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Review

Endothelial cell senescence in aging-related vascular dysfunction☆

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ABSTRACT

Increased cardiovascular disease in aging is partly a consequence of the vascular endothelial cell (EC) senescence and associated vascular dysfunction. In this contest, EC senescence is a pathophysiological process of structural and functional changes including dysregulation of vascular tone, increased endothelium permeability, arterial stiffness, impairment of angiogenesis and vascular repair, and a reduction of EC mitochondrial biogenesis. Dysregulation of cell cycle, oxidative stress, altered calcium signaling, hyperuricemia, and vascular inflammation have been implicated in the development and progression of EC senescence and vascular disease in aging. A number of abnormal molecular pathways are associated with these underlying pathophysiological changes including Sirtuin 1, Klotho, fibroblast growth factor 21, and activation of the renin angiotensin-aldosterone system. However, the molecular mechanisms of EC senescence and associated vascular impairment in aging are not completely understood. This review provides a contemporary update on molecular mechanisms, pathophysiological events, as well functional changes in EC senescence and age-associated cardiovascular disease. This article is part of a Special Issue entitled: Genetic and epigenetic regulation of aging and longevity edited by Jun Ren & Megan Yingmei Zhang.

1. Introduction

The vascular endothelium is a single layer of cells adjacent to the lumen of blood vessels and plays an important physiological role in vascular homeostasis including maintenance of blood fluidity, regulation of vascular tone, modulation of pro-inflammatory molecule production, pro-inflammatory immune responses, and neovascularization [1]. Risk factors such as obesity, insulin resistance, diabetes, smoking, and aging can induce alterations in endothelium morphology and endothelium dysfunction, which contribute to arterial stiffness, atherosclerosis, hypertension, stroke, and coronary artery disease [1]. In this regard, increasing aging in our population is a major risk factor and represents a major global health challenge in the pathogenesis of cardiovascular disease (CVD) [2]. Vascular cellular senescence is a process in which vascular cells cease dividing and undergo distinctive phenotypic changes such as profound chromatin and secretome alterations and tumor-suppressor activation [3]. The senescence of vascular endothelial cells (ECs) has been found to play a key role in vascular aging leading to the initiation, progress, and advancement of CVD [2]. Aged ECs usually become flatter and enlarged with an increasingly polypoid nucleus. These changes are accompanied by modulation in cytoskeleton integrity, angiogenesis, proliferation, and cell migration [4]. For instance, senescent ECs show attenuated endothelial nitric oxide (NO) production, increased endothelin-1 (ET-1) release, elevated inflammation and cell apoptosis [4]. Thus, EC senescence induces vascular structural and functional changes enhance thrombosis, inflammation, and atherosclerosis with impairment of vessel tone, angiogenesis, and vascular integrity, all of which contribute to development and progression of CVD [1]. For example, in a large randomized controlled substudy of the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) involving 541 men or women aged 70 to 82 years it was found that elevated levels of plasminogen activator and Von Willebrand factor, as markers of EC injury and dysfunction, were associated with lower cerebral blood flow in older adults at high risk for CVD [5]. Atorvastatin for Reduction of Myocardial Damage during Angioplasty-Acute Coronary Syndromes (ARMYDA-ACS) trial suggested that endothelial progenitor cells (EPCs) may promote endogenous vascular repair and contribute to cardio-protection and reduction of CVD

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morbidity and mortality [6]. However, the molecular mechanisms of EC senescence and the linkage to the underlying pathophysiological changes are not yet completely understood. In this review, we discuss the roles and mechanisms of EC senescence in the process of vascular aging and CVD.

