Review

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The emerging role of cell senescence in atherosclerosis

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Abstract: Cell senescence is a fundamental mechanism of aging and appears to play vital roles in the onset and prognosis of cardiovascular disease, fibrotic pulmonary disease, liver disease and tumor. Moreover, an increasing body of evidence shows that cell senescence plays an indispensable role in the formation and development of atherosclerosis. Multiple senescent cell types are associated with atherosclerosis, senescent human vascular endothelial cells participated in atherosclerosis via regulating the level of endothelin-1 (ET-1), nitric oxide (NO), angiotensin II and monocyte chemoattractant protein-1 (MCP-1), senescent human vascular smooth muscle cells-mediated plaque instability and vascular calcification via regulating the expression level of BMP-2, OPN, Runx-2 and inflammatory molecules, and senescent macrophages impaired cholesterol efflux and promoted the development of senescent-related cardiovascular diseases. This review summarizes the characteristics of cell senescence and updates the molecular mechanisms underlying cell senescence. Moreover, we also discuss the recent advances on the molecular mechanisms that can potentially regulate the development and progression of atherosclerosis.

Keywords: atherosclerosis; cell senescence; HVECs; HVSMCs; macrophages.

Abbreviations: AP-1, activator protein 1; AKT, protein kinase B; ALP, alkaline phosphatase; BMP, bone morphogenetic protein; BH4, tetrahydrobiopterin; C/EBPβ, CCAAT/enhancer binding protein β; CoQ10H2, coenzyme Q10; CCR2, monocyte chemoattractant protein-1 receptor; CDKs, cyclin-dependent kinases; CD44, cluster of differentiation 44; DAG, diacylglycerol; ET-1, endothelin-1; ETA, endothelin-1 A receptor; ETB, endothelin-1 B receptor; eNOS, endothelial nitric oxide synthase; FGE21, fibroblast growth factor-21; iNOS, inducible nitric oxide synthase; IP3, triphosphate; IL-1β, interleukin-1β; IL-18, interleukin-18; ICAM-1, intercellular cell adhesion molecule-1; LOX-1, lectin-like oxidized low density lipoprotein receptor-1; MMPs, matrix metalloproteinases; MCP-1, monocyte chemoattractant protein-1; NGAL, neutrophil gelatinase associated lipocalin; NF-κB, nuclear factor kappa-B; NADPH, triphosphopyridine nucleotide; NO, nitric oxide; $ONOO^-$, peroxide anion; OPG, osteoprotegerin; OPN, osteopontin; Ox-LDL, oxidized low-density lipoprotein; PPARγ, peroxisome proliferator-activated receptor γ; PMA, phorbol-12-myristate-13-acetate; P53, protein 53; P21, protein 21; PKC, protein kinase C; P16, protein 16; P38, protein 38; p62/SQSTM1, multifunctional ubiquitin-binding folded protein; P2X7R, purinergic ligand-gated ion channel 7receptor; PLA, phospholipase A; PIK3, phosphatidylinositol 3-kinase; PLC, phospholipase C; RB, retinoblastoma gene; RUNX-2, runt-related transcription factor; RAAS, renin-angiotensin-aldosterone system; ROS, reactive oxygen species; RANKL, receptor activators of the NF-κB ligand; SA-β-GAL, senescence-associated β-galactosidase; SIRT1, sirtuin 1; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecules-1; 4-HNE, 4-hydroxnonenal.

Introduction

Cell senescence is a physiological process of irreversible cell cycle arrest that contributes to various stress conditions, telomere shortening, DNA damage, reactive oxygen species (ROS) production, and mitochondrial dysfunction are main characteristic of cell senescence [1]. This process is classified as replicative senescence and stress-induced premature

