BBA - Molecular Basis of Disease 1865 (2019) 1810–1821

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Review

Vascular smooth muscle cell senescence and age-related diseases: State of the art☆

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ARTICLE INFO

Keywords:
Senescence
Vascular smooth muscle cells
Aging
Hypertension
Atherosclerosis
Diabetes

ABSTRACT

Aging is a worldwide challenge, and it is accompanied by the accumulation of senescent cells. Cellular senescence is traditionally defined as permanent cell growth arrest and currently includes the senescence-associated secretory phenotype (SASP). There are two main types of cellular senescence, including telomere-dependent replicative senescence and stress-induced premature senescence. The process of cellular senescence is mainly controlled by two effector pathways, namely, the p53-p21 and p16-retinoblastoma protein (pRB) pathways. Vascular smooth muscle cells (VSMCs) are integral parts of arteries and play an important role in vascular structure and function. VSMC senescence may be triggered by many factors, such as angiotensin II, oxidative stress, inflammation, DNA damage, and small molecule compounds. These inducers are able to genetically and epigenetically regulate VSMC senescence. The senescence of VSMCs together with the SASP contributes to chronic vascular inflammation, the loss of arterial function, and the development of age-related diseases. Current evidence suggests that the senescence of VSMCs might be harmful to individual health, whereas its influence on the lifespan is not clear. The purpose of this paper was to review the current knowledge regarding VSMC senescence and its relevance to hypertension, atherosclerosis, and diabetes, as well as the potential mechanisms responsible for VSMC senescence in these age-related diseases.

1. Introduction

Aging, as a global health concern, is a challenge to individual health and public health policy. In the aging process, tissue and organ functions are progressively lost, at least in part, thereby leading to a high risk of age-related diseases and an increased risk of mortality [1]. Specifically, cerebra-cardiovascular diseases are the leading cause of mortality among all age-related diseases and are responsible for more than half of all deaths in developed countries, such as Europe [2,3]. One characteristic of aging is the accumulation of senescent cells, which is the result of an imbalance between damage to cells and an insufficient clearance or repair of damaged cells in aged organisms [1]. Quantitative data have indicated that in lungs or livers, senescent cells in aged mice were approximately twice the percentage of those in young mice [4], and the accumulation of senescent cells in arterial walls has also been reported by numerous researchers [5]. With emerging data, cellular senescence, which has primarily been defined as cellular growth arrest or withdrawal from the cell cycle, has been implicated as an important contributor to age-related diseases [6].

Vascular smooth muscle cells (VSMCs), as basic ingredients of the vascular wall and the sole cell type in the arterial medial layer, play critical roles in vascular physiological functions. VSMCs and the extracellular matrix (ECM) synthesized by VSMCs are the major regulators of the contractile tone of arteries, thus contributing to the maintenance of arterial peripheral resistance, the regulation of blood pressure, the distribution and redistribution of blood flow, and the repair of arteries [7]. Changes in VSMCs and ECM, particularly in large arteries, such as the aorta and the carotid, would influence arterial compliance. Increased arterial stiffness suggests an impaired transformational function of arteries, in which blood flow cannot be changed

☆ This article is part of a Special Issue entitled: Genetic and epigenetic regulation of aging and longevity edited by Jun Ren & Megan Yingmei Zhang.

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https://doi.org/10.1016/j.bbadis.2018.08.015
Received 31 May 2018; Received in revised form 20 July 2018; Accepted 13 August 2018
Available online 16 August 2018

from pulsatile to steady [8]. Moreover, VSMCs are not only the regulator but also the effector in arteries. VSMCs were able to respond to many circulating vasoactive substances and act as a sensor of mechanical forces and transduce them into various biochemical signals [9]. VSMCs are the focus of substantial attention in vascular studies because of their importance in vascular structure and function.

The accumulation of senescent VSMCs raises a question regarding the specific role that senescent VSMCs play in age-related diseases. In this review, we emphasized the characteristics of VSMC senescence and focused on the relationship between VSMC senescence and age-related diseases (hypertension, atherosclerosis and diabetes), as well as the potential mechanisms responsible for VSMC senescence.

2. VSMC senescence: what is it?

Cellular senescence was first introduced by Hayflick and Moorhead [10] to describe the concept that cells can no longer divide (also referred to as the Hayflick limit). Campisi J and his colleagues [11] summarized four common characteristics of senescent cells: 1) growth arrest, 2) apoptosis resistance, 3) altered gene expression, and 4) changes in senescent markers. With the deeper knowledge of cellular senescence, senescence is currently beyond the initial growth arrest, representing complex cellular states in which both phenotypical and mechanistic changes are involved, and the relationships between cellular senescence and age-related diseases are generally identified [12].

VSMC senescence conforms to the common characteristics of senescent cells. Similar to other senescent cells, senescent VSMCs have a low ability of mitotic division [13] and changes in cell signaling pathways and senescent markers, for example, the senescence-associated beta-galactosidase (SA-β Gal) activity and the levels of p16, p38, p53-p21, and phospho-histone H2A.X [14]. Apart from these changes, there are special characteristics of VSMC senescence, including changes in the responsiveness of VSMCs to contracting and relaxing mediators, changes in VSMC phenotypes from contractile to synthetic during the senescent process, changes in specific signaling pathways in VSMCs, such as protein kinase G-1 (PKG-1) and voltage-dependent and Ca^{2+}-activated K^+ (BKCa) channels, and changes in the communications between VSMCs and the ECM [15]. Senescent VSMCs could directly influence the arterial tone with these special characteristics.

It is of note that cellular senescence includes not only growth arrest but also the senescence-associated secretory phenotype (SASP), which is defined as the expression and secretion of a series of proteins that influence both senescent cells and the local environment [14]. Proteins secreted in the SASP were all pro-inflammatory factors, such as cytokines, chemokines, growth factors and proteases. No anti-inflammatory factors were detected in the SASP [16]. Animal experiments confirmed that the SASP also existed in senescent VSMCs [17]. Gardner SE et al. [18] identified that senescent human VSMCs released multiple high-level pro-inflammatory proteins, which might upregulate inflammasome components and increase the metabolic burden of senescent VSMCs. These results suggested that the SASP was not only the result of VSMC senescence but also a promotor of VSMC senescence, thus creating a vicious circle in aging and age-related diseases [19]. This primary-senescent-cell-induced cellular senescence has another name referred to as paracrine senescence [6]. Current evidence suggests that senescent cells and the SASP might be participators or major drivers in chronic vascular inflammation, the loss of tissue function, and age-related diseases [20].

