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Review

Advances in epigenetic regulation of vascular aging

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Vascular aging is a major risk factor and driver of age-related cardiovascular diseases (CVD). Atherosclerosis, hypertension, and other CVD lead to vascular dysfunction that involves multiple pathological processes such as oxidative stress, endothelial dysfunction, inflammation, and autophagy. Epigenetics refers to genetic changes that occur when the DNA remains unchanged that include DNA methylation, histone modification, and non-coding RNA. It has been reported that epigenetics plays an effective regulatory role in CVD and affects cardiovascular repair function. Presently, drugs targeting epigenetics have applications in malignant tumors and inflammation. Therefore, exploration of epigenetic mechanisms in vascular aging will allow us to understand the pathogenesis of diseases related to vascular aging. This review focuses on the pathological changes in vascular aging and analyzes the relationship between vascular aging and epigenetics. Additionally, this review focuses on the pathogenesis of vascular aging related diseases from a new perspective in order to develop epigenetic-based treatment strategies for patients with age-related cardiovascular diseases.

Keywords

Vascular aging; epigenetics; endothelial dysfunction; oxidative stress; inflammation

1. Introduction

Vascular aging is a major risk factor of age-related cardiovascular diseases that lead to the gradual decline of vascular function (Katusic and Austin, 2014). It is necessary to explore the mechanism of vascular aging to diagnose and prevent CVD. Although aging is inevitable, pathological vascular aging is a modifiable risk factor (Laurent, 2012). Clinical studies have shown that vascular aging is associated with vascular endothelial dysfunction, hypertension, atherosclerosis (AS), coronary heart disease, and stroke (Lakatta, 2003). Many of these pathologies are related to endothelial cell injury, vascular smooth muscle cell proliferation, inflammation, fibrosis, and calcification (Cahill-Smith and Li, 2014). The pathogenesis of these age-related CVD; however, are not fully understood. In China, the aging population and chronic cardiovascular complications from health and economic burden has increased. It is necessary to investigate the impact of vascular aging on CVD that prevent and repair damaged aging arteries and reduce the risk of vascular age-related diseases (Harvey et al., 2015).

Structural and mechanical stress changes, such as vascular media thickness may be observed in senescent blood vessels. With the increase of age, endothelium-dependent vascular dilatation is continuously attenuated. This results in vascular structure changes that are accompanied by pathological changes such as lumen dilation, endothelial dysfunction, and diffuse intimal thickening. In addition, endothelial barrier permeability decreases, which allows vascular smooth muscle cells to migrate to the subendothelial space producing extracellular matrix proteins. Both the increased elastin degradation and collagen deposition results in vascular plasticity reduction, which leads to vascular remodeling and endothelial homeostasis change (Najjar et al., 2005). Age-related dysfunction of endothelial cells may in turn aggravate media thickness and vascular fibrosis resulting in hypertension, atherosclerosis and vascular obstruction (Brandes et al., 2005).

2. Pathologic mechanism of vascular aging

Clinical studies have shown that the mortality of CVD increases exponentially with age. Age-related intracellular homeostasis changes make elderly blood vessels more vulnerable to pathophysiological conditions. Exploration of age-related vascular pathophysiological mechanisms is helpful to reduce the mortality of vascular age-related diseases (Fig. 1) (Ungvari et al., 2010).

2.1 Oxidative stress and endothelial dysfunction in vascular aging

Studies have shown that vascular aging is mainly characterized by endothelial dysfunction and vascular stiffness. This is associated with decreased bioavailability of endothelial nitric oxide (NO) and increased production of reactive oxygen species (ROS) (Yeo et al., 2015). ROS can cause endothelial dysfunction, which in turn leads to coronary artery disease and stroke in the elderly (Donato et al., 2007). In vascular aging, NO bioavailability is seriously impaired and endothelial nitric oxide synthase (eNOS) expression is decreased. This decreased expression aggravates NO bioavailability that reduces tetrahydrobiopterin (BH4) (Hoffmann et al., 2001), intracellular L-arginine utilization (Berkowitz et al., 2003) and promotes vascular atherosclerosis.

