycle arrest, apoptosis or senescence [49]. P53 can directly activate P21 and mediate cell cycle arrest and senescence by transcriptional induction of P21 [50] [51] [52] [53]. When P21 binds to CDK, it forms a proliferating cell nuclear complex called P21-cyclin-CDK-PCNA, which can inhibit the binding of CDK and PCNA to other molecules and thereby inactivate them [54]. The phosphorylation of Rb protein by CDK is reduced and the phosphorylation of Rb is blocked. Rb can form a complex with E2F, further hindering the transcription of E2F and negatively regulating the cell cycle, thus making the cell unable to differentiate and proliferate [55]. Increased P21Waf1/Cip1 expression and/or Rb activity leads to cell senescence (**Figure 2**) [56].

Moreover, many studies have confirmed that expression of P53 can affect aging through different pathways. For example, the pharmacological activation of P53 can promote the increase of senescence skeletal muscle stem cells in the body [57]. The activation of 53 is inhibited by fibroblast growth factor 21 (FGF21) by improving mitochondrial biogenesis and in an AMPK (AMP-activated protein kinase)-dependent manner, thereby preventing Angiotensin II (Ang II)-induced

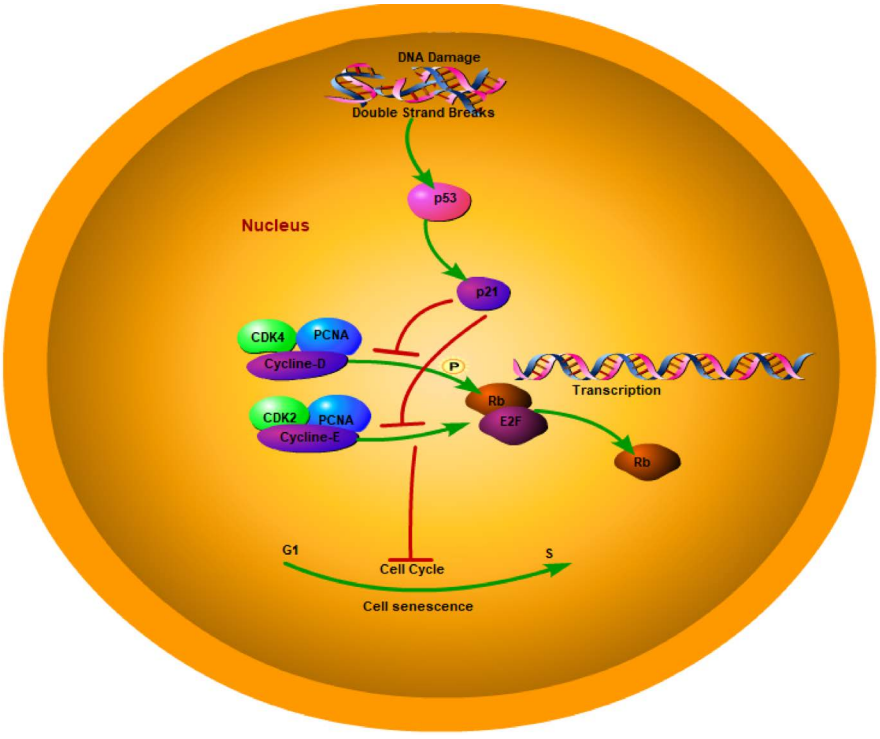

Figure 2. P53/P21 pathway in the process of aging.

cerebrovascular aging [58]. IL-10 induces activated hematopoietic stem cell (HSC) aging by increasing P53 protein expression, thereby reducing liver fibrosis in rats [59]. With regard to the effect of P53 in human aging, there is evidence that a relatively active class of individuals carry a common P53 polypeptide variant (proline, rather than arginine), and that leads to cell cycle arrest and senescence [60]. In vivo studies on the effects and mechanisms of BZBS on age-related hypogonadism, it was found that the expression of P53 was significantly increased in the aging group, and after the administration of BZBS, it was found that the hypogonadism was alleviated pathologically, and the expression of P53 also decreased to the expression level of normal mice [61]. In the study on the anti-aging effect of mint and thyme, it was found that the activity of β-galactosidase was decreased in senile cells, but the expression of P53 protein was significantly increased [62]. P53 regulates mitochondrial dynamics through the PKA-DRP1 pathway, thereby inducing cell senescence [63].

3.2. DNA Methylation in Aging

Recent research suggests that epigenetics, especially DNA methylation, play a mechanistic role in aging process. The DNA methylation age, or the epigenetic clock has been shown to be highly correlated with age. Epigenetic clocks can measure changes in hundreds of specific CpG sites and can accurately predict the age of various species, including humans [64].