3. How do VSMCs undergo senescence?

To address how VSMCs become senescent, three questions should be clarified, including the phases that cellular senescence experiences (modes), the signaling pathways that regulate the senescent progress (effector pathways) and the factors that cause the cellular senescence (inducers).

With the increasing understanding of cellular senescence, senescence is currently considered a multistep process rather than a static state of cells [12]. Deursen et al. [12] summarized that the process of senescence could be divided into four phases, namely, quiescence (initial temporary senescence), early senescence (stable growth arrest), full senescence (chromatin changes associated with senescence and the SASP), and late/deep senescence (phenotypic diversification). Moreover, in contrast to the traditional concept that cellular senescence was limited to mitotic cells, studies have also confirmed that senescence was present in postmitotic cells [21–23].

The well-accepted classical effector pathways have been summarized by Campisi et al. [11]. Regardless of whether the senescence was caused by advanced age (telomere-dependent replicative senescence) or other types of inducers (stress-induced premature senescence), senescence was predominately controlled by two tumor suppressor pathways, the p53-p21 and p16 pathways. During the aging progress, telomeres become short and dysfunctional, which leads to a DNA damage response (DDR) [24]. The DDR, as well as other numerous intra- and inter-cellular stressors (such as Ang-II, ROS and inflammation), could activate p53 and p16 alone or together. Activation of p53 could induce p21, a cyclin-dependent kinase (CDK) inhibitor, thus leading to the inhibition of cyclin E-CDK2 [25]. Activation of p16, another CDK inhibitor, was able to inhibit cyclin D-CDK4/6. Both p21 and p16 were able to maintain the retinoblastoma protein (pRB) in an active form, resulting in cellular senescence and subsequent effects [11] (Fig. 1). (Fig. 1). In addition to the previously described classical pathways, other senescence signaling pathways have been demonstrated. For example, Freund et al. [26] showed that p38 could independently regulate SASP when the long-term DDR occurred.

The inducers of cellular senescence are very complicated. As a result of the confluence of genes and environments, VSMC senescence could be triggered by many factors, which may regulate cellular senescence at the genetic and/or epigenetic levels. In 1990, telomere shortening was identified as an important cell senescence trigger [27]. This age- and proliferate-dependent cellular senescence could explain, in part, the accumulation of senescent cells in healthy elderly subjects. However, emerging data have suggested that VSMC senescence could also be caused by factors independent from proliferation, such as angiotensin II [28], oxidative stress and inflammation [29], DNA damage [30], oncogene activation [31], small molecule compounds, such as drugs [32], and collagenase-resistant collagen [33]. Here, we summarized three conventional senescence inducers that act on VSMCs (angiotensin II, oxidative stress, and inflammation) and two emerging regulators of VSMC senescence (epigenetics and autophagy).

3.1. Angiotensin-II in VSMC senescence

The renin-angiotensin-aldosterone system (RAAS) plays a critical role in vascular function, and inhibition of the RAAS is a frequently used treatment strategy among many age-related diseases, such as hypertension and chronic kidney diseases [34]. Here, we highlighted the effects of the RAAS (with a particular focus on Angiotensin-II [Ang-II]) on VSMC senescence. The reasons are as follows: 1) the RAAS and Ang-II are extensively investigated in vascular diseases, and the regulations, pharmacological actions and effects of Ang-II are well clarified; 2) accumulative evidence has proven that Ang-II is involved in VSMC senescence and vascular aging in cell or animal models [35]; 3) Ang-II is widely used in the research of vascular senescence both in vivo and in vitro, and the Ang-II-induced vascular/VSMC senescence model is effective and repeatable [36]; 4) Ang-II is associated with the epigenetic regulation of cellular senescence and age-related diseases [37].

The RAAS is one of the most important signaling pathways that trigger VSMC senescence. Ang-II is an essential substance that effectively activates the RAAS by acting on Ang-II type I and type II (AT1 and AT2, respectively) receptors. It has also been demonstrated that Ang-II could increase the expression of DNA methyltransferase in

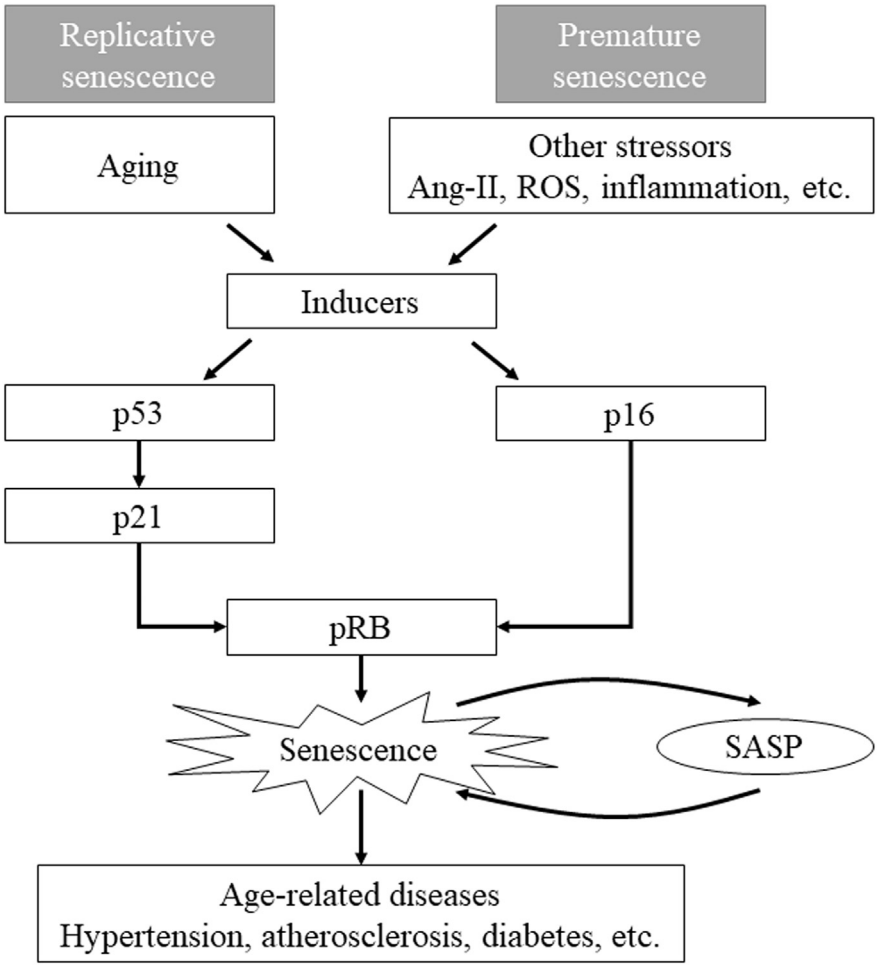

Fig. 1. Types of cellular senescence and effector pathways.