BH4 is a coenzyme of eNOS that regulates catalytic activity (Alp and Channon, 2004). The BH4 reduction in senescent blood vessels directly leads to the conformational change of eNOS from dimer to monomer state resulting in the loss of NO (Farah et

al., 2018). Clinical studies have shown that BH4 supplement can improve endothelial function in the elderly, suggesting that BH4 may be a potential target for improving the aging vascular function (Pierce and Larocca , 2008). In addition, there is increasing evidence that arginine II (Arg II) is down-regulated in senescent cells and involved in early vascular aging by changing the endothelial cell and vascular smooth muscle cell (VSMCs) phenotypes. In contrast, silencing Arg II inhibited the aging phenotype of human endothelial cells (Wu et al., 2015). Furthermore, the Arg II gene deletion reduces vascular inflammation in mice fed a high-cholesterol and high-fat diet, prevents atherosclerosis, and improves insulin sensitivity and glucose homeostasis (Pernow and Jung, 2013). These findings suggest that Arg II may be a co-determinant of metabolic disease, atherosclerosis, and aging (Yepuri et al., 2012).

In recent years, the gradual discovery of longevity genes, such as the Klotho gene, has been observed to accelerate the aging phenotype that is associated with vascular endothelial dysfunction and premature atherosclerosis (Kuro-o et al., 1997). Recent studies have shown that mitochondrial oxidative stress also plays an important role in vascular dysfunction caused by aging. Mitochondria-derived H_2O_2 contributes to the NF-κB activation, which leads to the pro-inflammatory transformation of endothelial gene expression profile. Apoptosis is another important link between mitochondrial ROS production and vascular aging (Ungvari et al., 2007). The mechanism of mitochondrial oxidative stress in vascular endothelial cell senescence may be multifaceted including reduced glutathione content and dysfunction of the electron transport chain (Ungvari et al., 2008). Recent studies have shown that the mitochondrial enzyme $p66^{Shc}$ protein plays an important regulatory role in the production of mitochondrial ROS linking oxidative stress with apoptos is (Napoli et al., 2003). Mice deficient in $p66^{Shc}$ showed a reduction of ROS, which was closely related to a 30% increase in lifespan and improved vascular endothelial function (Camici et al., 2008).

2.2 *Inflammation in vascular aging*

Recent studies have found that the interaction of inflammation, ROS and endothelial dysfunction plays an important role in the pathogenesis of cardiovascular aging (Wu et al., 2014). Chronic inflammation is closely related to aging and is the main factor to induce the atherosclerosis (Fulop et al., 2017). ROS could activate endothelial cell activation and secretion of inflammatory mediators. Even in physiological aging, there are inflammatory changes in the vascular gene expression profile such as up-regulation of inflammatory cytokines, chemokines, adhesion molecules, and inducible nitric oxide synthase (Csiszar et al., 2003). Plasma concentrations of various inflammatory markers (TNF-α, VCAM-1, IL-6, IL-18, MCP-1) were positively correlated with age and independent of other cardiovascular risk factors (Miles et al., 2008). High levels of inflammatory cytokines are involved in the development of inflammatory microenvironment and vascular dysfunction, therefore promoting senescent endothelial cell apoptosis (Csiszar et al., 2007). In addition, the NF-κB binding may increase the expression of adhesion-inducible nitric oxide synthase in coronary arteries and aorta (Cernadas et al., 1998), leading to atherosclerosis (Hajra et al., 2000). Removing the mitochondrial source of hydrogen peroxide from aging blood vessels decreases

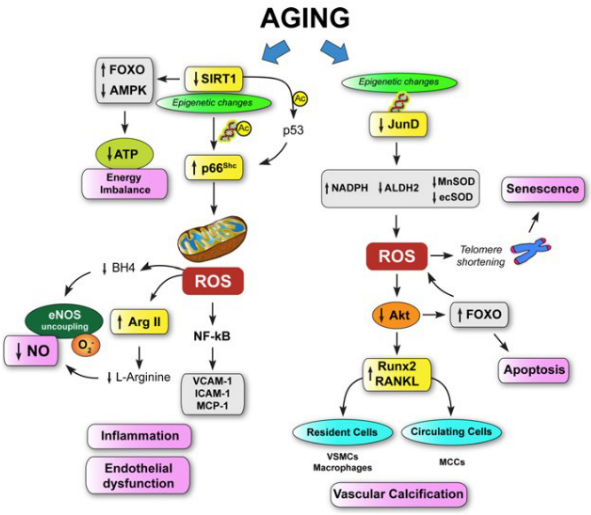

Figure 1. Vascular age-related molecular signaling pathway (Paneni et al., 2015). Aging, especially vascular aging, is associated with several signaling pathways. FOXO, forkhead box O; AMPK, AMP-activated protein kinase; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; MnSOD, manganese superoxide dismutase; ecSOD, extracellular superoxide dismutase.

NF-κB activation and suggests that mitochondrial oxidative stress can regulate the activity of NF-κB in senescent endothelial cells. An age-relted decline in mitochondrial function may lead to vascular inflammation (Ungvari et al., 2007).