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senescence. Replicative senescence is characterized by telomere shortening, whereas stress-induced premature senescence is mainly due to oxidative stress and oncogene activation. The main characteristics of cell senescence are (1) increased senescence-associated β-galactosidase (SA-β-GAL) activity [2]; (2) increased expression level of senescence-associated proteins (e.g., P53/P21 and P16); and (3) increased expression level of pro-inflammation cytokines (e.g., IL-18 and IL1β) and adhesion molecules (e.g., ICAM-1 and VCAM-1). In recent years, senescent-associated diseases, especially cardiovascular diseases, have endangered human health worldwide. Senescence-associated atherosclerosis is a major cause of cardiovascular diseases, which is considered the leading cause of mortality and morbidity. Senescence in atherosclerosis involves several cell types, including human vascular endothelial cells (HVECs), human vascular smooth muscle cells (HVSMCs), macrophages, and other cell types [3]. Accumulating evidence has shown that the development of atherosclerosis is closely linked with senescent cells, which participate in different disease stages. The early stage is characterized by the presence of needle-like crystals in the arterial intima, as well as yellow-striped lesions (type I lesions) of different sizes, which are called the striate stage. It is well-known that HVECs senescence mediates endothelial damage, which occurs at the initial stage of atherosclerosis [4]. In turn, senescent HVECs lead to endothelial dysfunction, which increases the expression level of inflammatory cytokines and promotes the progression of atherosclerosis. The second stage, fibrous plaque formation, is characterized by the increased deposition of lipids in the inner membrane, causing fibrous tissue proliferation and glass-like degeneration, forming gray-whitish plaques in the intima. At this stage, macrophages with senescence markers accumulate in the subendothelial space, where they cause pathology by increasing the expression of key atherogenic and inflammatory cytokines and chemokines [5]. In the third stage, atherosclerotic plaque formation, the fibrous tissue is large and necrotic, enriched with lipids, the lesion surface is thinner, and a few foam cells are present at the base and margin. In atherosclerotic lesions, HVSMCs migrate from the media to the intima, accumulate around the lipid core formed by necrotic foam cells, and switch from a contractile to a synthetic phenotype. Senescent macrophages that phagocytose lipids exhibit an abnormal or activated phenotype, which promotes pathological vascular proliferation [6]. At this stage, HVSMCs are dominated by proliferation but no aging occurs and formed a typical atherosclerotic plaque. The fourth stage involves secondary changes to atheromatous plaques, in which senescent macrophages promote plaque instability, elastic fiber degradation, and fibrous cap thinning, and increase the expression of metalloproteases and the formation of ulcers and thrombosis [5]. At this stage, foam cells induce the senescence of HVECs by releasing 4-hydroxynonenal (4-HNE) [7], which aggravates senescence and induces the development of atherosclerosis. Senescent HVSMCs differentiate to an osteogenic phenotype and express calcification factors, which in turn, lead to plaque calcification. Noteworthy, HVSMCs proliferate at the early stage of plaque formation. However, the proliferation rate of these cells is lower in advanced plaques than early lesions, indicating the possibility of cell senescence [8]. In addition, vascular injury and the phenotypic transformation of senescent HVSMCs also play a vital role by mediating vascular calcification [9]. Therefore, we concluded different cell types at different stages of atherosclerosis and discussed potential therapeutic targets in anti-senescence.

Causes of cell senescence in atherosclerosis

Atherosclerosis is a complex systemic, progression and chronic inflammatory disease that affects mainly in large and medium-sized arteries. Several theories have emerged trying to explain the pathogenesis of atherosclerosis, including hyperlipidemia [10], lipid peroxidation [11], thrombosis [12], cell senescence [13], injury response [14], and inflammation [15]. The critical role of senescence on atherosclerosis has gained prominence. Senescence is classified into replication and stress-induced premature senescence, and the main difference between these processes is telomere shortening [16]. Accumulating evidence has shown that both processes participate in atherosclerosis [17]. Several factors other than age cause premature cell senescence, including miRNA, homocysteine, hyperglycemia, hypertension, hyperlipidemia, hyperphosphatemia, and oxidative stress, by decreasing telomerase activity, increasing ROS production, and promoting vascular calcification, mitochondrial dysfunction, and DNA damage. Cell senescence is not the consequence of single cause, but there are multiple aspects which may induce senescence in cell (Table 1).

HVECs senescence in atherosclerosis

HVECs serve as a selective semi-permeable barrier between the interior space of blood vessels and underlying tissues. These cells are endocrine organ that releases anti-proliferative vasodilators and vasoconstrictors. HVECs senescence are shown to be associated with vascular dysfunction and onset of vascular diseases, is manifested by

increased levels of senescence-associated β-galactose glucosidase activity, excessive production of reactive oxygen species and inflammatory molecules production, impaired cellular proliferation, and cell cycle arrest, decreased in the activity of telomerase and the expression level of anti-senescence proteins etc. In this review, we summarize that several senescence-related molecules which increased in HVECs senescence and affected the occurrence and development of HVECs senescence, the expression level of nitric oxide (NO), endothelin-1 (ET-1), angiotensin II and monocyte chemoattractant protein-1 (MCP-1) increased in HVECs senescence (Figure 1). Conversely, some of these substances accelerate senescence and contribute to atherosclerosis. The next section explains how these mediators affect HVECs senescence and regulate the development of atherosclerosis.

Nitric oxide (NO)