2. Clinical aspects of EC senescence in aging-related arterial stiffness and systolic hypertension

Cellular senescence is a physiological or pathological processes throughout life [7]. In physiological condition, cell senescent is involved in embryo tissue development, tissue repair, as well as tumor suppression responses [7]. However, the accumulation of cell senescent can lead to loss of replicative capacity, cell apoptosis, and adverse structural changes, and associated CVD [7]. Cellular senescence is usually linked with aging and age-related disorders. In human coronary arteries, ECs with increased β-galactosidase activity associated with enhanced senescence are observed during aging, suggesting that aging is also associated with reduced EC regenerative and EC senescence that is associated with a reduction of EC dependent arterial relaxation [8]. To this point, a reduction of EPC is associated with the development of arterial stiffness in patients with psoriasis [9]. Studies have found that NO donors reduce arterial stiffness in healthy subjects and patients with hypertension and hypercholesterolemia [10]. These data support a role of EC senescence in the pathogenesis of CVD. However, while few clinical studies have explored the relationship between EC senescence, arterial stiffness and hypertension those done have shown that aging is closely associated with arterial stiffness and CVD. For instance, data from the Framingham study found that advancing age was significantly associated with a higher carotid-femoral pulse wave velocity and mean arterial pressure [11]. Meanwhile, arterial stiffness has been regarded as an independent predictor of CVD morbidity and mortality in the general population, aging, hypertensive patients, as well as patients with end-stage renal disease [12]. With aging and associated arterial stiffness, systolic pressure tends to increase whereas diastolic pressure tends to decline and this pathophysiological change elevates pulse pressure and aortic pulse wave velocity. Indeed, the prevalence of hypertension, particularly isolated systolic hypertension in aging population is increased [13]. Increased systolic pressure increases left ventricular afterload with associated increases in myocardial oxygen requirements. The falling diastolic pressure decreases the perfusion of coronary circulation during diastole. These consequences of arterial stiffness, the increased systolic pressure and decreased diastolic pressure further induce left ventricular hypertrophy, myocardial ischemia, remodeling and other cardiovascular complications in aging individuals [1]. Invasive methods including the quantitative angiography and intracoronary Doppler and non-invasive methods including venous occlusion plethysmography, flow-mediated dilatation in brachial artery, and peripheral arterial tonometry have been applied to investigate endothelial and vascular function [1].

3. Mechanisms of aging-related EC senescence

Factors including cell cycle dysregulation, oxidative stress, calcium (Ca^{2+}) signaling, inflammation, activated renin angiotensin-aldosterone system (RAAS), and hyperuricemia in aging induce EC senescence. However, accumulated EC senescence also aggravates aging-related inflammation, oxidative, and vascular dysfunction with a feedback manner (Fig. 1).

3.1. Cell cycle dysregulation in EC senescence

Cellular senescence is a characteristic of the irreversible growth arrest and cell apoptosis that results in impairment of cellular function and regeneration [3] (Fig. 1). Typically, cells move in an irreversible cell cycle arrest in the G1 phase and no longer respond to cellular growth stimuli. Cell cycle progression is controlled by cyclin-dependent kinases (cdk) and its inhibitors [3]. The inhibitor of cdk4 family members in cdk inhibitor 2B (p15), cdk inhibitor 2A (p16), cdk inhibitor 2C (p18) and cdk inhibitor 2D (p19) inhibit activity of cdk4 and cdk6 [3]. The kinase inhibitor protein family in cdk inhibitor 1B (p27) and cdk inhibitor 1C (p57) inhibit a broader spectrum of cdks [3]. Cellular senescence is also regulated by p53 and p16/retinoblastoma gene product (pRB) pathways. Expression and activity of p53 is primarily activated by telomere dysfunction and DNA damage and is increased in senescent cells [14,15]. The p16/pRB is linked primarily to cellular stress, chromatin disruptions, and mitogenic stress [14,15]. In aging murine ECs increased p21 and p16 in arterial tissues were associated with oxidative stress-induced inhibition of NO-dependent vascular endothelium relaxation [16]. In older adults, p16 expression was increased in atherosclerotic plaques [17], and p53 and p21 transcript levels were elevated [18].

3.2. Oxidative stress

Oxidative stress is well known to be an important contributor in age-associated EC dysfunction and arterial dysfunction (Fig. 1). There are a variety of sources of reactive oxygen species (ROS) within the cells, such as hydrogen peroxide, peroxynitrite, and hydroxyl radical. ROS are not only generated through mitochondrial respiration but also from peroxisomal β-oxidation of free fatty acid, xanthine oxidase, lipoxygenase, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), microsomal P-450 enzymes, cyclooxygenases, and prooxidant heme molecules [19]. Further, uncoupling of endothelial NO synthase (eNOS) also contributes to formation of peroxynitrite [20,21]. Although physiological ROS levels are required to maintain the normal cellular function, overproduction of ROS induces deleterious effects such as changes in DNA transcription, interruption of numerous redox sensitive signaling pathways, impairment of cellular structure and function, inflammation, as well as whole organ dysfunction [20,21]. NOX is one of the important enzymes that is responsible for much of the generation of ROS in CVD. NOX1, NOX2, NOX4 and NOX5 isoforms have been found to induce EC dysfunction, inflammation and apoptosis in atherosclerosis, hypertension, and diabetes [22]. In patients with acute coronary syndrome, endothelial-derived microparticles induce premature EC senescence through NOX- mediated activation of mitogen-activated protein kinases and phosphoinositide 3-kinase/protein kinase B [23]. The oxidative stress-induced cellular stress and injury are driving factors to promote premature EC senescence, vascular stiffness and development of age-associated CVD.

