Chapter 4

Targeting Endothelial SIRT1 for the Prevention of Arterial Aging

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73019

Abstract

Cardiovascular diseases are the leading cause of morbidity and mortality in the elderly population all over the world. Arterial aging is the earliest manifestation and a key risk factor for age-induced cardiovascular abnormalities. The longevity regulator Sirtuin 1 (SIRT1) is abundantly expressed in the endothelium of the arteries and elicits potent protective functions against arterial aging. Targeting endothelial SIRT1 represents a promising approach for the prevention and treatment of cardiovascular diseases. This chapter provides an overview of SIRT1's regulation and function in endothelial cells and discusses the potential applications of targeting endothelial SIRT1 for arterial aging-related cardiovascular diseases.

Keywords: sirtuins, endothelium, cellular senescence, vasodilatation, arterial remodeling, hypertension, atherosclerosis

1. Arterial aging

Chronological age is associated with a progressive alteration of arterial structure and function, herein referred to as arterial aging, which contributes to the development of a wide range of cardiovascular diseases including hypertension, atherosclerosis, heart failure, and stroke [1–3]. Arterial system is composed of three types of arteries including large elastic or conduit arteries, medium-sized muscular arteries, and small arteries referred to resistance arteries. Arterial aging is characterized by endothelial dysfunction and arterial remodeling, indicating a decline in arterial elasticity/distensibility, decreased arterial compliance, and increased arterial stiffness. Physiological alterations of the vascular wall are dynamic and occur throughout life [4]. During aging, gradual thickening of the arterial wall, changes in wall composition (i.e., elastin fragmentation and collagen deposition), and an increase of artery diameter are observed in conduit arteries [2]. Increased intimal-to-media thickness (IMT) is a valid

indicator of arterial aging supported by the finding that the IMT of the carotid artery increases twofold to threefold between 20 and 90 years of age [4]. Pulse wave velocity (PWV) is a noninvasive measure of vascular stiffness. Stiffening of the conduit arteries leads to increased aortic pulse pressure and increased PWV, which occurs in both sexes along aging [5].

The endothelium, a monolayer of flattened, polygonal cells lining the inner surface of arteries, plays an important role in regulating arterial structure and function. The endothelium can respond to pathophysiological signals by producing various factors that regulate vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation, and inflammation. During arterial aging, senescence, activation, and dysfunction of endothelial cells (ECs) represent the earliest abnormalities that lead to an impaired endothelium-dependent vasodilatation and adverse arterial wall remodeling [6]. Senescent ECs undergo permanent growth arrest, get enlarged and flattened in morphology, and also display positive staining for senescence-associated β-galactosidase (SA-β-gal) [7]. There are mainly two types of senescence. One is caused by successive cell duplication as a kind of natural aging process termed as "replicative senescence" and characterized by shortening of telomere [8]. The other is called "premature senescence" and induced by several stress conditions such as oxidative stress, radiations, and exposure to oncogenes [9]. Endothelial activation is defined as the initial event in atherogenesis. Circulating proinflammatory molecules including cytokines (i.e., tumor necrosis factor-α (TNF-α)) or modified lipoproteins (i.e., oxidized low-density lipoprotein (oxLDL)) activates ECs to express chemokines, cytokines, and adhesion molecules, thus attracting and recruiting inflammatory cells such as macrophages and T cells. Both endothelial senescence and activation can induce endothelial dysfunction which is reflected by impairment of endothelium-dependent vasorelaxation caused by a loss of nitric oxide (NO) bioavailability in the vessel wall and altered anticoagulant and anti-inflammatory properties of the endothelium. Impaired endothelium-dependent vasodilation in the coronary circulation of humans has profound prognostic implications in that it predicts adverse cardiovascular events and long-term outcomes [1, 2, 10, 11].