2.3 *Autophagy in vascular aging*

Autophagy occurs in age-related diseases; however, its mechanism is not clear. Studies have reported that mice are more prone to pulmonary hypertension after the knockout of autophagy related gene LC3-II (Lee et al., 2011). In addition, Beclin1 gene knockout in local vascular tissues resulted in severe vascular remodeling, decreased vascular reendothelialization, and increased apoptosis (Ye et al., 2014). Specific deletion of ATG7 gene in smooth muscle cells can promote intimal hyperplasia and atherosclerotic plaque formation (Grootaert et al., 2015). In contrast, studies have shown that excessive autophagy can damage cells. The autophagy inhibitor 3-MA inhibits autophagy and alleviates vascular endothelial cell injury. In addition, another autophagy inhibitor chloroquine showed protective effects in pulmonary arterial hypertension (Long et al., 2013).

3. Epigenetics in vascular aging

The mechanism of aging is complex and cannot be fully explained. Studies have defined aging as telomere shortening, protein homeostasis disorder, mitochondrial dysfunction, genomic instability, epigenetic modification, reduced nutritional sensitivity, cellular senescence, stem cell failure, and cell communication changes (Lopez-Otin et al., 2013). While most studies have focused on genetic aging, environmental factors may also influence age-related diseases (Costantino et al., 2018). The epigenetic modification in aging has recently been a topic of discussion.

Epigenetics is necessary for the maintenance of tissue-specific

gene expression development as well as the cardiovascular homeostasis and gene plasticity changes. The main content maybe divided into three categories: DNA methylation, histone modification, and non-coding RNA changes (Cencioni et al., 2013). Further understanding of the role of epigenetics in age-related cardiovas-cular diseases may provide additional insights into understanding age-related CVD and to seek relevant intervention strategies.

3.1 DNA methylation

DNA methylation is defined as covalently linking cytosine residues of CpG island with methyl bonds and inhibiting gene transcription by binding to the gene promoter region or by recruiting chromatin modifying enzymes (Fraineau et al., 2015). DNA methylation is catalyzed by three different methyltrans-ferases (DNMTs). DNMT1 maintains methylation during repli-cation and participates in de novo synthesis of DNA methylation along with DNMT3a and DNMT3b (Okano et al., 1999).

DNA methylation is an independent predictor of mortality in addition to some known risk factors for aging such as smoking, diabetes and hypertension (Marttila et al., 2015). Genome-wide studies of senescent stem cells have shown that the low level of gene promoter DNA methylation is associated with cell self-renewal (Marttila et al., 2015). In addition, the decrease of age-dependent DNA methylation level is also accompanied by the high methylation of some special gene loci, such as c-fos, IGF-2, and $p16^{INK4a}$. Down-regulation of DNMT3A and TET2 (main regulators of DNA methylation state) contributes to the increased stem cell number and decreased differentiation ability (Challen et al., 2011). Hypomethylation of eNOS gene promoter region induces up-regulation of eNOS gene level and increases enzyme activity. Studies have shown that the DNA methylation of $p66^{shc}$ and Jun D leads to endothelial injury and ROS production (Challen et al., 2011). Furthermore, hypomethylation of the promoter region also promotes gene expression associated with inflammation, obesity, cell dysfunction, and vascular injury (Liu et al., 2008).

3.2 Histone modification

Nucleosome is the smallest unit of eukaryotic chromatin, composed of DNA and core regions (H2A, H2B, H3 and H4). Histone modification regulates gene transcription and expression and post-translational modifications modify chromatin in different ways including acetylation, methylation, ubiquitination, and phosphorylation to regulate chromatin structure and function that affect gene transcription and expression (Kimura, 2013).

3.2.1 Histone acetylation and deacetylation

Histone acetylation is a special epigenetic marker that plays an important role in chromatin structure, function, gene expression, gene transcription, and chromatin remodeling. Histone acetyltransferase (HATs) and histone deacetylase (HDACs) are responsible for acetylation and deacetylation, respectively. His-tone acetylation modifications are the first identified histone post-translational modifications (Cooper and El-Osta, 2010). Studies have shown that DNA methylase (DNMTs), histone methyltrans-ferase (HMTs), and histone acetyltransferase (HATs) are closely related to chromatin remodeling under certain pathophysiological stimuli. HATs and HDACs are reported to regulate endothelial dysfunction and inflammatory responses in type 2 diabetes (Vil-leneuve and Natarajan, 2010).