3.3. Ca^{2+} signaling in EC senescence

EC dysfunction in aging is related to altered EC Ca^{2+} signaling (Fig. 1). Increased Ca^{2+} signaling regulates endothelium-dependent vasodilation through NO, prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF) [1]. In this regard, an important role for ECs is to transduce increases in intracellular Ca^{2+} and hyperpolarization via activation of small- and intermediate-conductance Ca^{2+}-activated potassium channels that provide electrical conduction between ECs and vascular smooth muscle cells (VSMCs) [1]. With advancing age, Ca^{2+} signaling and other related vasodilation signaling pathways are impaired and these abnormalities induce EC and vascular dysfunction. For example, local EC Ca^{2+} signaling was impaired with a reduction in internal elastic lamina holes in aged mice [24]. Meanwhile, in mesenteric arteries controlling splanchnic blood flow the frequency of spontaneous local EC Ca^{2+} signals was reduced by ~85% in old (24–26 months) vs young (3–6 months) mice [24], suggesting that reduced Ca^{2+} signaling between ECs and VSMCs contributes to impairment of resistance artery function with advancing age.

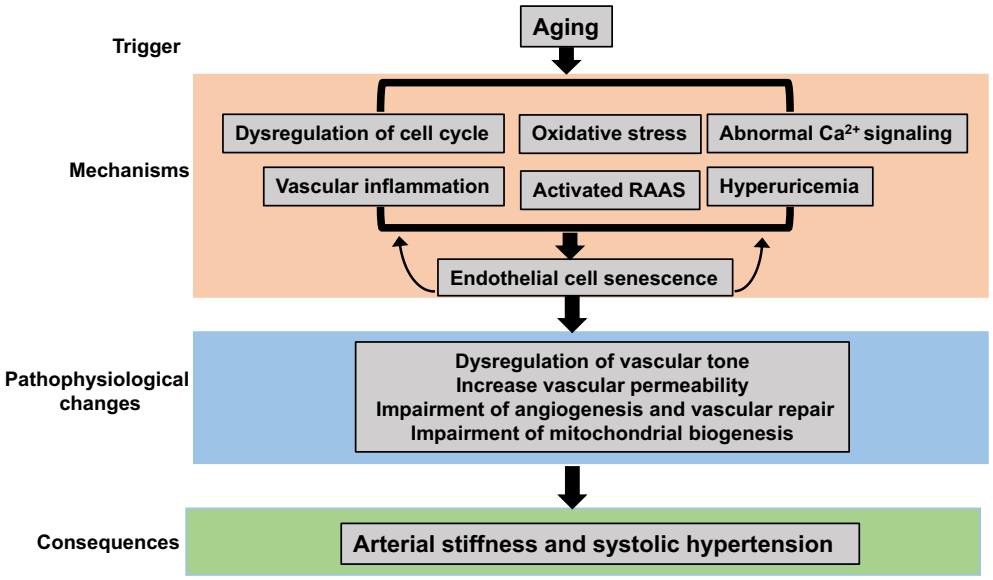

Fig. 1. Pathophysiological mechanisms of aging-related endothelial cell senescence and vascular dysfunction. Dysregulation of cell cycle, oxidative stress, abnormal Ca^{2+} signaling, vascular inflammation, activation of RAAS and hyperuricemia in aging induce endothelial cell senescence that are characteristic of dysregulation of vascular tone, increase endothelium permeability, impairment of angiogenesis and mitochondrial biogenesis, resulting in arterial stiffness and systolic hypertension. Abbreviations: RAAS, renin-angiotensin-aldosterone system.