arteries, and the inhibition of DNA methyltransferase could prevent vascular wall thickness [38]. Previous studies have shown that the expression of Ang-II, together with the AT1 receptor, increased with age. In contrast to this finding, the AT2 receptor decreased with age [39–41]. Based on studies from the AT1 and AT2 receptors, it is strongly proposed that Ang-II is associated with VSMC senescence mainly by acting on the AT1 receptor. Min et al. [42] have demonstrated that the deletion of the AT2 receptor could enhance the cellular senescence induced by Ang-II. Two well-accepted effects of Ang-II on VSMC senescence were migration and inflammation. Ang-II could modify many factors that influenced VSMC migration, including but not limited to monocyte chemotactic protein-1 (MCP-1), calpain-1, milk fat globule protein epidermal growth factor-8 (MFG-EGF8), and matrix-degrading metalloproteinases (MMPs) [43]. Furthermore, Ang-II was tightly associated with inflammation in VSMCs. Ang-II has been shown to be an effective activator or promotor of nuclear factor κB (NF-κB), transforming growth factor-β (TGF-β), MMP systems and reactive oxygen species (ROS) productions, thus making it a key contributor to arterial inflammation and cellular senescence [44]. Anti-inflammatory or antioxidant strategies, such as the use of resveratrol [45] or celastrol [46], activation of the nuclear factor-E2-related factor 2 (Nrf2) [47] or α-7 nicotinic acetylcholine receptor (α7nAChR) [48], or clearance of ROS derived from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 1 [28], could alleviate the effects of Ang-II on VSMC senescence.

In general, three signaling pathways have been considered to play a role in VSMC senescence determined by the RAAS, including the MMP, MCP-1, and TGF-β1 pathways. MMPs are a family of enzymes with critical roles in the degeneration of proteins in the extracellular matrix (ECM). The typical structure of MMPs contains a propeptide, a catalytic metalloproteinase domain, a linker peptide, and a hemopexin domain [49]. MMPs have been shown to exert multiple effects on VSMCs, including proliferation (MMP-9, stimulating VSMC proliferation), migration (MMP-1,2,9, facilitating VSMC migration and modulating VSMC-ECM adhesion), and relaxation (MMP-2,9, inhibiting VSMC contraction) [50]. Apart from these effects of MMPs, the activation of MMP-2 in VSMCs by Ang-II was able to induce age-related vascular calcification and fibrosis [51], and the increment of the MMP-9 level could result in vascular inflammation [35]. Most importantly, MMP-9 has been defined as the signature of inflammatory senescence [52]. Similar to MMPs, MCP-1, which is a member of the C-C subfamilies of chemokines and exhibits effects through the C-C chemokine receptor type 2 (CCR-2), also contributes to cellular senescence [53]. However, it should be noted that the link between MMPs (or MCP-1) and age-related diseases was mainly characterized by the aberrant vascular architecture (that is, ECM degeneration and vascular fibrosis) rather than directly inducing VSMCs to become senescent [54].

TGF-β1 is another molecule related to senescence. TGF-β1 is cleaved from pro-TGF-β1, a large molecular complex. The release of TGF-β1 from pro-TGF-β1 could be promoted by inflammation and/or MMPs (MMP-2/9) [55], and activation of TGF-β1 could be promoted by Ang-II [56]. TGF-β1 was able to increase the expression of collagenase and stromelysin and decreased the expression of the tissue inhibitor of metalloproteinases-1 (TIMP-1), thus leading to the senescence of cells in vitro [57]. Moreover, TGF-β1 was important in collagen deposition, and an increased expression of TGF-β1 was associated with enhanced

arterial stiffness [58]. Furthermore, TGF-β1, which could be secreted by senescent VSMCs in the SASP, was an important mediator in paracrine senescence and was able to initiate the senescence of other cells and reinforce the senescent phenotype of VSMCs [59].

3.2. Oxidative stress and inflammation in VSMC senescence

Current evidence has indicated two common mechanisms involved in nearly all age-related diseases, namely, cellular oxidative stress and inflammation [60]. We hereby summarized the effects of oxidative stress and inflammation on VSMC senescence.

Oxidative stress, which reflects an imbalance between the production of ROS and the ability to readily scavenge the reactive intermediates or repair the resulting damage, plays an important role in VSMC senescence and age-related diseases. It has been shown that the methylation and acetylation of histone were associated with age, the level of superoxide dismutase, and the production of ROS, the well-known contributors to cellular senescence and cardiovascular diseases [61]. It has also been demonstrated that histone modification was an important regulator of ROS. In naturally aged mice or H_2O_2-treated mice, H3-Ac, H3K9-Ac and H3K4-tri-Me could regulate the expression of $p66^{shc}$, a protein that could promote ROS production, thus resulting in cellular senescence [62]. H3 Lys-4 methylation and the excessive acetylation of H4 have also been identified in H_2O_2-induced senescent cells [63]. Moreover, compared with VSMCs in young mice, ROS production was significantly increased in senescent VSMCs, leading to an increase of mitochondrial DNA damage, and a decrease of endogenous antioxidant activity ensued [64]. ROS have also been associated with nuclear DNA damage, and both ROS and nuclear DNA damage were important inducers of premature senescence [65].