The cyclin dependent kinases1A (CDKN1A, p16 ink4a/Arf) is a cell cycle inhibitor whose expression is a mature aging marker [65], its expression is controlled by methylation and is frequently activated in various cancers [66]. Brain and muscle Arnt-like protein-1 (BMAL1) is a circadian rhythm gene associated with cell aging [67] and its expression is also regulated by epigenetic modification of DNA methylation and histone modification [68]. The study found that age-related changes in the major histocompatibility complex class 1 (MHC1) gene promoter and intra-gene methylation involved in immune function are closely related to changes in gene expression [69]. During aging, DNA methylation changes occur in tissues [70], The rDNA activity is decreased, and both its coding and promoter regions became increasingly methylated [71]. Age-related methylation was originally observed on the CpG island promoter of protein-coding genes [22] [72]. In mice, CpG sites with more than 20% CpG methylation in the whole genome showed age-related variations, and methylation and hypomethylation were observed [73] [74]. In humans, the blood methylation distribution of 32 pairs of mothers and their progeny were analyzed using Illumina's human methylation 450 bead chips, and it was found that the methylation level in CpG islands at the promoters of the three genes was significantly correlated with age [75]. Another research team used a different but similar DNAm-age indicator to prove that interventions related to aging (such as calorie restriction) can reduce methylation aging in rhesus monkeys [76]. Studies have analyzed the stem cells from human exfoliated deciduous teeth (SHED) and permanent teeth of young

(Y-DPSCs) and old (A-DPSCs) adults. In the elderly group, there is less methyl donor S-adenosylmethionine and hypomethylation of the aging marker P16 (CDNK2A) [77]. A study of 92 patients aged between 18 to 93 years old (mean age = 54.3 years, median age = 55.5 years) of human serum DNA samples was performed with bisulphite conversion and pyrophosphoric acid sequencing, including 44 males and 48 females. Age was found to be linearly correlated with DNA methylation levels at the CpG site. Among them, 19 showed hypermethylation of ELOVL2, TRIM59, KLF14, and FHL2, while 8 showed hypomethylation of MIR29B2CHG. Nine CpG loci of ELOVL2 gene showed the strongest correlation with age, and hypermethylation showed a significant linear correlation with age (R = 0.833 ~ 0.919). Importantly, DNA methylation levels were significantly increased at all CpG sites in the ELOVL2 gene. There were also four hypermethylated CpG loci in TRIM59 gene, which showed a significant linear relationship with age, and the methylation level increased with age [78].

3.3. Interactions between DNA Methylation and P53

The most important methyltransferase in DNA methylation is DNMT1, which also has the same function of regulating cell cycle as P53 [79]. P53 acts as a transcription factor in the nucleus, also mediates the regulation of DNMT1 gene expression. In the absence of genotoxic stress, P53 locates in the nucleus and binds to the common site of DNMT1 promoter, thereby blocking DNMT1 gene expression. But, when DNA damage occurs, P53 signaling pathways are activated by modification after translation or after the interaction with other transcription factors to eliminate the P53 inhibition of DNMT1, leading to a rise in DNMT1 expression [80], RB protein as downstream gene P53 pathway, not the phosphorylation of RB and E2F union, will raise to the HDAC complexes and prevent the cyclin E, PCNA and E2F-1 cell cycle protein expression level to maintain DNMT1 [81]. In addition, some researchers found the relationship between DNMT1 and P53 is not only the inhibition of the expression of DNMTA by P53, but also the interaction between DNMT1 and NEAT1, thus inhibiting the expression of P53, in the pathogenesis of lung cancer [82].

4. Expectation and Unresolved Questions

In the past few decades, our research on aging has never stopped; The P53-mediated effects play a vital role in the aging and the healthy aging process [83], and regulate various organs and overall aging via many ways [84] [85] [86]. Therefore, P53 may be of great value in the future research on aging and aging-related diseases. Epigenetics research has never stopped in the field of aging; there have been many studies on its relationship with aging. It has been proposed that the epigenetic changes in the aging process will lead to the decline of physical and cognitive functions; moreover, the accelerated aging of epigenetics is related to disease and all-cause mortality in old age [87]. DNA methylation is an important part of epigenetics. People are also curious about how it relates to aging. Studies

on aging have found that the CpG of many genes has different levels of methylation associated with aging. If we can regulate the methylation of CpG sites, we can regulate aging. The regulation of cell cycle by P53 pathway is one of the most classical pathways in aging research. In addition, proteins related to the regulation of DNA methylation can interact with P53. However, at present, the specific genes and mechanisms of age-related DNA methylation and gene expression are not detailed and clear enough, but it is the most promising molecular marker for monitoring aging at present and warrants further investigation.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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