3.4. Vascular inflammation

There is a shift towards a pro-inflammatory phenotype with increased expression of inflammatory cytokines, adhesion molecules, and chemokines from ECs in aging (Fig. 1). These pro-inflammatory cytokines include interleukin (IL)-6, IL-1β, cellular adhesion molecules, tumor necrosis factor-alpha (TNF-α) and monocyte chemoattractant protein-1. The pro-inflammatory response is related to activation of nuclear factor-kappa B (NF-κB) signaling that is an important nuclear transcription factor in promoting inflammation cytokine expression in in endothelial dysfunction and CVD [1]. The NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome is a new molecular marker in vascular inflammation. Activated NLRP3 undergoes oligomerization and induces the recruitment of procaspase-1 and activation of caspase-1 serves as enhancer of multiple pro-inflammatory pathways involving NF-κB, chemokines, and ROS [25]. Meanwhile, enhanced NF-κB signaling induces a positive feedback which further prompts NLRP3 inflammasome assembly, pro-IL-1β processing and maturation [25]. Indeed, circulating inflammatory biomarkers such as C-reactive protein, IL-6 were elevated and this was associated with brain structure change in the elderly [26]. Prolonged TNF-α exposure induces EC premature senescence and this is prevented by inhibition of NF-κB activation, suggesting that inflammation drives premature EC senescence [27]. Therefore, human aging is a chronic, systemic, and low-grade pro-inflammatory status and this phenomenon has been defined as “inflammaging” [28].

3.5. RAAS

An activated RAAS contributes to the vascular structural and functional changes that occur with aging (Fig. 1). On the one hand, angiotensin II (Ang II) increases ROS production, inflammation, extracellular matrix remodeling, and vessel tone through Ang II type 1 receptor (AT1R) [1]. On the other hand, activation of Ang II type 2 receptor (AT2R) inhibits cell proliferation, inflammation, and fibrosis and exerts series positive roles in the prevention of CVD [1]. One study found that expressions of Ang II, and AT1R, NOX2, collagen IV, and fibronectin were increased whereas expressions of Mas receptor and AT2R were decreased in 24 month aging mice [29]. The angiotensin converting enzyme inhibitor (enalapril) and AT1R inhibitor (losartan) decreased aging-associated declines in mitochondrial uncoupling protein 2 and the capacity for energy production [30]. Moreover, treatment with AT1R blocker losartan increased the lifespan by improving EC function and alleviating complications of hypertension in the young spontaneously hypertensive rats [31]. Reduction of phosphorylation of eNOS and NO production are also involved in the pathological changes in aging mice [29]. Further, Ang II promotes increases in senescence-associated beta-galactosidase activity, a biomarker for cellular senescence in cultured human ECs [32]. Therefore, abnormal RAAS activation is associated with EC senescence and dysfunction in aging.

3.6. Hyperuricemia

Increase in the levels of serum uric acid levels are considered to one of the risk factors for EC senescence, endothelial dysfunction, and associated CVD [33,34]. In this regard, high serum uric acid levels are seen in aged individuals with higher values in women compared to men at older age [35]. Moreover, increased consumption of foods rich in refined carbohydrates such as sucrose and fructose in obese subjects is associated with increased serum uric acid levels and endothelial dysfunction [36]. We have reported increased aortic xanthine oxidase activity and oxidative stress in Western diet (high in saturated fat and refined carbohydrates)-fed mice [37,38]. Suppression of aortic stiffness and associated maladaptive immune and inflammatory responses by the xanthine oxidase inhibitor allopurinol decreased arterial stiffness and impairment of endothelial dependent arterial relaxation [37,38]. Moreover, uric acid has been shown to cause EC senescence through increased expression of endothelial activation of angiotensin signaling and oxidative stress [33,36].

4. Pathophysiological changes in aging-related EC senescence

4.1. Dysregulation of vascular tone

The vascular endothelium plays a key role in the regulation of vascular tone by releasing vasoconstrictor and vasodilator substances and thus regulates blood flow (Fig. 1). Endothelin 1 (ET-1) is a vasoconstrictor and has been implicated in the development and progression of various vascular disorders including arterial stiffness, hypertension and atherosclerosis [1]. Increased ET-1 mediated senescence in human ECs [39], contributes to aging related impairment of shear stress-induced vascular relaxation [40]. Therefore, the delicate balance between vasoconstriction and vasodilation is regulated by ET-1 and other vasodilator substances including NO, prostacyclin, and EDHF. Aging individuals have decreases the flow-mediated vascular dilation and a decrease in systolic volume and NO production [41]. Meanwhile, ECs