Age-related loss of arterial functions has been demonstrated, and underlying mechanisms were studied in human studies. Reduced NO bioavailability in older age was reported by observing diminished forearm vasoconstrictor response to infusion of NO-synthase inhibitor L-NMMA in resistance arteries [12]. In older adults, supplementation of NO precursor, L-arginine, improves coronary artery blood flow response to acetylcholine [13] and skin blood flow response to whole body heating [14]. Moreover, age-related decline in synthesis of tetrahydrobiopterin, a co-factor in NO production, provides further evidence for impairment of vasodilation NO-pathway during aging [15]. In the aspect of vasoconstrictor pathways, a greater lower limb vasodilatation response to endothelin (ET)-receptor blockade in old men was reported [16]. A small but significant age-related impairment in vascular smooth muscle function was also observed in conduit and resistance arteries in a meta-analysis [17].

2. SIRT1 in endothelial cells: expression and regulation

Sirtuin 1 (SIRT1) is the mammalian orthologue of the yeast longevity regulator Sir2 [18]. Members of the Sirtuin family share a highly conserved core domain to catalyze deacetylation,

ADP-ribosyltransferation, desuccinylation, and demalonylation [19]. Sirtuins regulate energy homeostasis, stress resistance, circadian rhythmicity, mitochondrial functions, and embryonic development, which in turn contribute to increased lifespan [20].

Human SIRT1 gene is located at chromosome 10q21.3 containing 11 exons with a total length of 33,715 base pair [21]. SIRT1 is composed of 747 amino acids including a core catalytic domain consisting of 275 amino acids and both N- and C-terminal extensions spanning about 240 amino acids [22]. There are two nuclear localization signals and two nuclear exportation signals located in the extensions whose balanced functionality determines the presence of SIRT1 in either the nucleus or cytoplasm and explains the distinct location of SIRT1 among different cell lines and tissues [23].

Regulation of SIRT1 enzymatic activity occurs at various levels including post-translational modification, protein complex formation, transcriptional regulation, and concentrations of enzymatic substrates [19, 24]. Phosphorylation of SIRT1 represents the major form of post-translational modifications. Independent studies report multiple phosphorylation sites by distinct proteins, including c-Jun N-terminal kinase 1 (Ser27/47), cyclin B/cyclin-dependent kinase 1 (Thr530, Ser540), casein kinase 2 (Ser659/661), and adenosine 5′-monophosphate-activated protein kinase (AMPK) (Thr344) [25–28]. Additional post-translational modifications include methylation by SET7/9 [29], nitrosylation by glyceraldehyde-3-phosphate dehydrogenase [30], and sumoylation by sentrin-specific protease 1 [31]. In addition, several endogenous protein-binding partners of SIRT1 are found to regulate its function via forming protein complex. For example, the active regulator of SIRT1 can bind to amino acids 114–217 in the N-terminus of SIRT1 and stimulate deacetylation of p53 in vivo [32]. On transcriptional level, SIRT1 was reported that nicotinamide phosphoribosyltransferase (NAMPT) upregulated the expression of SIRT1 and SIRT1 antisense long noncoding RNA, thus regulating senescence, proliferation, and migration of endothelial progenitor cells (EPCs) [33]. SIRT1 activity is also thought to be affected by the levels of intracellular co-substrate nicotinamide adenine dinucleotide (NAD+) and its product nicotinamide [34].

With age, SIRT1 expression in ECs is progressively downregulated. Overexpression of SIRT1 in the endothelium prevents cellular senescence, enhances vasodilatory responses, and attenuates aging-induced vascular damages [35–37]. The subsequent review will summarize the recent progresses related to the molecular regulation of SIRT1 expression in ECs and the anti-vascular aging effects of SIRT1 by focusing on endothelial dysfunction and arterial remodeling.

3. SIRT1 in endothelial cells: molecular targets and biological functions

Apart from histones, SIRT1 can mediate the deacetylation of various signaling substrates to exert vasoprotective functions. SIRT1 is abundant in ECs mediating postnatal blood vessel growth via Foxo1 and helps to maintain endothelial function [38]. In vitro experiments showed that downregulation of SIRT1 using small interfering RNA (siRNA) uniquely inhibited endothelial sprout formation via a three-dimensional assay, while other mammalian sirtuin family members (SIRT2–SIRT7) could not [38]. In addition, the reduction of matrix metalloproteinase-14

