Biochimica et Biophysica Acta 1852 (2015) 1311–1322

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Sirtuins in vascular diseases: Emerging roles and therapeutic potential

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ARTICLE INFO

Article history:
Received 18 December 2014
Received in revised form 20 February 2015
Accepted 4 March 2015
Available online 10 March 2015

Keywords:
Sirtuins
Endothelium
Vascular dysfunction
Endothelial progenitor cell
Cardiovascular disease

ABSTRACT

Silent information regulator-2 (Sir-2) proteins, or sirtuins, are a highly conserved protein family of histone deacetylases that promote longevity by mediating many of the beneficial effects of calorie restriction which extends life span and reduces the incidence of cancer, cardiovascular disease (CVD), and diabetes. Here, we review the role of sirtuins (SIRT1-7) in vascular homeostasis and diseases by providing an update on the latest knowledge about their roles in endothelial damage and vascular repair mechanisms. Among all sirtuins, in the light of the numerous functions reported on SIRT1 in the vascular system, herein we discuss its roles not only in the control of endothelial cells (EC) functionality but also in other cell types beyond EC, including endothelial progenitor cells (EPC), smooth muscle cells (SMC), and immune cells. Furthermore, we also provide an update on the growing field of compounds under clinical evaluation for the modulation of SIRT1 which, at the state of the art, represents the most promising target for the development of novel drugs against CVD, especially when concomitant with type 2 diabetes.

1. Introduction

The structural and functional changes of the vasculature are strictly linked to vascular aging processes and other risk factors for cardiovascular diseases (CVD), such as hypercholesterolemia, hypertension, smoking, and diabetes. Specifically, the complex phenomenon of vascular dysfunction involves aging-related deterioration of the vasculature and secondary stresses, such as metabolic disorders, altered nitric oxide (NO) pathway, and increased inflammation and oxidative stress [1–5]. Particularly, the reduction of NO bioavailability caused by its diminished synthesis and/or by its augmented scavenging due to oxidative stress, along with the increased platelet aggregation, cytokines production, and adhesion molecule and chemokine expression, are the main intracellular events leading to endothelial dysfunction and vascular damage [1–5].

The epigenetic changes of histone and non-histone protein deacetylation catalyzed by sirtuins, or silent information regulator 2 (Sir2) proteins, take part to the mechanisms regulating vascular dysfunction related to aging, CVD and, most of all, vascular complications during diabetes [1,6–9]. Beyond controversy about the influence of Sir2 on lifespan extension in *Caenorhabditis elegans* and *Drosophila melanogaster* [10–12], the first evidence showing the connection between sirtuins and aging is the observations that overexpression of the sirtuin member Sir2 was able to extend lifespan in yeast, *C. elegans*, and in *D. melanogaster* [13]. Sirtuins, firstly described as modulators of energy metabolism, DNA repair, and oxidative stress responses [13] are known to exert protective effects against age-related diseases such as CVD, cancer, diabetes, and neurodegenerative diseases [13–15].

Mammals hold seven members of the sirtuin family, from SIRT1, the most extensively characterized for its role in aging, to SIRT7, all possessing a common highly conserved catalytic domain and nicotinamide adenine dinucleotide (NAD^+)-binding site. They have different subcellular localization, tissue specificity, activity, and targets [16].