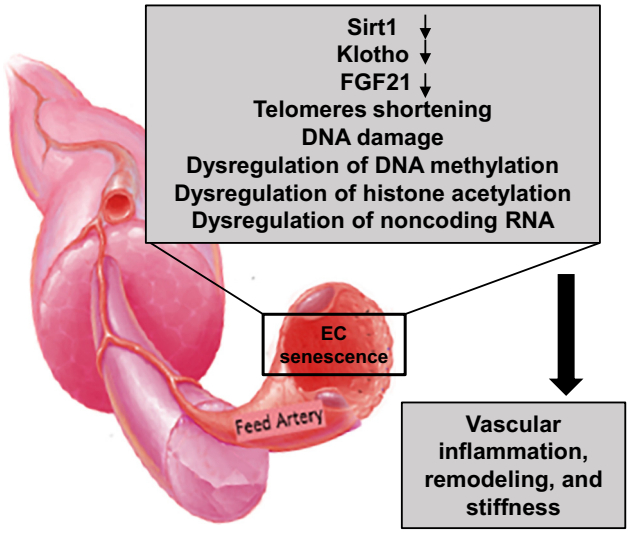

Fig. 2. The molecular mechanisms in endothelial cell senescence-induced vascular dysfunction. Reduction of Sirt1, Klotho, FGF21, DNA damage, and dysregulation of DNA methylation, histone acetylation, and noncoding RNA induce vascular inflammation, remodeling, and stiffness. Abbreviations: Sirt1, Sirtuin 1; FGF21, Fibroblast growth factor 21.

synthesize and release EDHF to promote relaxation of VSMC. In this regard, increased Ca^{2+} influx in ECs increases Ca^{2+}-sensitive small and intermediate conductance potassium channels, which induce VSMC hyperpolarization and VSMC relaxation through myo-endothelial gap junctions [1]. Compared to younger controls, mesenteric arteries demonstrated hyper-contractility in response to phenylephrine in aged rats [42]. Conversely, carotid arteries displayed hypo-contractile responses to both phenylephrine and ET-1 in aged guinea pigs [43], suggesting that vascular contraction is altered in aging as well. Mechanisms for these abnormalities are incompletely understood, but the different expression and activation of NO, EDHF, Ca^{2+} are involved in altered age-related contractile and dilation responses.

4.2. Vascular permeability

The normal endothelium acts as a mechanical barrier to regulate migration of the small molecules, inflammation cells and nutrients from the circulation and vascular wall [22]. The gaps between EC junctions regulate vascular permeability in responding to oxidative and inflammation stimulation. Impairment of EC integrity increases vascular permeability to allow for the flow of small molecules and inflammation cells in and out of the vessel [22]. One study found that EC senescence impaired the barrier integrity and contributes to blood-brain barrier breakdown [44]. Furthermore, endothelial concentrations of TNF-α were significantly increased in aged mice compared to young mice [45], suggesting that inflammation is associated with disruption of tight junction complex assembly and endothelium barrier integrity (Fig. 1).

4.3. Impairment of angiogenesis and vascular repair

It is well known that expression of vascular growth factors are attenuated in elderly individuals (Fig. 1). Factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor, NO, as well as EPCs are involved in this physiological process. To this point, VEGF is one of the important regulators of physiological and pathological angiogenesis. Studies have found that the expression of VEGF, muscle capillary contacts, and capillary-to-fiber perimeter exchange index are lower in old mice compared to younger mice [46,47], suggesting that skeletal muscle capillarization and VEGF expression are lower with aging. Aging-induced reduction of bioavailability of NO not only inhibits vascular relaxation but also increases EC apoptosis, resulting in EC dysfunction and impairment of angiogenesis [48]. Indeed, eNOS has been identified as an angiogenic signaling factor in the healing of skin wounds and genetic deletion of eNOS induced impairment of angiogenesis in a mouse model of hindlimb ischemia [49]. EPCs can be recruited from the bone marrow to sites of ischemia that induce EC differentiation and neovascularization. The reduction of EPC numbers is regarded as a consequence of impairment of differentiation in the bone marrow from aging individuals [50]. EPCs presented a characteristic of decreases in EC survival, migration, and proliferation in vitro from elderly persons [50]. Interestingly, delivery of young bone marrow ECs along with EPCs following irradiation improved hematopoietic stem cell engraftment and enhanced survival, suggesting that bone marrow ECs play an important role in regulating hematopoietic aging and support restoring hematopoietic stem cell function in aged mice [51].