(MMP-14), a membrane-anchored MMP essential for tip cell activity during sprouting angiogenesis, was found in siRNA-SIRT1-treated endothelial sprouts [39]. Decreased expression of SIRT1 either by mRNA silencing or pharmacological inhibition could induce premature-senescence-like phenotypes in ECs [40, 41]. SIRT1 displays anti-senescence activity in ECs by inducing the deacetylation of diversified signaling substrates [42]. For example, SIRT1 can deacetylate tumor suppressor protein p53 to downregulate its stability and activity as to promote cell survival in response to cellular stress [43]. SIRT1 also plays an important role in enhancing the endothelial NO synthase (eNOS) transcription and translation by deacetylating eNOS on lysine 496 and 506 to generate more NO, thus enhancing vessel dilatation, mediating vessel tone regulation, and providing athero-protective effects [44, 45]. Recent study demonstrated that SIRT1 activation could help reduce traction forces and reorganize actin localization (increased peripheral actin) in aged ECs, which is also a sign of anti-senescent effect [46]. Moreover, while senescent porcine aortic ECs (PAECs) showed decreased expression of SIRT1 compared to young PAECs, the protein level of liver kinase B1 (LKB1), a serine/threonine kinase and tumor suppressor, was dramatically increased as well as the phosphorylation of its downstream target AMPK (Thr172). In this case, SIRT1 can antagonize LKB1-dependent AMPK activation by promoting the deacetylation, ubiquitination, and proteasome-mediated degradation in order to retard PAEC senescence which also correlated with the Akt survival signaling pathway [41]. Furthermore, it was reported that SIRT1 can bind to the DOC domain of HERC2 [HECT and RLD domain containing E3 ubiquitin protein ligase 2] and then ubiquitinate LKB1 in the nuclear compartment of ECs [37]. SIRT1 can also negatively modulate Notch signaling in ECs via deacetylation of the Notch1 intracellular domain (NICD), in which loss of endothelial SIRT1 activity leads to impaired growth and sprout elongation [47]. Intracellular NAMPT-NAD+-SIRT1 cascade was shown to improve post-ischemic neovascularization through modulation of Notch signaling pathway [48]. Adapter protein p66Shc which can directly stimulate mitochondrial reactive oxygen species (ROS) generation was discovered downregulated by SIRT1 in mice with hyperglycemia-induced endothelial dysfunction [49]. Moreover, in vitro experiments using human aortic ECs (HUVECs) demonstrated that SIRT1 can deacetylate RelA/p65 to diminish tissue factor expression and suppress nuclear factor-κB (NF-κB) signaling, thus preventing atherothrombosis [50]. In EPCs, SIRT1 was implicated to protect against oxidative stress-induced apoptosis by inhibiting Foxo3a via ubiquitination and degradation [36]. microRNA-34a (miR-34a), regulated by p53 and able to control cell cycle arrest, has been reported to promote cardiac, endothelial, and EPC senescence via downregulation of SIRT1 [51]. Also, visfatin (an adipocytokine closely associated with human cell senescence) was reported to attenuate the oxLDL-induced senescence of EPCs by upregulating SIRT1 expression through the PI3K/Akt/ERK pathway [52].

4. Endothelial SIRT1 prevents arterial aging

Various animal studies demonstrated that SIRT1 plays a vital role in anti-endothelial senescence and anti-atherogenesis. Infiltration of monocyte-derived macrophages into the subendothelial space is a crucial step in atherogenesis [53]. SIRT1 can decrease cholesterol uptake especially oxLDL and prevent macrophage foam cell formation via suppressing the expression

of scavenger receptor Lox-1 [54] and reducing the expression of various pro-inflammatory molecules including TNF-α, monocyte chemotactic protein-1, and interleukins [55]. A recent discovery showed that treatment of the SIRT1 activator SRT3025 decreased plasma levels of LDL cholesterol and total cholesterol and attenuated atherosclerosis, owing to reduced secretion of hepatic Pcsk9 and enhanced protein expression of LDL receptor in apolipoprotein E-deficient ($ApoE^{-/-}$) mice [56]. In the meantime, SIRT1 was demonstrated to promote reverse cholesterol (mainly HDLs) transport into macrophages by directly deacetylating and subsequently regulating the transcriptional activity of liver X receptors, which play a significant role in lipid homeostasis and inflammation and can help express ATP-binding cassette transporter 1 that transport cholesterol into pre-βHDL particles [57].