Abbreviations: ACE, angiotensin converting enzyme; AICAR, 5-amino-4-imidazolecarboxamide riboside; AMPK, AMP-activated protein kinase; Ang II, angiotensin II; ApoE, apolipoprotein E; AT1R, angiotensin II type 1 receptor; ATM, ataxia telangiectasia mutated; ATP, adenosine triphosphate; CaMKKβ, Ca2+/calmodulin-dependent protein kinase β; COPD, chronic obstructive pulmonary disease; CSE, cigarette smoke extract; CVD, cardiovascular disease; DDAH2, dimethylarginine dimethylaminohydrolase; DDR, DNA damage response; EC, endothelial cells; ELAV, embryonic lethal abnormal vision; eNOs, endothelial nitric oxide; EPC, endothelial progenitor cells; FGFR 21, fibroblast growth factor 21; FOXO, forkheadbox O transcription factor; GHD, glutamate dehydrogenase; HIF, hypoxia-inducible factor; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; IGC, intensive glycemic control; IL, interleukin; KDR, kinase-domain insert containing receptor; LDL, low density lipoprotein; LKB1, serine/threonine kinase B1; MCP-1, monocyte chemoattractant protein-1; MnSOD, manganese superoxide dismutase; NAD^+, nicotinamide adenine dinucleotide; NAM, nicotinamide; NAMPT, nicotinamide phosphoribosyltransferase; NBS1, repair protein Nijmegen Breakage Syndrome-1; NF-κB, Nuclear factor-kappaB; NO, nitric oxide; PAI, plasminogen activator inhibitor; PCI, percutaneous coronary intervention; PDH, pyruvate dehydrogenase complex; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPAR-α, peroxisome proliferator-activated receptor alfa; RFX5, regulatory factor for X-box; ROS, reactive oxygen species; Sir2, silent information regulator 2; SIRT, sirtuin; SIN-1, 3-morpholinosydnonimine; SMC, smooth muscle cells; SNP, single nucleotide polymorphisms; STEMI, ST-segment elevation myocardial infarction; TIMP3, tissue inhibitor of metalloproteinase 3; TNF-α, tumor necrosis factor-α; UCP, uncoupling protein; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor

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http://dx.doi.org/10.1016/j.bbadis.2015.03.001
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SIRT1 is localized both in the nucleus and cytosol and the signal from nucleus to cytosol occurs following specific conditions [17]. SIRT2 is cytosolic but also nuclear in certain phase of the cell cycle [18]. SIRT3, SIRT4 and SIRT5 are mitochondrial [19], while SIRT6 and SIRT7 are nuclear and nucleolar, respectively [20,21]. As for the catalytic activities, SIRT1, SIRT2, SIRT3, SIRT5 SIRT6 and SIRT7 act as deacetylase enzymes using NAD^+ to cleave acetyl groups from ε-acetyl lysine residues of target proteins in a reaction that generates nicotinamide (NAM), 2′-O-acetyl-ADP-ribose, and deacetylated substrates [22] (Fig. 1).

SIRT4, as well as SIRT6, acts as a mono-ADP-ribosyltransferase, in a reaction where the ADP-ribosyl moiety of NAD^+ is transferred to a substrate protein [19,23]. It has been demonstrated that SIRT4 acts as lipoamidase that regulates the pyruvate dehydrogenase complex (PDH) and, importantly, its catalytic efficiency for lipoyl- and biotinyl–lysine modifications is superior to its deacetylation activity [24]. As for SIRT6, this deacetylase can locate at the endoplasmic reticulum and controls protein lysine fatty acylation [25]. Indeed, as revealed by crystal structure, SIRT6 possesses a large hydrophobic pocket able to accommodate long chain fatty acyl groups and efficiently remove them from lysine residues [25]. Beside the deacetylase activity, SIRT5 also shows demalonylase and desuccinylase activity [26] (Fig. 1).

Sirtuin chromatin-associated functions are also exerted through the modulation of epigenetic information by direct deacetylation of specific histone acetylation marks [27]. Among mammalian sirtuins, SIRT1 and SIRT6 are the most functionally important deacetylase of histone (H) 3 acetylated (Ac) on lysine (K) 9 (H3K9Ac). SIRT1 deacetylation of H3K9Ac and H4K16Ac is directly associated with its capacity to coordinate the formation of constitutive and facultative heterochromatin [27]. Other histone substrates of SIRT1 are H1K27Ac, H3K9Ac, H3K14Ac, H3K18Ac, H3K56Ac, H4K12Ac, and H4K6Ac. SIRT6 H3K9Ac deacetylase activity is important for modulating telomere structure and DNA repair of double-strand breaks [27].