NO is a multifunctional signaling molecule produced by HVECs and is involved in the maintenance of metabolic and cardiovascular homeostasis. NO is also a potent endogenous vasodilator associated with key processes that suppress the formation of vascular lesions [35] and protects against atherosclerosis by inhibiting oxidation, scavenging free radicals, inhibiting the oxidation of low-density lipoprotein (LDL) in blood vessels, preventing the production of oxidized LDL (Ox-LDL), inhibiting the activation of nuclear factor κB (NF-κB) [36], preventing abnormal constriction (vasospasm) of coronary arteries etc. NO also inhibits the infiltration and adhesion of white blood cells (macrophages) [37]. However, HVECs senescence significantly decreases NO production, and it is well known that under the action of tetrahydrobiopterin (BH_4), endothelial nitric oxide synthase (eNOS) accepts electrons from NADPH and transfer them to L-arginine for oxidation, leading to the production of NO. HVECs senescence impair the synthesis of BH_4 or reduces its bioavailability [38]. However, activated NOS accepts electrons from NADPH, stores them in keto alcohols, and transfers them to O_2, resulting in the production of superoxide anion. Superoxide anions combine with NO and produce the highly toxic peroxynitrite anion ($ONOO^-$), and the rapid oxidation of BH_4 by $ONOO^-$ further decreases the level of BH_4 [39]. The change in the BH_4/NOS and BH_4/dihydrobiopterin (BH_2) ratios uncouples NOS, which aggravates oxidative stress and forms a vicious circle. BH_4 (exogenously or endogenously) increases the bioavailability of NO and repairs endothelial function [40]. Therefore, it is essential to maintain BH_4 levels by removing factors that affect HVECs senescence and ensure the high bioavailability of NO for the optimal function of HVECs.

Table 1: Factors associated with replication senescence and stress-induced premature senescence.

Type	Factor	Pathway	Outcome	Reference
Replicative senescence	Age	Cell division	Telomere shortening; Decreased telomerase activity	[18, 19]
	Homocysteine	Upregulate $p16^{INK4A}$ and p21Cip1	Decreased telomerase activity; Cell cycle arrest	[20, 21]
	MiR-22	Upregulate CDK6, SIRT1, Sp1, AKT3	Cell cycle arrest; DNA damage	[22-24]
	MiR-34a	Downregulate SIRT1, upregulate SASP	Vascular calcification	[25]
	Hyperglycemia	Alu Hypomethylation; PI3K/AKT	DNA hypomethylation; Increase the level of ROS	[26, 27]
	Hypertension	Upregulate ROS, downregulate NO	Mitochondrial dysfunction	[28]
Stress-induced premature senescence	Hyperlipidemia	NOX	Increase the level of ROS	[29]
	Hyperphosphatemia	Upregulate ET-1,increase ROS production and activates AP-1 transcription factor	Increase P16 expression and SA-β-GAL activity	[30]
	Hypoxia	Upregulate HIF1alpha	Cell cycle arrest and DNA damage	[31]
	H_2O_2	Upregulate NMT1, p16, miR-192-5p/P53, NF-κB	Premature senescence and DNA damage	[32, 33]
	Ox-LDL	Upregulate NOX2 and caveolin-1/p47phox, p53/P21/P16; downregulate ABCA1/ABCG1/LXR-α	Increased oxidation reaction and the level of ROS; cell cycle arrest	[34]

CDK6, cyclin-dependent kinases 6; SIRT1, sirtuin 1; Sp1, transcription factors; AKT, protein kinase B; NOX, NADPH-oxidases; SASP, senescence-associated secretory phenotype; HIF, hypoxia-inducible factor 1; SA-β-GAL, senescence-associated β-galactosidase; ROS, reactive oxygen species; NMT1, N-myristoyltransferase 1; ABCG1, ATP binding cassette G1; ABCA1, ATP-binding cassette transporter A1; LXR, liver X receptor.