There are mainly four sources of ROS generation, including ROS from mitochondria during oxidative phosphorylation and from NADPH oxidases, xanthine oxidase, and uncoupled NO synthase. The effects of the first two sources on VSMC senescence and age-related diseases have received widespread attention [66]. With an increasing age, the expressions of mitochondrial biogenesis factors and cytochrome C oxidase (COX) were significantly decreased, thus contributing to the increment of mitochondrial oxidative stress [67]. NADPH oxidases (NOXs), particularly NOX-4, were identified as enzyme proteins that regulated ROS generation. Activation or increased expression of NOX-4 was able to enhance the hydrogen peroxide production and change VSMCs into the pro-inflammatory phenotype [68]. Vendrov et al. [69] showed that NOX-4 but not NOX-1 or NOX-2 was responsible for the increased production of ROS. NOX-4 could be epigenetically regulated. It was the direct target of miRNA-146a, miRNA-23b or miRNA-25. These miRNAs could upregulate the NOX-4 expression and ROS level, thereby leading to cellular senescence [70]. Furthermore, histone deacetylases (HDAC), particularly HDAC3, could decrease the expression of NOX4. The inhibition or knockout of HDAC3 resulted in the increase of NOX4 and ROS, thus contributing to cellular senescence [71]. Interestingly, Przybylska et al. [72] reported that a low level of NOX-4 could also induce VSMC senescence, which was independent of DNA breaks or DDR, thus suggesting the multiple functions of NOXs in cellular senescence.

In general, there are two types of inflammation, acute inflammation and chronic inflammation. Acute inflammation, which is caused by infection or injury, participates in pathogen destruction, damage reparation, and tissue function restoration [73]. The inflammatory response is typically well regulated and will end following homeostasis reestablishment. However, some conditions, such as the unsuccessful elimination of harmful agents or failure in inflammation regulation, will lead to a chronic inflammatory state [74], particularly in individuals with advanced age because of their impaired ability of inflammation resolution [75]. A meta-analysis has confirmed that most age-related gene expressions are characterized by the overexpression of inflammation and immune response genes [76]. Low-degree chronic inflammation contributes to the development of age-related diseases and is constantly present during the entire process of age-related diseases. Moreover, this process, caused by the interaction between environmental and genetic factors, has been defined as 'inflamm-aging' in recent years, which makes cells, including VSMCs, enter the senescent state [77]. Smoking might represent a good example to explain the interaction between environmental and genetic factors. Kuilman et al. [78] reported that longtime exposure to smoke could increase the level of NF-κB and the acetylation of RelA/p65, thereby accelerating cellular senescence. Data from human studies have shown that both the number of senescent VSMCs and the level of plasma inflammatory proteins, including C-reactive protein (CRP), interleukin-6 (IL-6) and NF-κB, were positively associated with age in an elderly population [73].

Oxidative stress and inflammation are closely linked to each other. Signaling by ROS is essential in the activation of inflammation. Various inflammatory factors, including many cytokines and chemokines, are regulated by ROS via transcriptional factors [79]. Moreover, these inflammatory factors may result in an inflammatory response in other cells, which increases ROS production. Thus, it generates a vicious circle between oxidative stress and inflammation and deteriorates the pathological conditions. Anti-inflammatory strategies, such as the stimulation of the vagus nerve and/or activation of α7nAChR, could reduce oxidative stress [80–82], and vice versa [83].

In conclusion, these three inducers of VSMC senescence (Ang-II, oxidative stress, and inflammation) are altered with age. The age-related increment of Ang-II and the Ang-II/AT1-receptor ratio, oxidative stress, and inflammation contributed to the senescence of VSMCs. Moreover, senescent VSMCs, particularly the SASP, were able to secrete pro-inflammatory factors in the local environment, which deteriorated to arterial dysfunction and vascular aging.

3.3. Epigenetics in VSMC senescence

Epigenetics play important roles in VSMC senescence and vascular aging. However, current data regarding epigenetics and VSMC senescence are very limited. DNA methylation is an important form of epigenetics. Studies have shown that DNA methylation was globally decreased in the genome of centenarians [84], whereas other studies have indicated that DNA methylation increased with age [85]. Based on this background, it was not surprising to find that the activation of DNA methylases [86] or DNA demethylases [85] could attenuate cellular senescence and vascular aging. Histone methylation and acetylation have also been related to VSMC senescence and vascular aging. The methylation of histone 3 (H3) lysine K4 and K27 was increased with age, and a longer life was observed with H3K27 demethylase inhibition [87]. Histone acetylation was more directly linked to VSMC senescence. The inhibition of HDAC8, a histone deacetylase located in VSMCs, could induce senescence [88]. Furthermore, miRNAs have also been considered to play important roles in VSMC senescence. Badi et al. [89] reported that miRNA-34a induced VSMC senescence via the down-regulation of SIRT1, and Nicholson et al. [90] showed that miRNA-203 induced VSMC senescence through cytoskeleton pathways. Zheng et al. [91] reported that miR-200c regulated VSMC proliferation, and Lin et al. [92] proposed that miRNA-135a suppressed VSMC senescence and calcification. Taken together, these findings suggested that epigenetics regulated VSMC senescence and vascular aging. However, the current data are limited, and future studies regarding epigenetics, VSMC senescence and vascular aging are required.

3.4. Autophagy in VSMC senescence

As previously discussed, Ang-II, oxidative stress and inflammation contributed to VSMC senescence, whereas the role of epigenetics in VSMC senescence might be accelerating or preventing. Emerging evidence has shown that autophagy, which was defined as the natural, regulated, destructive process of cells that disassembled unnecessary or

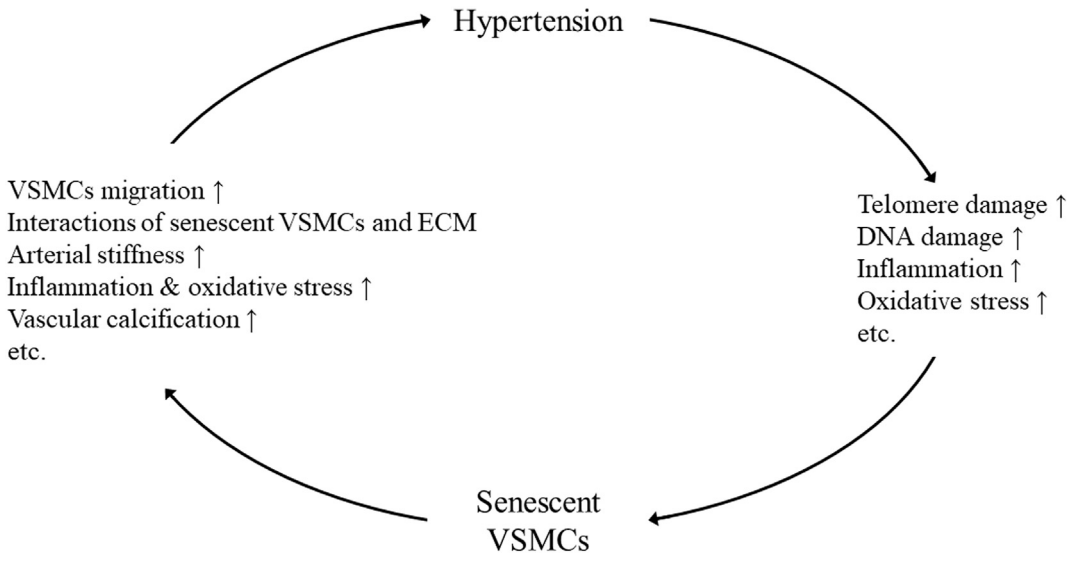

Fig. 2. Vascular smooth muscle cell (VSMC) senescence in hypertension.