Given the array of potentially beneficial effects of sirtuin modulation on cardiovascular health, the interest in developing specific modulators is keeping increasing [28–30]. Indeed, although the role of SIRT1 on longevity *per se* is not fully convincing because transgenic mice overexpressing SIRT1 did not show to live longer than controls, to date, the efficacy of SIRT1 in protecting mice against age-associated diseases unveils an important role in improving health span and preventing CVD [14,31]. At vascular level, given that all seven sirtuins are expressed in vascular endothelial cells (EC), SIRT1 is the only member of the sirtuin family shown to uniquely regulate EC physiology by promoting vasodilatory and regenerative functions of the vascular wall through the modulation of endothelial nitric oxide synthase (eNOS) activity, forkhead box O1 (FOXO1), p53, and angiotensin II (Ang II) type 1 receptor (AT1R) [28,32,33].

Over the recent years, in addition to SIRT1, the function of other sirtuins in vascular physiology has been investigated and some of them are likely to have roles in the normal and diseased blood vessels. Among these, recent advances have been made on the role of SIRT3, a

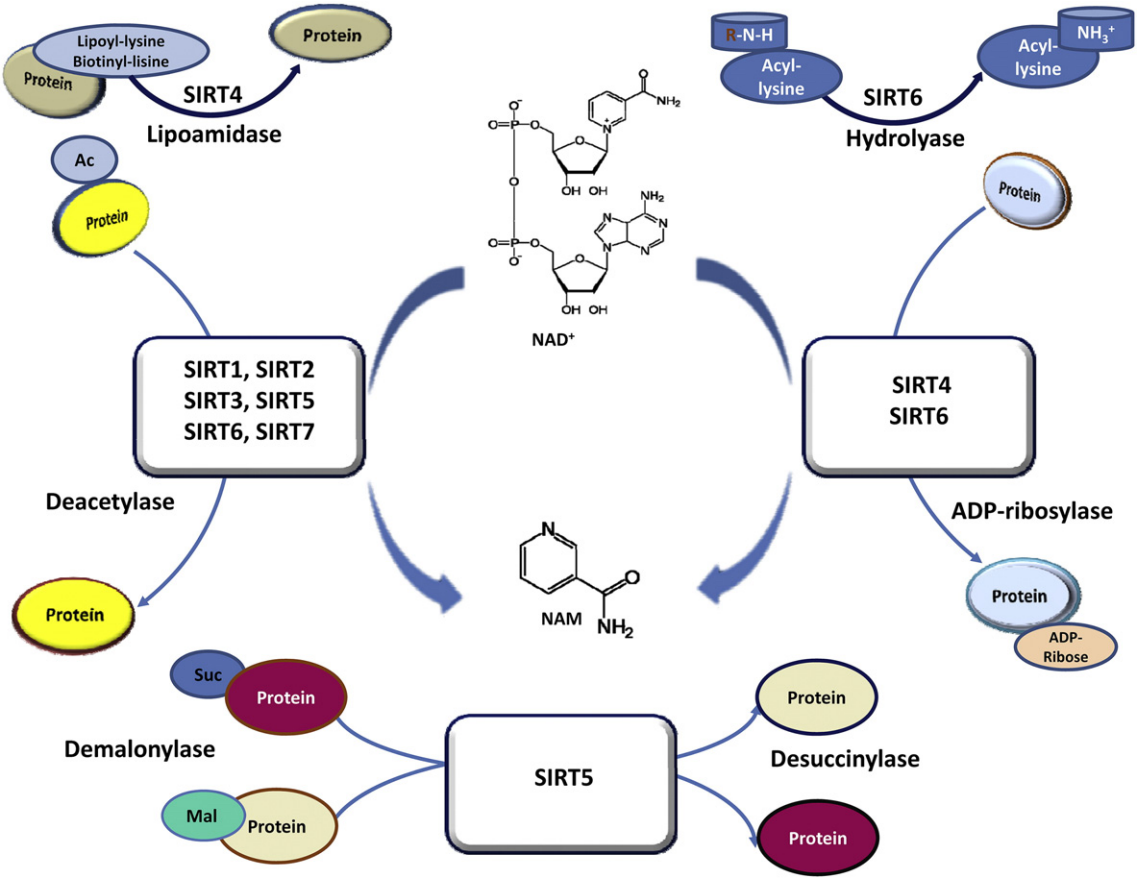

Fig. 1. Enzymatic activities of sirtuins. The seven sirtuins are categorized into four classes, class I (SIRT1, SIRT2, and SIRT3), class II (SIRT4), class III (SIRT5), and class IV (SIRT6 and SIRT7). Mammalian sirtuins have primarily two different NAD^+-consuming activities. SIRT1, SIRT2, SIRT3, SIRT5 SIRT6 and SIRT7 act as deacetylase enzymes using NAD^+ to cleave acetyl groups from ε-acetyl lysine residues of target proteins in a reaction that generates NAM and 2′-O-acetyl-ADP-ribose. SIRT4 and SIRT6 act as a mono-ADP-ribosyltransferase, in a reaction where the ADP-ribosyl moiety of NAD^+ is transferred to a substrate protein. SIRT6 has both enzymatic activities and, also, efficiently removes long chain fatty acyl groups from lysine residues. Moreover, SIRT4 acts as a cellular lipoamidase and SIRT5 acts as demalonylase and desuccinylase, by removing malonyl (Mal) or succinyl moiety (Suc) from target proteins. NAD^+, nicotinamide adenine dinucleotide; NAM, nicotinamide; ADP: adenosine diphosphate.

mitochondrial sirtuin associated with the development of metabolic syndrome and, most of all, on SIRT6, a chromatin-associated sirtuin which transmits its signals through histone deacetylation [9,23,34].