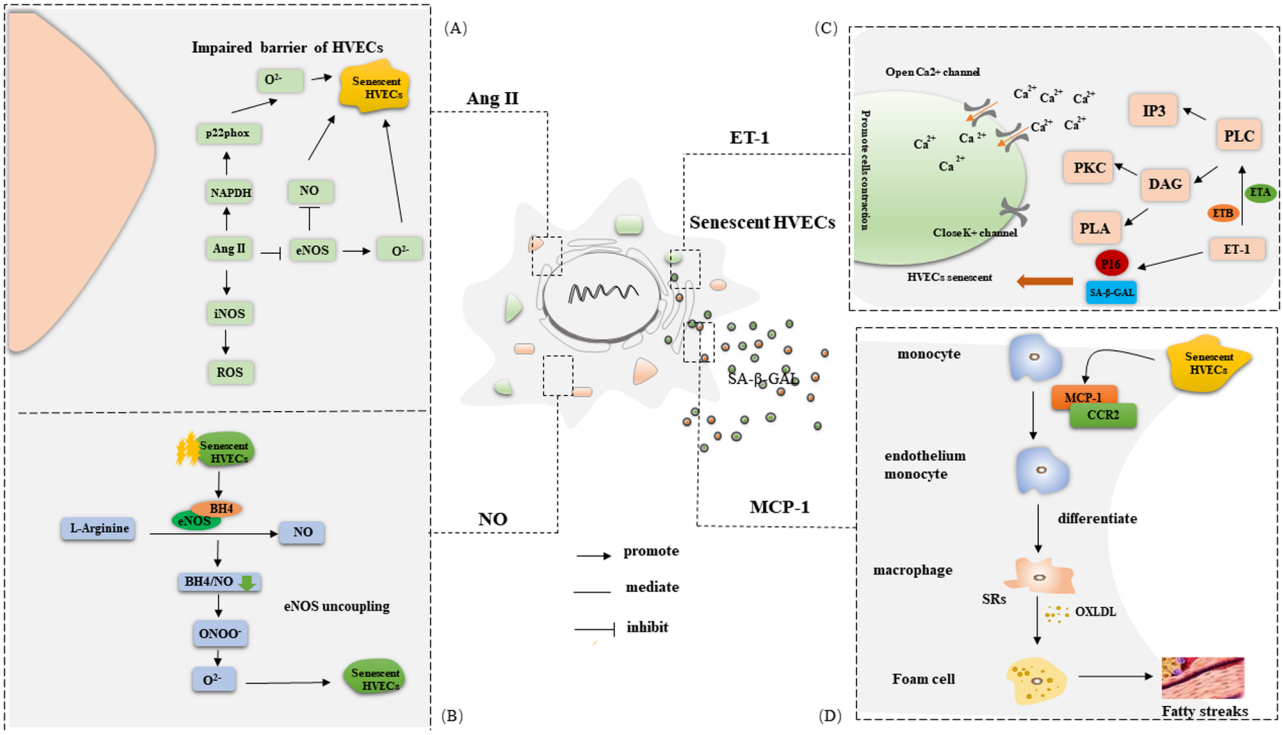

Figure 1: Senescence-related molecules in HVECs.
(A) Angiotensin II impairs the HVECs barrier by increasing the levels of $O2^-$ and decreasing the levels of NO; (B) Senescent HVECs lead to a decrease in BH4 or NO and an increase in $ONOO^-$, and the rapid oxidation of BH4 by $ONOO^-$ further decreases the level of BH4. (C) ET-1 increases phospholipase C (PLC) activity, leading to the production of signaling molecules, including inositol triphosphate (IP3) and diacylglycerol (DAG). DAG activates protein kinase C (PKC) and phospholipase A (PLA), which activate voltage-dependent calcium channels and close ATP-sensitive potassium channels, respectively, and increased the concentration of Ca^{2+} and caused cell shrinkage; (D) Senescent HVECs increases the expression of MCP-1, which recruits circulating monocytes to the endothelium; monocytes differentiate into macrophages in the submucosa; subsequently, macrophages mediate the oxidative modification of LDL cholesterol and form Ox-LDL; receptor A phagocytoses Ox-LDL, resulting in intracellular lipid accumulation; macrophages phagocytose Ox-LDL and form foam cells, which induce the form of fatty streaks.

Endothelin-1

ET-1 is a 21-amino-acid peptide with a powerful vasoconstrictor effect in conductive and resistive arteries [41]. The balance between ET-1 and NO provides an endothelial barrier to lipoprotein influx and macrophage recruitment, stimulating cell proliferation and contributing to endothelial dysfunction. ET-1 activates endothelin-1 A receptor (ETA) and endothelin-1 B receptor (ETB), these receptors mediate essential functions, including cardiovascular remodeling, vasoconstriction, cell proliferation and differentiation, production of extracellular matrices, and the control of water and sodium secretion [42]. Barton et al. [43] revealed a marked increase in total ET-1-binding density in the vascular wall and atheromatous plaque due to augmented ETA receptor density. Other study has shown that hyperphosphatemia induces senescence in human endothelial cells by increasing endothelin-1 production, and HVECs senescence promoted the expression of ET-1 and endothelin-converting enzyme 1 [44]. However, the increased production of ET-1 inhibited the endothelial release NO, impairing endothelium-dependent relaxation, and promoting atheroma formation. ET-1 binds to receptors and increases phospholipase C (PLC) activity, which stimulates the production of signaling molecules such as diacylglycerol (DAG) and inositol triphosphate (IP3). DAG activates protein kinase C (PKC) and phospholipase A (PLA), which activates voltage-dependent Ca^{2+} channels and closes ATP-sensitive K^+ channels, respectively, and the opening of Ca^{2+} channels cause extracellular calcium influx, which increases intracellular Ca^{2+} concentration; Ca^{2+} binds to calmodulin and activates the muscle reflex protein, causing cell constriction and increasing vascular tension. The intensity of vascular tension increased may be the basis of cardiovascular diseases such as hypertension and atherosclerosis [45]. Study found that ET-1 caused vessel constriction and had pro-inflammatory action [46]. However, it is known that the production of inflammatory factors is the basis for the onset of atherosclerosis. Therefore, HVECs senescence, together with hyperphosphatemia, mechanical damage, and oxidative stress, promote the expression of ET-1, which

participates in atherosclerosis by increasing vascular tension and releasing pro-inflammatory cytokines. Conversely, increased expression level of ET-1 may contribute to vascular dysfunction via multiple pathways, including direct hemodynamic effects, oxidative stress, and inflammation, and induced HVECs senescence by increasing p16 expression and SA-β-GAL activity, demonstrating that ET-1 has a complex and crucial role in regulate HVECs senescence and atherosclerosis.