Some studies regarding upstream regulators of SIRT1 including cathepsin, caspase-1, and cyclin-dependent kinase 5 (CDK5) elucidate beneficial roles of SIRT1 in anti-endothelial senescence and anti-atherogenesis [58–60]. The cysteine cathepsins belong to the leaked lysosomal contents with the viability in cleavage and degradation of SIRT1, which lead to stress-induced premature senescence [58]. Studies on $ApoE^{-/-}$/caspase-$1^{-/-}$ double knockout mice have shown promising evidences that early hyperlipidemia promoted endothelial activation via a Caspase-1-SIRT1 pathway [59]. In this case, researchers found that inhibition of caspase-1 resulted in SIRT1 accumulation in the $ApoE^{-/-}$ mouse aorta and $ApoE^{-/-}$/caspase-$1^{-/-}$ mice had attenuated early atherosclerosis, decreased aortic expression of proinflammatory cytokines, and reduced aortic monocyte recruitment, as well as decreased endothelial activation [59]. Another upstream regulator of SIRT1 is CDK5, which was proved to increase the phosphorylation of SIRT1 especially at S47 during cellular senescence [60]. In this study, replacing S47 with nonphosphorable alanin (S47A) elevated, while mutation of S47 to phospho-mimicking aspartic acid (S47D) abolished the beneficial effects of SIRT1 such as anti-senescence, growth promotion, and downregulation of LKB1 expression [60]. Interaction between SIRT1 and telomeric repeat-binding factor 2-interacting protein 1 was abolished when S47 was phosphorylated. NF-κB signaling pathway is activated to induce endothelial inflammation and leads to endothelial senescence and atherosclerosis. Downregulation of CDK5 by either knockdown (by siRNA) or inhibition (by roscovitine) reduced percentage of senescent ECs and attenuated inflammatory gene expression. Meanwhile, long-term treatment of $ApoE^{-/-}$ mice with the CDK5 inhibitor, roscovitine, resulted in attenuated atherosclerosis in aortae [60]. As CDK5R1(p35/p25) is the crucial activator mediating the kinase activity of CDK5 [60, 61], further research will be conducted to unveil the underlying mechanism of CDK5-p35/p25-SIRT1 pathway in ECs.

Limited information is available concerning the role of endothelial SIRT1 in vascular remodeling. In eNOS-deficient mice, overexpression of endothelial SIRT1 prevents hypertension and age-related adverse arterial remodeling [37].

Laminar shear stress is an important stimulus for the endothelium-dependent control of vascular tone and of vascular remodeling processes. In cultured ECs, laminar flow increases both the expression and activity of SIRT1, whereas oscillating flow decreases SIRT1 expression [62]. In mouse arteries, the formation of neointima is accompanied by a progressive downregulation of SIRT1 expression [63]. SIRT1 inhibition in ECs increases the expressions of p53

and its downstream target, plasminogen activator inhibitor-1 (PAI-1), which promotes the formation of neointima and vascular remodeling in response to vascular injury [40].

Loss of vascular smooth muscle cell (VSMC) function is an alarming sign of vascular disease. During the aging process, VSMCs undergo increased dysregulation, apoptosis, and senescence [64]. In VSMCs, SIRT1 can act as a modulator of neointima formation (associated with repression of activator protein-1 (AP-1) activity [63]) and protect against DNA damage. Aging-related loss of SIRT1 expression correlates with lower capacity for vascular repair, abolished stress response, and elevated senescence [63].