Herein, we review the current knowledge on the roles of sirtuins in vascular homeostasis and diseases, with emphasis on the molecular mechanism underlying their protective role at vascular level. Moreover, we also examine the potential clinical application of sirtuin modulators in the development of novel therapeutic strategies to treat endothelial dysfunction in CVD and vascular complications of type 2 diabetes.

2. Role of sirtuins in vascular homeostasis

2.1. SIRT1

To date, among all sirtuins, SIRT1 is the most critical modulator of the vascular function. The prominent role of SIRT1 in the regulation of vascular homeostasis and diseases is exerted through its action at multiple cellular levels. Indeed, as described below, this sirtuin is involved in the promotion of vasodilatory and regenerative functions at EC, EPC, and SMC levels, as well as in the modulation of monocyte adhesion and foam cell formation through the regulation of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) expression.

2.1.1. Endothelial cells

SIRT1, highly expressed in EC, maintains normal endothelial function and controls the angiogenic potential [28,32,33] (Figs. 2, 3). Under pathological conditions, SIRT1 shuttles between the nucleus and the cytoplasm to modulate several molecular signaling involved in the protection of EC against oxidative stress [35]. In particular, SIRT1 prevents hydrogen peroxide-induced premature senescence of EC, by deacetylating the tumor suppressor p53, and protects blood vessels from hyperglycemia-induced endothelial dysfunction through a mechanism involving the downregulation of p66Shc expression and transcriptional regulation of eNOS (Fig. 3) [36]. In human EC, the decreased expression of SIRT1 during high-glucose treatment also induces the activation of p53 by increasing its acetylation [37]. This event, in turn, accelerates EC senescence and induces functional abnormalities. On the other hand, the activity of SIRT1 has been shown to be rescued by several compounds, such as resveratrol [6,28], unacylated ghrelin [38], ginsenoside Rb1 [39], a steroid glycoside found in ginseng, and paeonol [40]. In particular, unacylated ghrelin, the most abundant form of circulating ghrelin, protects EC from ROS imbalance in hind limb ischemia subjected *ob*/*ob* mice, with a reduction of *in vivo* EC senescence *via* SIRT1-mediated p53 and H3K56Ac deacetylation [38]. The molecular basis of the protective effect of paeonol, a phenolic compound isolated from cortex Moutan (tree Peony bark), against the hydrogen peroxide-induced premature senescence in human umbilical EC is attributed to an increase in cell proliferation through the downregulation of p53 and a decrease of the hydrogen peroxide-induced upregulation of H3K14Ac and H4K16Ac [40].