4.4. Impairment of mitochondrial biogenesis

With aging there is a decrease in endothelial mitochondrial biogenesis trough transcriptional coactivator peroxisome proliferators activated receptor gamma coactivator 1 (PGC-1) (Fig. 1). PGC-1, a coactivator of nuclear transcription factors in nuclear respiratory factors 1 and peroxisome proliferators activated receptors, is regarded as an inducible integrator of transcriptional circuits controlling mitochondrial biogenesis and function in cardiovascular tissues [52]. PGC-1α is also regulated by eNOS and bioavailable NO [53]. In aged endothelial cells the expression of PGC-1, complex I, III, IV, and cytochrome c oxidase are significantly decreased and these abnormalities further increase ROS production and impair vascular function in the aged vessels [54].

5. Molecular signaling related EC senescence

5.1. Sirtuins

Sirtuins (Sirts), a group of silent information regulator 2 proteins, possess nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase activity and ADP-ribosyltransferase activity [55] (Fig. 2). Seven Sirt proteins have been identified and Sirt1 plays the most important roles during the processes of DNA repair, EC senescence, cell cycle regulation, organism longevity, stress resistance, gene silencing, apoptosis and inflammation in vascular since Sirt1 is highly expressed in ECs from artery, vein and capillary [55]. For example, Sirtuin 1 (Sirt1) was found to increase the expression and activation of eNOS and NO production and thus prevent oxidative stress-induced cellular senescence in human umbilical vascular ECs [56]. The activity of Sirt1 was decreased in the aging population and pharmacological activators of Sirt1 significantly increased anti-aging cardiovascular effects [57]. However, inhibition of Sirt1 significantly decreased the endothelium-dependent relaxation induced by acetylcholine and this change was associated with increases in vascular superoxide production, NADPH oxidase activity, and expression of its subunits p22 (phox) and NOX4 [57]. Recent work has found that Sirt1 is a master regulator of $p66^{Shc}$ transcription that modulates acetylation of histone H3 to bind the $p66^{Shc}$ promoter [58]. Moreover, Sirt1 prevented DNA damage, cell cycle arrest, oxidative stress, and arterial aging through inhibition of the Forkhead box O detrimental pathway [59]. Therefore, Sirt1 is an important regulator of vascular endothelial aging.

5.2. Klotho

Klotho is regarded as anti-aging gene recent (Fig. 2). Klotho delays the aging process and preserves longevity through regulation of phosphate homeostasis, Wnt signaling, and insulin signaling [60]. Klotho is expressed mostly in the kidney, parathyroid gland, and choroid plexus [60]. The soluble Klotho protein also exists in the urine, blood, and

cerebrospinal fluid [60]. Clinical trials have found that higher plasma Klotho concentrations were independently associated with a lower risk of CAD, stroke, heart failure, and peripheral arterial disease in community-dwelling adults [61]. Conversely, reduced serum Klotho levels were associated with the presence and severity of CAD independently of other established CVD risk factors including dyslipidemia, diabetes, and hypertension [62]. Moreover, the serum Klotho level independently predicts arterial stiffness [63]. To this point, overexpression of Klotho prevented aging-induced renal and cardiovascular dysfunction and prolonged lifespan in experimental mice [64]. Further, mouse Klotho knockout presented the premature aging features, such as altered calcium/phosphate metabolism, vascular calcification, and reduced lifespan [65]. These data support the notion that high levels of soluble Klotho may protect EC and cardiovascular function and extend human lifespan.

5.3. Fibroblast growth factor 21

Fibroblast growth factor 21 (FGF21) is mostly secreted in the liver, and it is also expressed in the adipocytes, skeletal muscle and vascular tissues. The primary functions of FGF21 include improved lipid metabolism, increased insulin sensitivity, and reduction of glucose levels [66]. For example, injection of FGF21-mimetic antibody decreased the body weight, triglycerides, plasma insulin, and glucose during tolerance testing in obese monkeys [66]. Also, FGF21 prevented atherosclerosis by suppression of hepatic sterol regulatory element-binding protein-2 and induction of adiponectin in apolipoprotein E knockout mice [67]. FGF21 deficiency induced a severe hypercholesterolemia, remarkable hypo-adiponectinemia, and development of atherosclerotic plaque formation [67]. Most recently, FGF21 was found to regulate EC senescence and the anti-aging process (Fig. 2). In this regard, FGF21 prevented high glucose-induced cellular damage and inhibition of eNOS activity in cultured human umbilical vein ECs [68]. Further, FGF21 prevented Ang II-induced cerebrovascular aging through inhibiting p53 activation and improving mitochondrial biogenesis in an AMP-activated protein kinase-dependent manner [69], suggesting that FGF21 plays an important role in EC senescence and aging-related CVD.