Decreased expression of SIRT1 in VSMCs exerts its proatherogenic effects by the failure to deacetylate histones in DNA repair, response to oxidant stress and LDL, and therefore leads to VSMC senescence and apoptosis [63, 65]. As to atherosclerotic plaques, SIRT1 activity has been suggested to deacetylate the regulatory factor for X-box (RFX5) and antagonized repression of collagen type I (COL1A2) transcription in VSMCs, consequently stabilizing the plaque and avoiding rupture [66]. Another most recent finding relevant to destabilization of atherosclerotic plaque is that SIRT1 participated in downregulation of platelet-activating factor receptor (PAFR) in VSMCs through β-arrestin 2-mediated internalization and degradation, resulting in the inhibition of PAF-induced matrix metalloproteinase (MMP-2) generation [67]. In addition, inhibition of miR-138 was found to increase SIRT1 expression in VSMCs separated from diabetic (db/db) mice and in SMC lines C-12511 in recent study, which indicated miR-138 as another potential inhibitory target to attenuate the proliferation and migration of VSMCs and cure atherosclerosis [68]. Furthermore, SIRT1 was also found to inhibit angiotensin II-induced VSMC hypertrophy in rat embryonic aortic VSMCs [69]. Later on, SIRT1 demonstrated antihypertensive activity in transgenic mice with selective overexpression of SIRT1 in VSMCs (SV-Tg). Alleviated vascular remodeling in mouse thoracic and renal aortae induced by angiotensin II is observed, along with significantly decreased transforming growth factor-β1 (TGF-β1) expression, ROS generation, vascular inflammation, and collagen formation in the arterial wall of SV-Tg mice [70]. Similar to contribution in ECs, overexpression of miR-34a can upregulate p21 level and inflammation through SIRT1 downregulation and cause senescence-associated secretory phenotype factors induction (including pro-inflammatory molecules such as cytokines, chemokines, proteases, growth factors, soluble receptors, etc.), promoting VSMC senescence and leading to arterial dysfunction [71].

5. Targeting endothelial SIRT1 for the prevention of arterial aging

Slowing down the vascular aging requires early intervention, lifelong treatment, and site-specific approaches. To reduce arterial stiffness, pharmacological agents, including angiotensin-converting enzyme inhibitors, angiotensin II type 1 blockers, aldosterone antagonists, and statins, are currently available and in clinical use [35]. The evident vasoprotective effects of SIRT1 definitely pose great opportunities and challenges for drug discovery targeting endothelial dysfunction. So far, a few natural and synthetic substances have been demonstrated as SIRT1 activators to promote vascular health. The first potent activator of SIRT1 is resveratrol, a small polyphenol discovered in red wine, which could protect ECs against inflammation,

apoptosis, and oxidative stress [72]. It was found to dramatically lower the incidence of cardiovascular diseases in spite of high saturated fat diet, which was termed as "French paradox" [73]. Several natural ingredients extracted from various traditional Chinese herbs were also found to activate SIRT1. Tetramethylpyrazine is proved to reverse high-glucose-induced endothelial dysfunction via SIRT1 [74]. Polydatin can attenuate hemorrhagic shock by upregulating SIRT1 [75]. Quercetin is capable of inhibiting oxidized LDL-induced EC damage by SIRT1 activation [76]. Some other natural polyphenols including fisetin and butein can also activate SIRT1 [77]. Vitamin D protects ECs from irradiation-induced senescence and apoptosis by modulating MAPK/SirT1 axis [78]. On the other hand, a series of SIRT1 activators like SRT2183, SRT1460, SRT1720, SRT2379, SRT501, SRT2104, SRT3025, and BMT0-512 have been synthesized and developed as potential drugs to protect against vascular aging [77, 79].

Despite the fact that SIRT1 is as an optimal therapeutical target for cardiovascular diseases, the dosage of upregulation of SIRT1 should be considered seriously and titrated cautiously in clinical practice. It was reported that 2.5- to 7-fold overexpression of SIRT1 prevented heart from oxidative stress via SIRT1/FOXO, while 12.5-fold overexpression of SIRT1 increased apoptosis and hypertrophy and decreased cardiac function, suggesting that only low to moderate doses of SIRT1 can exert beneficial effects [80]. The aforementioned findings call for more careful evaluation of dosage and possible adverse effects in drug development targeting endothelial SIRT1.

In light of all the above studies, there have already been several potential drugs to target the anti-vascular aging effects of endothelial SIRT1. The entry of SIRT1 activators into human trials is exciting but also highlights the necessity to better understand the SIRT1 specificity, clinical effects, and side effects of these promising activators in vivo.

Acknowledgements

This work was financially supported by Hong Kong Health and Medical Research Fund 13142651.

Conflict of interest

No interest of conflict was declared.

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