dysfunctional components [93], might be an essential factor to protect VSMCs from senescence. Autophagy is of substantial importance in regulating the proliferation and cellular phenotype of VSMCs [94]. Activation of autophagy could alleviate VSMC senescence, and inhibition of autophagy produced the opposite effects [95]. The mammalian target of rapamycin (mTOR) pathway is a classical signaling pathway that regulates autophagy. Activation of the mTOR pathway, which could inhibit autophagy, induced VSMC senescence, and inhibition of the mTOR pathway could reduce VSMC senescence [96]. Another novel link between autophagy and VSMC senescence includes key autophagy-related (Atg) genes and proteins. Defective autophagy caused by the deletion of the Atg-3 gene [97] or the oxidation of Atg-3 and Atg-7 proteins [98] could induce VSMC senescence. Agents such as emodin [99] and celastrol [46], or the activation of silent information regulator 1 [100] could activate autophagy and alleviate VSMC senescence, suggesting the bright future of pharmacological intervention in autophagy and senescence.

4. VSMC senescence in age-related diseases

Aging, as one of the most important risk factors for age-related diseases, is an inevitable process in life. As previously discussed, the number of senescent VSMCs increases with aging. To date, the methods used to characterize senescent cells in vivo are limited. Studies have demonstrated that in contrast to diseases that are irrelevant to age, age-related diseases often occurred in the background of senescent cell accumulation and the subsequent chronic tissue/organ function loss [101]. Numerous studies have shown that VSMC senescence and age-related diseases are positively related. However, the causal relationship between them is not clear. Based on current evidence, cellular senescence in arteries, the consequences of which vary in different situations, seems to be harmful in age-related vascular diseases [11]. To differentiate whether cellular senescence is under the natural progression of aging (normal) or a condition of diseases (abnormal), Childs et al. [101] termed cellular senescence as primary senescence and secondary senescence. Primary senescent cells were based on 'normal' changes, which indicates this type of senescence was the fate of cells as the result of aging, tissue repair, etc. The secondary senescence seemed to be the 'abnormal' change as it occurred from the initiation of the diseases and continued through the entire course, which was in addition to the primary cellular senescence. Both primary senescence and secondary senescence could induce or aggravate age-related diseases. In this review, we focused on the current understanding of VSMC senescence in these age-related diseases: hypertension, atherosclerosis and diabetes.

4.1. VSMC senescence in hypertension

In general, hypertension is regarded as an age-related disorder as many clinical studies have indicated a significant relationship between aging and hypertension. The high prevalence and significant contribution of hypertension to various diseases make it a public health challenge among developing and developed countries [102]. The mechanisms that underlie hypertension are under investigation. From the physiological point of view, arterial compliance is a determinant of systolic blood pressure, and total peripheral resistance is essential for diastolic blood pressure. Both arterial compliance and total peripheral resistance are, at least in part, determined by the muscle tone in the smooth muscle tissue, that is, mainly by VSMCs [103]. The contractile state of the vascular tunica media is regulated by hormones, vasoactive peptides and ROS. As the main components of the tunica media, evidence indicated that VSMCs were involved in cellular mechanotransduction when blood pressure stretches the vessel wall, which suggested that the degradation of mechanosensing or the mechanoresponse function of VSMCs may be important for the genesis of hypertension [9].

Emerging data demonstrate that hypertension is significantly associated with VSMC senescence (Fig. 2). As previously discussed, the effector pathway of VSMC senescence is mainly controlled by the p53-p21 and p16-pRB pathways. In this part, we focused on the role of DNA damage, inflammation, and oxidative stress on VSMC senescence and hypertension.

It has been demonstrated that compared with normotensive individuals, the uncapping of telomeres, as a hallmark of DNA damage, was two-fold greater in hypertensive patients. However, there was no significant difference in the telomere length between normotensive individuals and hypertensive patients [104]. Studies based on hypertensive animal models have indicated that the accumulation of senescent VSMCs was more serious in hypertensive animals than in normotensive animals. These results were consistent in various hypertensive animal models, including spontaneously hypertensive rats [105], the deoxycorticosterone-acetate-salt treated hypertensive model [106], and specific-gene-mutated models with hypertension, such as Col1a1 gene mutation mice [33]. Furthermore, senescent VSMCs might comprise a risk factor or one mechanism responsible for hypertension. Noureddine et al. [107] reported that senescent VSMCs stimulated the migration and growth of normal VSMCs with the SASP effect, thus

contributing to the vascular remodeling and development of pulmonary hypertension. Moreover, Yin et al. [14] reported that aging ECM together with the subsequent senescent VSMCs might be an important driver of hypertension. These findings have suggested that DNA damage in hypertension might contribute to VSMC senescence, which, in turn, aggravated hypertension.

Similar to other age-related diseases, inflammation and excessive oxidative stress were also present in hypertension. Clinical studies have shown that plasma levels of inflammatory factors, such as C-reactive protein (CRP) [108], IL-6 and IL-1β [109], and tumor necrosis factor alpha (TNF-α) [110], were significantly increased compared with the concentrations in normotensive participants. Antihypertensive treatment could decrease the levels of these inflammatory factors with the use of RAAS blockades or calcium channel blockers [111–113], or with the application of renal sympathetic denervation [114]. In various hypertensive animal models, it has also been reported that dysfunction of the cholinergic anti-inflammatory pathway resulted in increased pro-inflammatory cytokines, damaged VSMCs and impaired target organ functions [115]. H_2O_2, which is considered a major component and an important signaling molecule of ROS, contributed to oxidative stress and tissue damage pathologically [116]. Touyz et al. [117] indicated that compared with normotensive individuals, the concentration of H_2O_2 in VSMCs in hypertensive patients was increased potentially because of phospholipase D pathway activation. Many studies have investigated the molecular mechanisms of inflammation, oxidative stress, VSMC senescence and hypertension, and numerous results have built up a complex network among them [118–120]. These findings suggested that hypertension and VSMC senescence were closely associated, and oxidative stress and inflammation might be the important links between them.