6. Genetic and epigenetic regulation in EC senescence

6.1. DNA damage and repair

The Genetics of Healthy Ageing Study has identified a number of loci or genes associated with a longevity trait in human [70]. For example, Loci at the chromosomes 3 (3q24-22), 4 (4q22-25), 8 (rs9650241, kgp6080058, and rs2901448), 9 (9q31-34), 14 (14q11.2), 17 (17q12-q22), and 19 (19p13.3-13.11, 19q13.11-13.32, 19q13.33) were involved in the longevity trait [70]. The genes in apolipoprotein E, forkhead box protein O1, IL-6 and Sirt6 are functionally associated with EC senescence [70]. In young and aging ECs, 2351 genes were altered under static and shear stress conditions [71]. Among these genes, argininosuccinate synthase 1, NOX4, aquaporin 1, p15, and p16 have significant correlation between transcriptional expression and senescence [71]. The role of DNA damage is further highlighted in progeria syndromes in human aging population. To this point, Werner syndrome, with premature onset of clinical signs of aging including osteoporosis and CVD, there is a Werner gene mutation that is involved in DNA replication, recombination, transcription, repair, as well as telomere maintenance [72]. As a protective response, accumulating unrepaired DNA damage triggers a series of biological pathways to avoid the harmful consequences of genomic instability through cell proliferation arrest, apoptosis and cellular senescence. Genes involved in DNA repair such as poly (ADP-ribose) polymerase 1 (PARP1), p53, DNA-dependent protein kinase, and apurinic/apyrimidinic endonuclease 1/redox factor 1 have been found to increase in carotid endarterectomy specimens [73], suggesting that DNA repair systems play an important role in maintaining normal physiological function (Fig. 2).

6.2. Telomeres and EC senescence

Telomere changes is one of the important mechanisms in vascular cell senescence (Fig. 2). Telomeres are chromatin complexes composed of noncoding double-stranded repeats of G-rich tandem DNA sequences (TTAGGG repeats) and various telomere-binding proteins that are located in the ends of chromosomes and maintain the genome stability by protecting this region from recombination and degradation [74]. Telomere shortening has a positive correlation in the pathogenesis of CVD because of the imperfect duplication of the extreme terminals of the chromosomes by DNA polymerase [75]. As a result, cell division shortens telomeric DNA and cells lose capping function at the chromosomal ends. Eventually, progressive telomere shortening triggers senescence and apoptosis and reduces the proliferative capacity of cells [75]. In a clinical study from a cohort of 193 patients ($\geq$70 years of age), leukocyte telomere length associates significantly with vascular cell senescence, hypertension, and atherothrombotic events [76]. Disturbed flow accelerates EC senescence at sites of the iliac artery bifurcation, where the telomeres are demonstrably shorter [74,75] and histological analysis presents an increased number of senescent ECs, suggesting an association between reductions in telomerase activity and corresponding vascular aging in human ECs [77,78]. Increased telomerase activity and expression reversed ECs' senescent characteristic to a normal EC phenotype that has the ability to generate NO and maintain of EC migratory, proliferative, and survival capacities [79]. Therefore, the interaction of telomerase activity regulates EC function and CVD.

6.3. DNA methylation

DNA methylation occurs in the cytosine-paired-with-guanine (CpG) dinucleotide gene sequences through attachment of methyl group to the carbon-5. DNA methylation has been studied from the different age population and data showed that there were specific DNA methylation patterns including DNA binding and transcription regulation associated with aging and longevity [80,81] (Fig. 2). DNA methylation is usually catalyzed by three different DNA methyltransferases (DNMTs). While DNMT1 maintains methylation status in the process of replication, DNMT3A and DNMT3B regulate de novo methylation [80,81]. Age-dependent DNA hypomethylation occurring in an aging is often associated with a decrease in the activity of DNA methylation enzymes and hypermethylation in specific gene loci including IGF-II, c-fos, and p16 [82]. DNA methylation in long interspersed nucleotide elements −1 repetitive element is associated with circulating vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and C-reactive protein on the blood DNA samples from 742 male participants in the Boston area Normative Aging Study [83]. Further, Inhibition of DNA methylation in GATA-2/3 and eNOS promoters enhances the differentiation of cultured EPCs into mature ECs [84].