Angiotensin II

Angiotensin II is a naturally-occurring octapeptide hormone from the renin–angiotensin–aldosterone system and a potent vasoconstrictor [47]. This peptide participates in atherosclerosis by inducing endothelial dysfunction, endothelial cell apoptosis, lipoprotein peroxidation, promoting macrophage uptake Ox-LDL, and stimulating the production of oxygen free radical etc. [48] Study has found that senescent HVECs could increase the expression level of angiotensin II [49], and Hu et al. [50] have demonstrated that angiotensin II could upregulate the expression expression of inducible nitric oxide synthase (iNOS), increase ROS production and cell apoptosis, and downregulate the expression of phosphorylated-endothelial nitric oxide synthase (eNOS). Furthermore, study has confirmed that angiotensin II-induced senescence of HVECs senescence and gene expression of $p16^{INK4a}$ [51], angiotensin II-mediated production of vascular superoxide anion could contribute to endothelial dysfunction, hypertension, and atherosclerosis. A NAD(P)H oxidase has been found to be a major endothelial source of superoxide anions [52], angiotensin II upregulated the transcription of NAD(P)H oxidase p22phox, which increased the production of O^{2-} [53], which in turn, might be one of the main contributors to HVECs senescence.

Monocyte chemoattractant protein-1

MCP-1 is a member of the chemokine CC subfamily, it can chemotaxis and activates immune cells such as monocytes and T lymphocytes [54]. MCP-1 attracted its receptor CCR2 on monocytes [55], which recruited monocytes to the endothelium, monocytes pass through the specific binding of MCP-1 to CCR2, mononuclear cells migrate to the intima under the chemotaxis of MCP-1. Studies have shown that the expression of MCP-1 was upregulated in senescent HVECs and HVSMCs [56, 57]. Furthermore, MCP-1 attracts monocytes to the endothelium and promotes the proliferation and migration of HVSMCs. However, although monocyte chemotaxis to the endothelium is well known, the mechanism by which MCP-1 regulates atherosclerosis is poorly understood. In summary, senescent HVECs could increase the expression of MCP-1 and subsequently recruit circulating monocytes, which differentiate into macrophages in the submucosa and join in cholesterol metabolism in atherosclerosis.

HVSMCs senescence in atherosclerosis

HVSMCs are highly specialized cells that maintain vascular tone and participate in vessel remodeling [58]. As it is known, HVSMCs undergo telomere-based senescence in atherosclerosis, HVSMCs senescence were affected by oxidative stress and age, leading to shorten of telomerase and mediating cell cycle arrest. Studies have confirmed that double-strand DNA breaks (DSB) and increased ROS level are the main culprit of senescence, which reduce telomerase activity and impair DNA, and affects the expression level of P53, P21, and P16, subsequently mediates pRB phosphorylation and causes cell cycle arrest [59]. In addition, the phenotypic transition of HVSMCs is the main cause of atherosclerosis [60], which mediates vascular calcification and plaque instability. A SASP also plays an indispensable role in atherosclerosis via secretion of pro-inflammatory cytokines such as interleukins (e.g., IL18 and IL1β) and matrix metalloproteinases (MMPs) (Figure 2).

Calcification regulatory factors

It is well-known that the phenotypic transformation and senescence of HVSMCs play an integral role in the formation of atherosclerotic lesions. Senescent HVSMCs promote vascular remodeling by secreting vascular remodeling factors. However, osteogenic transformation is a form of phenotypic remodeling that occurs during cell senescence. Senescent HVSMCs increased calcium deposition [61] and promoted the expression of calcification regulatory factors, including bone morphogenetic protein (BMP), osteopontin (OPN) and runt-related transcription factor (Runx-2), leading to the calcification of blood vessels and increasing plaque instability.

Bone morphogenetic protein-2

BMPs are prominent growth factors that induce new bone formation [62]. BMP-2 is a member of the BMP family and

enhances the migration and proliferation of HVSMCs via the actin/CD44/MMP-2 molecular pathway [63]. BMP-2 is upregulated in senescent HVSMCs, and a study showed that TNF-α induced cell senescence and promoted monocyte recruitment via secretion of BMP-2 [64]. In addition, hyperphosphatemia could increase the expression of BMP-2 in mouse smooth muscle cells [65]. Furthermore, clinical and experimental studies have shown that hyperphosphatemia is a risk factor for vascular calcification [66], suggesting that atherosclerotic plaque instability increased in HVSMCs due to hyperphosphatemia, and the expression of BMP-2 was increased in calcified atherosclerotic plaque [67] etc. These studies provided solid evidence for BMP-2 mediates vascular calcification and induces the development of atherosclerosis.