Another key factor between VSMC senescence and hypertension is arterial stiffness. Arterial stiffness, also referred to as arteriosclerosis (often confused with atherosclerosis), is defined as the lack of distensibility of arteries. Thus, the arterial lumen area cannot adequately respond to a specific change in blood pressure [8]. Arterial stiffness is a well-accepted valuable predictor of cardiovascular events in hypertensive patients [121]. Mitchell, the former leader of the vascular group of Framingham study [122], raised a theory of 'arteriosclerosis-related organ damage', which proposed that the distensibility mismatch between large arteries and small arteries induced the high pulse pressure in elderly hypertensive patients. Stiffer arteries were associated with more energy from blood pressure in target organs, leading to the higher rate of hypertensive target organ damage. It is accepted that arterial stiffening is a complex process that involves many changes. Hypertension and aging independently promote arterial stiffness. Initially, arterial stiffness was a result of structural changes in the arterial walls and the dysfunction of endothelia cells. However, increasing data have indicated that VSMC senescence participated in the progress of arterial stiffness. Basically, there are two types of VSMC phenotypes, namely, synthetic VSMCs (synthesizing ECM proteins) and contractile VSMCs (responding to agonists). These two VSMC phenotypes could switch to each other [123]. VSMC senescence was able to be added to both of these two VSMC phenotypes, thereby influencing vascular function and structure [8]. Cardiotrophin-1 may represent a regulator of arterial stiffness and VSMC senescence because of its contribution to vascular fibrosis [124]. Moreover, the serum levels of inflammatory biomarkers, such as IL-6 and CRP, were positively associated with pulse wave velocity, which worked as the current golden standard of noninvasive arterial stiffness measurement, suggesting the role of inflammation in arterial stiffness [125]. Recent studies regarding VSMC stiffness and its contribution to arterial stiffness have received substantial attention. Several regulators of VSMC stiffness were identified. Zhou et al. [126] reported that the activity of Rho kinase (ROCK) was significantly increased in hypertensive rats, and the inhibition of ROCK could reduce VSMC stiffness, arterial stiffness and blood pressure. ROCK might play a role through the serum response factor (SRF)/myocardin pathway, as the inhibition of ROCK decreased the expression of SRF/myocardin, and the inhibition of the SRF/myocardin pathway had similar effects as ROCK inhibition [126]. Wirth et al. [127] reported that activation of G-protein signaling could increase the vascular tone, and inhibition of G-protein signaling could decrease the age-dependent blood pressure elevation in mice. Moreover, TGFβ-1 has been shown to increase the expression of integrin receptors in fibronectin and the cytoskeletal stiffness in VSMCs, and both contributed to the molecular tension reinforcement in VSMCs [128]. Apart from these factors, vascular calcification, particularly medial artery calcification, could accelerate arterial stiffness and is closely associated with hypertension and heart failure [129]. It was believed that senescent VSMCs could significantly enhance vascular calcification, and this calcification induced by senescence was potentially mediated through runt-related transcription factor-2 (RUNX-2) by increasing the expression of alkaline phosphatase [130]. Moreover, markers of VSMC senescence, such as prelamin-A and IL-6, were identified in calcified VSMCs [8]. Kim et al. [131] demonstrated that the mineralocorticoid receptor in VSMCs, which was a direct regulator for blood pressure [132], also played a role in arterial stiffening with aging. This finding was consistent with a previous finding that the activation of the mineralocorticoid receptor in VSMCs changed the VSMCs into a pro-inflammatory phenotype [133]. Finally, it should be emphasized that although hypertension and aging independently promote arterial stiffness, their influences on the process of stiffening might be different. Both the changes in the ECM and VSMCs contributed to the development of arterial stiffness [134]. However, in the process of stiffening, hypertension only increased VSMC stiffness and adhesion without affecting aortic collagen and elastin, whereas aging not only changed the quantities of collagen and elastin but also had synergistic effects with hypertension on VSMC stiffness [135]. Cellular senescence caused by aging would gradually decrease the ability of contraction and the reprogramming potential of VSMCs, which increased the arterial stiffness as a complement [7]. Although the relationship among VSMC senescence, arterial stiffness and hypertension is rather complicated, it may be true that VSMC senescence contributes to arterial stiffness and thereby promotes hypertension.

4.2. VSMC senescence in atherosclerosis

Atherosclerosis is a worldwide, age-related health challenge. It is the most common pathological cornerstone of cardiovascular diseases, and its complications result in the most mortality and disability in many countries [136,137]. When an atherosclerotic plaque ruptures, the subsequent thrombosis is the top cause of acute coronary syndrome (ACS), leading to more than 70% of ACS [138]. Furthermore, more than 50% of cerebral ischemic strokes occur in patients with carotid plaque [139]. Atherosclerosis is a typical age-related disease. Population studies have shown that age is a strong risk factor for atherosclerosis, which is independent of other conventional cardiovascular risk factors, and more than 80% of individuals who are over 65 years old exhibit intracranial carotid artery atherosclerosis [140]. Pathologically, atherosclerosis is a complex phenomenon that involves the initial overload of lipids, accumulation of foam cells, and formation of plaques, including a fibrous cap and lipid-rich necrotic core, and subsequent potential changes, such as calcification, ulceration, intra-plaque hemorrhage, and rupture [141]. As the stromal cells of arteries and fundamental components of the fibrous cap, VSMCs play an important role in atherosclerosis.

There is strong evidence for the accumulation of senescent VSMCs in atherosclerosis. Studies from both humans and animals have indicated that senescent VSMCs were increased in atherosclerotic lesions, including fatty streaks and plaques [14,142–144]. Moreover, inhibition of cellular senescence regulators could reduce VSMC senescence in atherosclerosis. For example, with the inhibition of p21, the number of senescent VSMCs induced by Ang-II was significantly decreased [145].