6.4. Histone acetylation

Histone acetylation is another epigenetic mark regulating chromatin accessibility and gene expression (Fig. 2). Reduction of histone modifications such as acetylation of histone 3 at lysine 9 and trimethylation of histone 3 at lysine 27 was found in older rat [85]. Further, Sirt1 was able to bind to the PAI-1 promoter to inhibit the acetylation of histone H4 lysine 16 on the promoter region of plasminogen activator inhibitor-1 and exerted a protective effect in vascular EC senescence [86]. Interestingly, Ang II induced protein kinase B-dependent phosphorylation and lysine acetylation of PGC-1 through the histone acetyltransferase of general control nonderepressible 5, resulting in decreasing PGC-1 activity and catalase expression in vascular cells [87]. The enzymes of histone deacetylases (HDACs) and histone acetyltransferases (HATs)

operate the modification of histone acetylation. For example, HDAC3 was stabilized and activated by laminar flow and VEGF through the VEGF receptor 2/protein kinase B pathway and thus induced proliferation of EPCs and EC differentiation. Deletion of HDAC3 abolished VEGF-induced EPC proliferation and differentiation [88]. Meanwhile, shear stress increased HATs activity and induced the EC lineage differentiation the in mouse embryonic stem cells [89]. These data suggest that histone acetylation plays an important role in EC senescence and associated vascular dysfunction.

6.5. Noncoding RNAs regulation in EC senescence

MicroRNAs (miRNAs) is the single-stranded RNAs of about 25 nucleotides that regulate the pathobiology of EC senescence and CVD (Fig. 2). For instance, several miRNAs such as miR-17, -29, -30, -31, -34a, -43a, -126, -143, -145, -146, -216, -217 and -299 are involved in the vessel growth angiogenesis, inflammation and fibrosis [90]. To this point, MiR-29 was increased in cultured senescent ECs and aortas in aging mice [91]. Further, upregulation of miR-29 in aging were involved in the downregulation of type IV collagen and accelerated tissue aging in a mouse aging model with Klotho gene deletion [92]. Also, miR-34a was found to elevate in older animals and overexpression of miR-34a repressed EC proliferation and induced senescence by inhibition of Sirt1 [93]. Indeed, MiR-34a is a tumor suppressor and a potent trigger of senescence through regulation of cdk4/6, Bcl-2, cyclin D1/E2, as well as hepatocyte growth factor receptor [94]. Long noncoding RNAs (lncRNAs) are > 200 nucleotides noncoding RNA and have been found to regulate cell cycle and senescence. For example, lncRNA MALAT1 controls the expression of cell cycle genes such as p53 and B-Myb and thus regulates cellular proliferation and senescence [95]. The mitochondrial lncRNA ASncmtRNA-2 was increased in aortas of old mice and over-expressing ASncmtRNA-2 induced cell cycle arrest in in the G2/M phase and replicative senescence in ECs [96], suggesting that these non- coding RNAs are involved in the regulation of EC senescence, vascular function and CVD.

7. Conclusions

EC senescence is an important contributor in aging-induced vascular dysfunction. Cardinal features of vascular aging include increases in arterial stiffness, and systolic pressure. Abnormal expression and activation of NO, EDHF, Ca^{2+} signaling, increased endothelium permeability, impairment of angiogenesis and vascular repair, and reduction of EC mitochondrial biogenesis contribute to the pathophysiological changes in EC senescence and aging associated vascular dysfunction. Cell cycle regulation, oxidative stress, increased ROS, altered Ca^{2+} signaling, hyperuricemia, and vascular inflammation are involved in the pathophysiological process. Molecular proteins and signaling pathways including Sirt1, Klotho, FGF21, activation of RAAS contribute to these pathophysiological changes. Further, accumulation of genetic damage and epigenetic alterations change the normal gene expression and activity, resulting in cellular senescence and vascular dysfunction. While cellular senescence impacts vascular function and induce CVD, cellular senescence also plays a beneficial role in prevention of human disease including cancer. Indeed, cellular senescence is a physiological event in repair of adult tissues. Therefore, further studies of EC senescence are necessary in order to better understand the precise mechanisms and to develop potential strategies in prevention of age-related CVD.

Transparency document

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

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Conflict of interest

The authors declare no competing interests.

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