Osteopontin

Osteopontin (OPN) is a phosphorylated acidic non-collagenous bone matrix glycoprotein, which promotes bone resorption by enhancing osteoclast attachment [68]. OPN regulates calcium release from vascular deposits and inhibits the growth of hydroxyapatite crystals, consequently affecting vascular calcification in atherosclerosis. Furthermore, OPN is regulated by the SASP regulator C/EBPβ [69], and Lee et al. [70] suggested that hydroxynonenal (HNE) induced OPN expression in HVSMCs via signaling pathways involving AP-1 and C/EBPβ, increasing HVSMCs proliferation and vascular remodeling. Another study demonstrated that hypertension and senescence had a compounding effect on the vascular system and induced OPN expression via the PI3K/AKT/eNOS and Ras/Raf/MAPK signaling pathways [71]. These results indicate that hypertension may be mediated by senescent HVSMCs, which in turn promotes the expression of OPN.

Runt-related transcription factor

Runt-related transcription factor (Runx-2) is a key transcription factor for osteogenic differentiation and plays an important role in cardiovascular ectopic calcification. Research found that senescent HVSMCs acquired osteoblast-like features with enhanced expression of Runx2, and type I collagen, and exhibited exacerbated HVSMCs mineralization

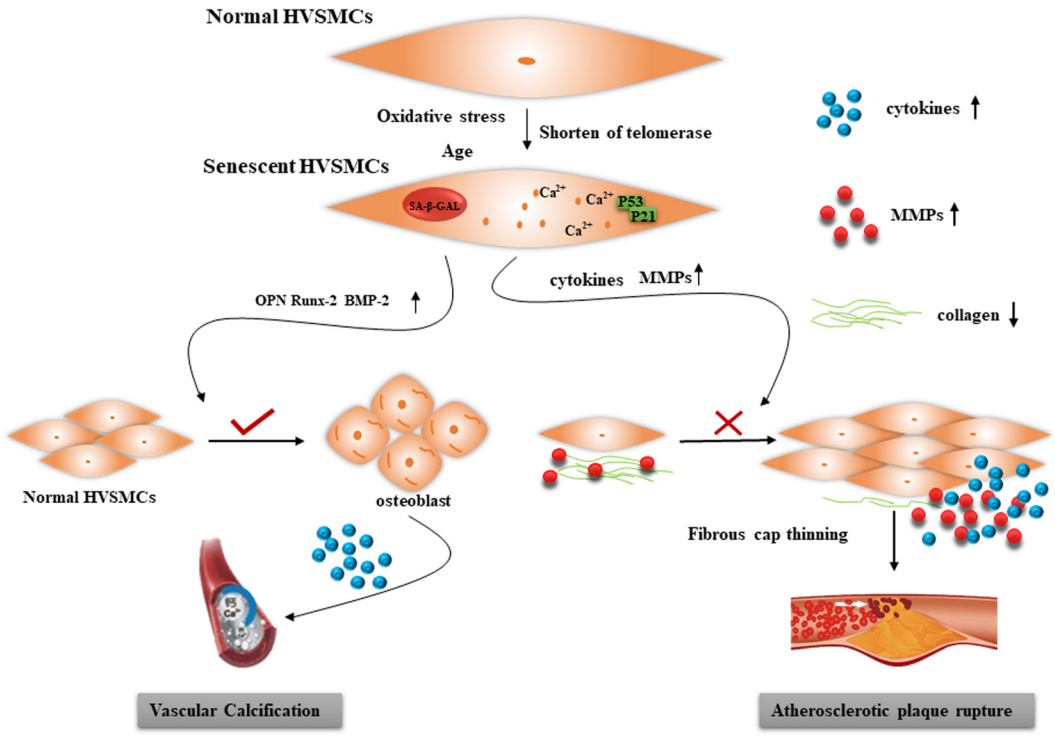

Figure 2: Expression of senescent-related molecules in HVSMCs.
Normal HVSMCs become senescent by oxidative stress and aging, leading to telomerase shortening, increased expression of senescence-associated β-galactosidase and accumulation of calcium ions. Senescent HVSMCs secreted OPN, Runx-2, and BMP2, which induce an osteogenic phenotype in HVSMCs and vascular calcification. In addition, HVSMCs secrete inflammatory cytokines and MMPs, which bind to collagen and lead to fibrous cap thinning, ultimately causing the rupture of the atherosclerotic plaque.

[72], and Cao et al. [73] also found that a reduction in Runx-2 inhibited calcification by the effect of FGF21 and suppressed the expression level of ALP in senescent HVSMCs, suggesting that Runx-2 was involved in senescence-mediated osteoblastic transition. These findings strongly indicated that senescent HVSMCs play important role in the pathophysiology of senescence-associated medial calcification by upregulating the expression of Runx-2, and the inhibition of osteoblastic transition could be a new therapeutic approach for preventing this type of calcification.

Proinflammatory cytokines

One of the main features of senescent HVSMCs is the SASP [74], characterized by secreting proinflammatory cytokines, such as interleukins and matrix metalloproteinases, which are critical in the development of atherosclerosis. Study has concluded that aged HVSMCs significantly increased secretion of interleukin-1β, MCP-1, and tumor necrosis factor α compared with young control cells, secretion of interleukin-6 also tended to increase in aged HVSMCs [57]. In addition, MMPs bind to collagen and lead to plaque rupture by weakening the fibrous cap [75], and study has shown that patients with atherosclerosis have higher levels of neutrophil gelatinase-associated lipocalin (NGAL) and MMP-9/NGAL complexes [76]. Additionally, in mouse senescence models, senescent cells secrete bioactive molecules, including ROS and pro-inflammatory cytokines (IL-18 and IL-1β), so we concluded that increased chronic low-level inflammation may be causal factors in senescence.