Following p53 inhibition, the proliferation ability of senescent VSMCs in atherosclerotic lesions was significantly increased [146]. Salpea et al. [147] reported that the telomere length of VSMCs in atherosclerosis was shorter than normal. Of note, the occurrence of senescent VSMCs in atherosclerosis might be only in the intima and not in the media [148], which suggests the special role of VSMC senescence in the vulnerability of plaques. Consistent with this finding, it was demonstrated that the degeneration of ECM in fibrous caps of plaques was positively associated with VSMC senescence, which made plaques become unstable and easy to rupture [149]. It should be noted that senescent VSMCs might have little contribution to the formation of plaques; however, they imposed important effects on the size and phenotype of plaques [150], and the switch of the VSMC phenotype was able to influence the plaque stability [151]. These findings suggested that VSMC senescence existed in atherosclerosis and might exert a special role in plaque vulnerability.

Oxidative stress is considered an important link between VSMC senescence and atherosclerosis [152]. An increased ROS level was identified in aged VSMCs, and Nox4 NADPH oxidase might be one of the key regulators for VSMC phenotype changes, leading to the loss of stability in atherosclerotic plaques [153]. During the progress of atherosclerosis, oxidative stress was significantly enhanced, which contributed to DNA damage, telomere shortening and the down-regulation of telomerase activity, leading to VSMC senescence [144].

Inflammation also plays an important role in VSMC senescence and atherosclerosis. In contrast to oxidative stress, current studies regarding inflammation have suggested that inflammation was more likely to be the consequence rather than the initiator of VSMC senescence in atherosclerosis. It has been reported that breaks of DNA double-strands in VSMCs increased the level of IL-6, which was tightly associated with VSMC senescence, with no influence on p53 or pRB [154]. Data from VSMCs isolated from aged mice confirmed that the expression of IL-6 together with other atherogenic factors, such as intracellular adhesion molecule 1 and Toll-like receptor 4, were elevated [155]. Moreover, Gardner and his colleagues [18] reported that senescent VSMCs in atherosclerosis would switch to the SASP, thus secreting cytokines and chemokines via an IL-1-dependent manner and inducing chemotaxis and inflammation. Furthermore, the SASP could activate immune cells, which participated in the development of plaques [156]. The senescence-induced inflammation, as part of the complex network of inflammation in atherosclerosis, would subsequently contribute to the progress of atherosclerosis.

It is well-established that DNA damage is a key inducer of cellular senescence. Both DNA damage and DNA damage reparation have been extensively investigated in VSMC senescence and atherosclerosis. With respect to which type of DNA damage that senescent VSMCs suffered, the results from different labs were relatively different. Studies showed that VSMCs in atherosclerosis became senescent only after persistent DNA damage, such as the breaking of the DNA double-strand [154], whereas other studies indicated that VSMCs could become senescent with short-time DDR or persistent DNA damage [150,157]. However, regardless of the specific type of DNA damage, the DNA damage of senescent VSMCs was typically sublethal, resulting in VSMC growth arrest and phenotype changes [158]. Regarding DNA damage reparation, previous data indicated that the activation of telomerase could inhibit the progress of VSMC senescence in atherosclerosis [144]. Gray et al. [150] reported that senescent VSMCs in plaques exhibited a significant increase in oxidative stress and DNA damage. Inhibition of Nijmegen breakage syndrome protein 1, a DNA-reparation-associated protein, aggravated the growth arrest of VSMCs [150]. Wang et al. [157] reported that telomeric repeat-binding factor-2 (TRF2), a protective protein of telomere, could accelerate DNA damage reparation, thus leading to the reduction of the plaque core and the stabilization of the fibrous cap. Moreover, statins, the important foundation for the prevention and treatment of atherosclerosis, could accelerate the reparation of DNA damage without changing the initial level of DNA damage [159]. This might be one reason that statins are used to 'stabilize the plaque' in clinical practice. Apart from nuclear DNA damage, mitochondrial DNA damage and mitochondrial dysfunction also contributed to VSMC senescence in atherosclerosis via a ROS-dependent or ROS-independent mechanism [160]. Fibroblast growth factor 21, a protector of mitochondrial function, was able to reduce the ROS increment and VSMC senescence induced by Ang-II [161]. Alpha lipoic acid, a potent antioxidant, was able to improve mitochondrial function, reduce ROS production and induce telomerase reverse transcriptase, thus protecting against vascular senescence [162].

Other links between VSMC senescence and atherosclerosis have been reported. Autophagy has recently received the attention of researchers. Grootaert et al. [97] indicated that VSMC senescence was accelerated in arterial walls in autophagy-gene-deleted mice, and defective autophagy of VSMCs could promote neointima formation and atherogenesis. A recent study showed that the inhibition of autophagy resulted in a shorter lifespan and more severe cardiac aging than the control [163]. The underlying mechanism of autophagy for protecting against VSMC senescence and plaque rupture was considered to be related to the mTORC1/ULK1/ATG13 signaling pathway [95] or the dephosphorylation of p38 and mitogen-activated protein kinase [164]. Toll-like receptor 4 was also identified as a regulator of autophagy [165]. Moreover, coagulation factor Xa, which is produced and activated by the plaque locally, might contribute to VSMC senescence and the progression of atherosclerosis [166]. Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1, which regulates mitosis and induces centrosome amplification, was also considered to be a modulator of VSMC senescence [167].

Although VSMC senescence and atherosclerosis are tightly related, the interpretation of the role of VSMC senescence in atherosclerosis should proceed with caution. Using p53 knockout [168] or p21 knockout [169] mice, it was shown that the atherosclerotic lesion developed faster than the wildtype, although the DNA damage and death of VSMCs were reduced compared with the control, which might be a result of the multiple functions of p53 and p21 genes. Therefore, we suggest that similar to cellular senescence in tumors, the role of VSMC senescence might vary in different stages of atherosclerosis [170].

4.3. VSMC senescence in diabetes

Diabetes is a type of metabolic disorder characterized by a high blood glucose level for a long period of time. Type-2 diabetes, which is characterized by insulin resistance and a potential insulin secretion reduction, is the common type of diabetes and is regarded as an age-related disease [171]. It has been demonstrated that cellular senescence played an important role in diabetes. However, first, most studies involving diabetes have focused on endothelial cell senescence [172], pancreatic beta cell senescence and fatty cell senescence [101], and VSMC senescence was typically investigated in diabetes-related atherosclerosis. Second, current studies were involved in the influence of diabetes on VSMC senescence, and there was no direct evidence for VSMC senescence causing diabetes.