Sirtuins, members of the deacetylase family, are widely studied to be key regulators of the aging process in yeast and mammals. SIRT1 is reported to regulate gene expression, metabolism, and aging by deacetylation of a series of enzymes and transcriptional switches such as PGC-1α, NF-κB, eNOS, FOXO, p53, p300/CBP, H3K9 and H3K56 (Beltrami et al., 2012). Studies have shown that SIRT1 overexpression increases endothelial cell migration, while loss of SIRT1 activity leads to down-regulation of key angiogenic factors including Flt1 and CXCR4 (Potente et al., 2007). Besides, SIRT1 is down-regulated in umbilical cord blood endothelial progenitor cells of premature infants, while SIRT1 overexpression alleviates EPCs aging and dysfunction (Vassallo et al., 2014). In addition, SIRT1 improves hyperglycemia induced endothelial dysfunction by inhibiting the vascular p66Shc gene transcription. SIRT1 decreases oxidative stress and inflammation in the vessel by inhibiting NF-κB and PARP activation (Zheng et al., 2012). In adipose tissue of obese patients, SIRT1 is down-regulated leading to histone hyperacetylation, recruitment of macrophages, and increased expression of pro-inflammatory factors such as IL-6, IL-1β, TNF-α, IL-13, IL-10 and IL-4. Collectively, these factors result in inflammation (Gillum et al., 2011). SIRT3 is an important regulator of senescent stem cells (Brown et al., 2013). VEGF expression decreased in $SIRT3^{-/-}$ mice results in angiogenesis and clone formation defects. Interestingly, SIRT3 overexpression reverses these abnormalities. Recent studies have shown that H3K9 deacetylase SIRT6 has a protective effect on senescent endothelial cells (Mostoslavsky et al., 2006). In macrophages, HDAC3 regulates the expression of inflammatory genes, while HDAC2 inhibits inflammation (Zeng et al., 2006). In addition, HDACs are also involved in angiogenesis. HDAC7 promotes endothelial cell migration and lumen formation by activating platelet-derived growth factor B and its receptors (Mottet et al., 2007). *In vivo* and *in vitro*, HDAC6 and HDAC9 also play a pro-angiogenic role by inducing endothelial cell migration and the formation of functional cavities (Kaluza et al., 2013). In contrast, HDAC5 was found to play an anti-angiogenic role in endothelial cells (Urbich et al., 2009).

3.2.2 Histone methylation and demethylation

Chromatin status and function are different due to the different number of methylation sites and groups. Histone methyltransferase is responsible for the methylation of histone lysine and arginine with different modification sites that drive different effects of gene expression. H3K4 methylation induces gene activation while H3K9 and H3K27 methylation inhibits gene expression. In addition, modification of polymethyl groups on histone lysine leads to different levels of methylation that may have different biological significance. For example, H3K9me and H3K9me2 modifications are often found in genes whose expression is inhibited, while H3K9me3 forms are found in heterochromatin. PDGF-BB-induced changes in the VSMCs phenotype were also associated with histone modification. Recently, with the development of single-cell epigenetic technology, the modification status of single VSMCs in vascular plaque tissues can be tracked (Gomez et al., 2015).

It has been reported that both H3K4me3 and H3K27me3 modification is closely related to lifespan extension. In senescent HSCs, H3K4me3 and H3K27me3 are increased, but H3K9me3 level is

down-regulated in MSCs isolated from elderly patients (Goodell and Rando., 2015). Other studies have shown that H3K4 methylation plays an important role in angiogenesis, which is mediated by the SET domain containing enzymes (e.g. SET1A/B, SET7, MLL1/2, MLL3/4) (Ernst and Vakoc, 2012). SET7-mediated lysine methylation regulates important longevity genes such as SIRT1, FoxO3, NF-κB and p53 (Paneni et al., 2013), but negatively regulates the transcription HIF-α thereby inhibiting retinal and tumor angiogenesis. Another histone methylase MLL1 leads to H3K4me3, which plays an important role in embryonic hematopoiesis, hematopoietic stem cell renewal, endothelial progenitor cell differentiation, and endothelial cells (Diehl et al., 2007). In addition, Histone methyltransferase G9a and SUV39H1 agonists could induce H3K9 methylation and inhibit VEGFA expression in HEK293 cells while treatment with G9a small molecule inhibitor, endothelial progenitor cells improved angiogenesis and myocardial repair after myocardial infarction (Snowden et al., 2002). Inhibition of H3K27 demethylase increased lifespan extension by targeting the insulin/IGF-1 signaling pathway (an important longevity pathway). In endothelial cells, VEGF recruits EZH2 to induce H3K27me3, which affects downstream angiogenesis. Interestingly, EZH2 silencing increases endothelial cell migration and promotes capillary network formation, which is considered as a potential therapeutic strategy to promote angiogenesis. Furthermore, EZH2 was recruited by MTA2 and bounded to the promoter region of the TSC2 gene to inhibit the transcription of TSC2 (Wei et al., 2015). Inhibition of histone methyltransferase HYPB/SETD2 induced down-regulation of H3K36me3 and further damaged the migration and cavity formation suggesting that HYPB/SETD2 plays an important role in the vascular reconstruction of embryos (Hu et al., 2010). Other studies have shown that H3K36me3 may be another important target for biological regulation in regenerative medicine. As a result, drugs targeting histone modification enzymes may have an important application in clinical tumor treatment and vascular remodeling-related diseases (Li et al., 2011; Usui et al., 2014).