Macrophages senescence in atherosclerosis

Atherosclerosis is an abnormal inflammatory response to lipoprotein accumulation in the arteries, resulting in lipid accumulation, cell apoptosis, and inflammation, ultimately leading to macrophage accumulation in the vascular wall [77]. Macrophages mediate the oxidative modification of LDL cholesterol that infiltrates into the vascular endothelium and forms Ox-LDL, and phagocytose Ox-LDL through the scavenger receptor, resulting in intracellular lipid accumulation. In addition, macrophages phagocytose lipid particles and form foam cells that produce fatty streaks [78]. Noteworthy, some studies found that senescent macrophages impaired cholesterol efflux and promoted senescent-related diseases, such as atherosclerotic heart disease, cancer, and macular degeneration [6]. Yang et al. [79] found that miR-216a induced macrophage senescence characterized by increased SA-β-GAL activity and p53 and p16 expression. Similarly, study has found that Ox-LDL significantly inhibited macrophage proliferation and migration, induced cell senescence, and promoted the secretion of TNF-α, MCP-1, and IL-1β [80], in turn, they increased the inflammatory response under the endothelium and aggregated the development of atherosclerosis. These research indicated that senescent macrophages may influence the development of atherosclerosis by impairing cholesterol efflux and promoting inflammatory response.

Potential therapeutic targets of cell senescence

Anti-senescence via protecting cells from oxidative stress

Nowadays, several potential therapeutic targets of cell senescence have been researched, include anti-senescence proteins Klotho, Pin1, FGE21, SIRT1, SIRT6, coenzyme Q10 (CoQ10H2), and the plant of salidroside et al. (show in Figure 3), which can potentially delay senescence by repairing telomere, correcting mitochondrial dysfunction, and repairing DNA. Klotho inhibits the expression of lectin-like oxidized LDL receptor-1 (LOX-1) via the PI3K/AKT/eNOS pathway [81], and the Klotho-induced inhibition of insulin/IGF-1 signaling increases the resistance to oxidative stress, potentially improving the anti-senescence properties of Klotho [82]. The level of Pin1 protein is decreased in human atherosclerotic tissues, and adenoviral-mediated Pin1 overexpression rescued cellular senescence in atherosclerotic HVSMCs, with the concurrent downregulation of P53, P21, phosphorylated retinoblastoma (p-pRB), and P65, and the upregulation of cyclin subfamilies (cyclin B, D, and E) and cyclin-dependent kinase subfamilies (CDK2, 4, and 6), whereas Pin1 knockdown has the opposite effects [83], indicating that these factors can be potent modulators of HVSMCs senescence. Similarly, FGF21 [84], SIRT1 and SIRT6 [85, 86], and CoQ10H2 [87] protected cells from oxidative stress-induced cell damage, including premature cell senescence, intracellular accumulation of ROS, and increased DNA damage. In addition, some authors reported that salidroside [88] reduced cell senescence by increasing the phosphorylation of RB, alleviating the hypophosphorylation of pRB by $P16^{INK4A}$ and inhibiting the expression of CDK4 and CDK6, consequently promoting cell cycle progression from G0/G1 to S phase via promoting RB phosphorylation.