More than thirty years ago, Goldstein et al. [173] determined that the lifespan of cultured VSMCs from diabetic individuals was significantly reduced compared with normal ones, and VSMCs obtained from insulin-dependent or insulin-independent diabetes easily became senescent. Compared with age, it was believed that diabetes might be more important in VSMC senescence [173]. In addition, the nonenzymatic glycation in diabetes was significantly increased with age, which resulted in the decrement of protein function and subsequent cellular senescence and tissue dysfunction [174].

Both oxidative stress and inflammation might play important roles in VSMC senescence and diabetes. High glucose was able to independently increase the level of oxidative stress and inflammation both in vivo and in vitro [175]. In response to high glucose, VSMCs from aged mice displayed significantly higher levels of NF-κB and

protein carbonyls, an indicator of oxidative stress, than VSMCs from young mice [176]. The nitration of krueppel-like factor 5 contributed to the inflammatory gene expressions of VSMC induced by high glucose [177]. AKT2 might play a key role in mitochondrial dysfunction and ROS production [178], and the ablation of AKT2 could even prolong the lifespan in mice [179]. Excessive oxidative stress was believed to be a strong inducer of DNA damage. In diabetes, oxidative DNA damage was identified, which led to VSMC senescence, vascular dysfunction and accelerated atherogenesis [180]. It was also shown that the decreased activity of deacetylases contributed to the increment of the ROS level and senescent cells [181].

With aged VSMCs, activator protein-1 was able to bind more DNA and subsequently activated extracellular signal-regulated kinases (ERK) together with TNF-α, which was not observed in young VSMCs [182]. Moreover, glycated matrix protein was able to be induced by chronic high blood glucose stimulation, which subsequently activated MMP-2 and contributed to arterial stiffness in diabetes [183]. Similar to endothelial cells, Pandolfi et al. [184] showed that VSMCs could synthesize nitric oxide synthases (NOS), and the function of VSMCs to release NOS was changed with chronic high blood glucose exposure. Specifically, this change might be caused by two mechanisms: 1) changes in the VSMC phenotype and 2) the reduction of VSMCs with the ability of proliferation and NOS synthesis [184]. These findings suggested that senescent VSMCs played a role in vascular function in diabetes.

Antidiabetic treatment might reduce senescent VSMCs in diabetes. Gliclazide, a commonly used oral antidiabetic drug, could dose- and time-dependently inhibit the phosphorylation of AMPK activated by hyperglycemia and decrease VSMC proliferation [185]. Metformin, via AMPK activation, was able to protect against cellular senescence and ameliorated vascular function by improving mitochondrial function [186]. Activation of peroxisome proliferator-activated receptor, a target of thiazolidinediones, would influence cellular senescence and help prevent against atherosclerosis in diabetes [187].

5. Future directions

In the future, one question, which must be answered, is “does cellular senescence influence the individual health and lifespan?” As every coin has two sides, the effects of cellular senescence are double-sided. Senescent cells cannot undergo neoplastic transformation because of the disability of proliferation, making it an important safeguard of tumors [12]. However, senescent cells, particularly the SASP, may significantly increase inflammation in the local environment, resulting in more cells that become senescent. This long-term low-grade inflammation might be harmful in age-related diseases, such as hypertension, atherosclerosis, and diabetes [188]. With respect to VSMC senescence, current studies regarding antisenescent strategies have focused more on the endpoints, which were easy to measure, for example, the reduction of atherosclerosis [189] or the protection of arterial compliance [120]; in contrast, data regarding lifespan changes are scare. Based on the relationship between the proliferation potential of VSMCs and the donor age, it was calculated that the lifespan of human beings might be limited to less than 120 years [190]. It was also determined that VSMCs from *Peromyscus leucopus*, whose lifespan was twice that of *Mus musculus*, had approximately twice the proliferation activity than VSMCs from *Mus musculus* [191]. It was suspected that the decreased state of VSMC senescence might extend the lifespan. An animal study showed that the deletion of cardiotrophin 1 was able to decrease VSMC senescence and extend the lifespan of mice [124]. More studies, particularly studies in naturally aged animals, are required to identify the effects of the antisenescent therapies on aging, age-related diseases, health and the lifespan.

Another question is “does prevention of cellular senescence by gene modification or drug treatment improve health or lifespan?” BubR1-insufficient mice displayed cellular senescence. Animal studies with BubR1-hypomorphic mice (a premature animal model) have shown that antisenescent treatment by *CDKN2A* deletion [192] or *INK-ATTAC* transgene [193] was able to extend the healthspan; however, it had no significant influence on the lifespan. It was also demonstrated that killing senescent cells with cytotoxic drugs in mice was able to improve the life quality and alleviate age-related chronic diseases in mice [194]. These findings provide clues related to the mystery of improving health or the lifespan via the prevention of cellular senescence.

6. Conclusion

VSMC senescence initially referred to cells that cease to divide, representing permanent growth arrest and the SASP. There are mainly two types of cellular senescence, including telomere-dependent replicative senescence and stress-induced premature senescence. The former is an ineluctable fate of cells because of aging and the accompanied generally shortening telomere, whereas the latter results from severe insults, such as Ang-II, oxidative stress and inflammation. Senescence is currently considered a multistep process, including quiescence, early senescence, full senescence, and late/deep senescence. The process of cellular senescence is mainly controlled by the p53-p21 and p16-pRB pathways. VSMC senescence is closely associated with age-related diseases. Although the role of senescence might be different in various situations, it seems to be clear that the accumulation of senescent VSMCs is able to decrease arterial function and increase the risk of vascular diseases. Additional studies are necessary to determine the role of senescent VSMCs in age-related diseases; however, preventing against VSMC senescence and/or killing senescent VSMCs pharmacologically and/or genetically will provide substantial promise in improving the health and lifespan of individuals in the future.

Conflicts of interest

No.

Transparency document

The Transparency document associated this article can be found, in online version.

Acknowledgements

This study was supported by grants from the Shanghai Municipal Health and Family Planning Commission Foundation (No. 2016ZB0306) and the National Natural Science Foundation of China (No. 81773719, No. 81703590).

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