3.3 MicroRNAs

Studies have shown that noncoding RNA, microRNA (miRNA) and long non-coding RNA (lncRNA), play an important role in the post-transcriptional regulation of gene expression. The microarray approach was used to reveal multiple miRNAs involved in endothelial cell senescence and angiogenesis. MiRNA expression was significantly up-regulated during aging leading to post-transcriptional inhibition of age-related genes in endothelial phenotypes (D'Andrade and Fulmer-Smentek, 2012). It has been reported that upregulation of mir-34a in senescent endothelial cells and endothelial progenitor cells inhibited cell proliferation that progressed cellular senescence and decreased mice lifespan (Boon et al., 2013). In response to DNA damage, Mir-29 and mir-17-92 are key regulators of chromatin related proteins such as chk1/2, g-H2AX, and ATM. (Ugalde et al., 2011). In addition, mir-29 was up-regulated in senescent endothelial cells and aortic tissue of elderly mice resulting in a decrease in extracellular matrix deposition. Interestingly, mir-29 was down-regulated in myocardial microvascular endothelial cells isolated from type II diabetic rats and promoted angiogenesis by regulating the expression of IGF-1 (Boon et al., 2011). Mir-20a inhibits endothelial cell migration by the inhibition of MKK3 and the activation of p38 MAP kinase (Deng et al., 2017). Downregulation of mir-126 led to cell apoptosis, decreased proliferation, and endothelial cell migration (Mocharla et al., 2013). By analyzing the MicroRNA expression profile of elderly mice, it was found that mir-10a, mir-21, and target genes are key drivers of endothelial progenitor cell dysfunction. Inhibition of these miRNAs resulted in regeneration of endothelial progenitor cells and improved *in vivo* and *in vitro* angiogenesis (Zhu et al., 2013). The increased age-dependent mir-217 level impaired endothelial angiogenesis by down-regulating SIRT1, FOXO1, and eNOS. On the contrary, inhibition of mir-217 in senescent endothelial cells alleviated aging and increased angiogenesis. The senescent endothelial cells are also associated with the decreased expression of mir-146a (Vasa-Nicotera et al., 2011).

In summary, epigenetics is an important mechanism of aging involving multiple signaling pathways such as inflammation, oxidative damage, mitochondrial dysfunction, and apoptosis. Vascular aging is an important risk factor of CVD and various types of epigenetic modifications interact with each other. For example, the expression of histone modifications such as H3K9Ac and H3K27me3 are down-regulated in aging. Age-related DNA hypermethylation is closely related to the inhibitory histone marker H3K27me3/H3K9me3 (Bracken et al., 2007); DNA hypomethylation is highly enriched at the chromatin marker H3K4me1 (Fernandez et al., 2015). Furthermore, calorie restriction plays an important role in promoting lifespan by regulating epigenetic mechanisms such as DNA methylation and histone modification (Rizza et al., 2014).

4. Concluding Remarks

Nowadays, a large range of environmental stimuli (pollution, high-fat diets, and cardiovascular risk factors) may bring epigenetic alterations such as DNA methylation, histone modification, and non-coding RNA. Gene expression, cell phenotypes, and biological functions could change upon these epigenetic alterations resulting in hypertension, atherosclerosis, restenosis, pulmonary hypertension, and other cardiovascular diseases. The aim of this review was to investigate the specific mechanism of epigenetics in vascular aging. Formulation of epigenetics treatment strategies is one of the major challenges of age-related cardiovascular diseases. With the development of high-throughput sequencing technology, the changing trends of genome-wide DNA methylation, histone modification and non-coding RNA is easier to detect, it is expected to develop epigenetic-based treatment strategies for patients with age-related cardiovascular diseases in the future (Costantino et al., 2018).

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Conflict of Interest

The authors declare no competing interests.

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