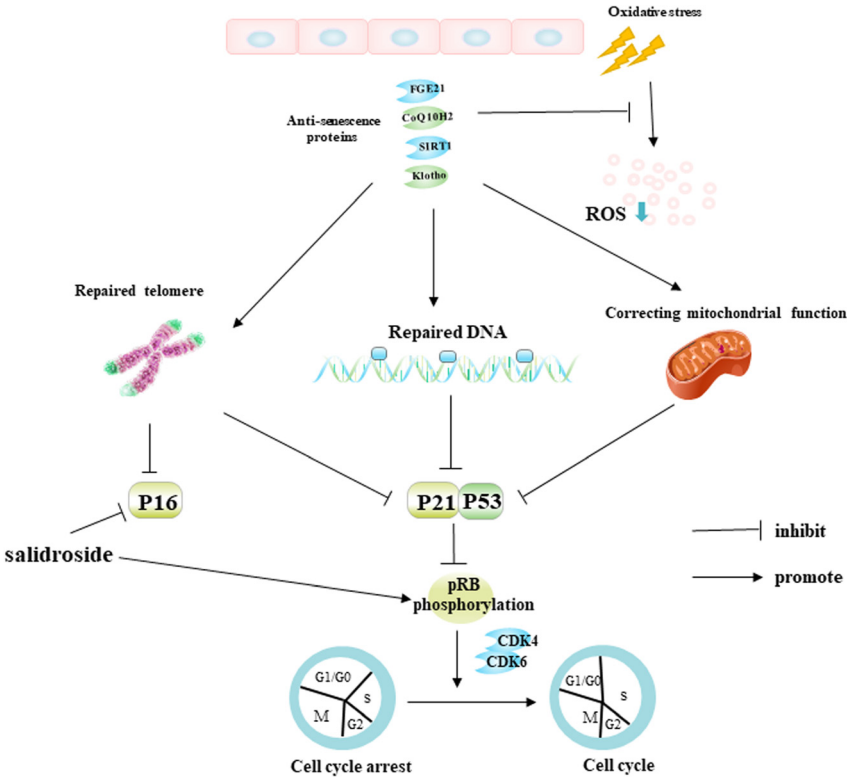

Figure 3: Potential therapeutic targets for cell senescence.
Many anti-senescence proteins such as FGF21, SIRT1, CoQ10H2, and Klotho can reduce oxidative stress by decreasing the level of ROS, and have a crucial role in repaired telomerase, repairing DNA, and reducing mitochondrial dysfunction by inhibiting the expression of P16, P21, and P53. In addition, salidroside can reduce cell senescence by inhibiting the expression of P16 and promoting pRB phosphorylation, consequently promoting cell cycle progression from G0/G1 to S phase.

Anti-senescence via inhibiting vascular calcification and reducing the production of inflammatory factors

Atherosclerotic plaque is prone to rupture in the advanced stage of atherosclerosis, cause of vascular calcification and the production of inflammatory factors by multiple cell types, so it is particularly important to stabilize plaque for delay the occurrence of cardiovascular events. Study has demonstrated that phosphate binders prevent phosphate-induced cellular senescence of vascular smooth muscle cells and vascular calcification in a modified [89], and Sirtuin 1 retards hyperphosphatemia-induced calcification of vascular smooth muscle cells [90]. Additionally, early in 2010, study has found that resveratrol as an anti-inflammatory and anti-senescence agent [91, 92], and peroxisomal Acyl-CoA oxidase type1 made an effort in anti-inflammatory and inti-senescence [93].

Anti-senescence via promoting cholesterol efflux in macrophages

Well known that lipid metabolism disorder is an important cause of atherosclerosis. Oxidative modification of LDL to form Ox-LDL is considered to be a key initiating factor for atherosclerosis [94]. Monocytes and LDL in the blood enter the subintima through damaged vascular endothelium to accelerate the form of lipid streaks. So it is important to retard the development of atherosclerosis via promoting cholesterol efflux in macrophages and accelerating the process of reverse transport cholesterol, a previous study [95] found that telomerase reverse transcriptase (TERT) deficiency in macrophages induced a senescence phenotype, and TERT was highly expressed in atherosclerotic plaques; TERT expression during inflammation may prevent macrophage senescence. These results show that TERT (1) may participate in a feed-forward loop for inflammatory gene expression, (2) extends lifespan without increasing telomere length, (3) is an NF-κB target gene in macrophages, and (4) is activated during atherosclerosis. The induction of TERT expression prevents macrophage senescence and may have important implications in atherosclerosis.

Discussion

Atherosclerosis is a common outcome of a variety of cell senescence and pathophysiological processes. This review summarized the changes in the levels of molecules involved in cell senescence and the mechanisms regulating the development of atherosclerosis, including the senescence of HVECs, HVSMCs, macrophages, which form a

complex regulatory network. In this review, we concluded that senescent HVECs promote the progression of atherosclerosis via regulating the levels of ET-1, NO, angiotensin II, and MCP-1, and HVSMCs participated in the formation of fibrous cap and vascular calcification, macrophages mediated cholesterol metabolism in the advanced stage of atherosclerosis. Noteworthy, some proteins were found that they can make an effort in anti-senescence via reducing oxidative stress, repairing DNA, repairing telomere, and correcting mitochondrial function. Otherwise, some molecules have been found that could suppress vascular calcification, inhibit the production of inflammatory factors and promote cholesterol efflux, which are highly concerned regard as therapy targets for treatment or prevention of atherosclerosis. In addition, the causes of cell senescence are multiple, and this review just focused on oxidative stress, hypertension, and hyperlipidemia. Other contributing factors are miRNA and homocysteine etc., which not be claimed thoroughly. An increasing number of studies have shown that cell senescence participates in atherosclerosis, which is a significant contributor to cardiovascular disease. Therefore, elucidating the emerging role of cell senescence in atherosclerosis, which will bring a great gift for patient with atherosclerosis and it is important to promote the research of cardiovascular diseases. However, the diseases caused by cell senescence has been plagued for a long time, the mechanism underlying cell senescence in atherosclerosis is not fully elucidated. Nowadays, the main strategies targeting cell senescence are anti-senescence proteins and drugs that reduce oxidative stress and silence senescence genes. Notwithstanding, additional studies are necessary to elucidate the mechanisms of cell senescence and identify novel potential therapeutic targets.

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