Numerous studies describe the protective role of SIRT1 against endothelial senescence [41–45] and SIRT1-mediated regulation of several senescence-regulating molecules, such as eNOS and FOXOs, in response to oxidative stress (Fig. 3) [46,47]. SIRT1 mRNA and protein levels progressively decline during the development of endothelial senescence which is accelerated by oxidative stress associated with risk factors for CVD [48]. Overexpression of SIRT1 and the concomitant increased deacetylation of stress-responsive serine/threonine kinase B1 (LKB1) prevented the EC senescence *in vitro*, as well as the stress-induced senescence in mice [36]. Consistent with these findings, a mouse model of vascular senescence created by genetically ablating exon 4 of *Sirt1* in EC (Sirt1-endo$^{-/-}$) [49] showed impaired endothelium-dependent vasorelaxation, angiogenesis, and fibrosis under basal conditions.

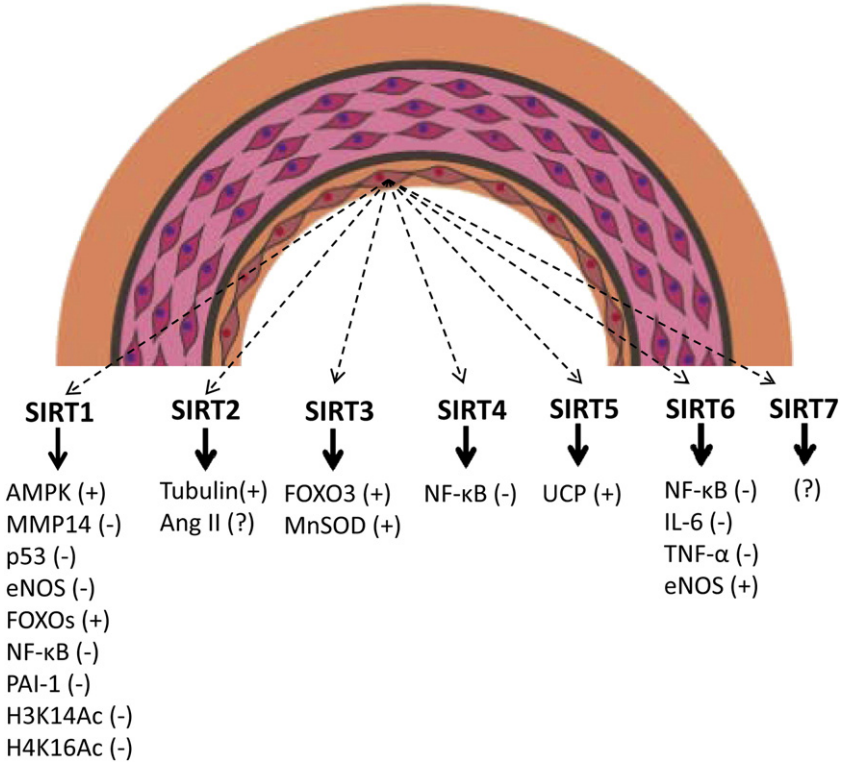

Fig. 2. Endothelial targets regulated by sirtuins. Sirtuins, by acting on specific endothelial targets, regulate several processes including inflammation (IL-6, TNF-α, NF-Kb, MMP-14), senescence (p53, eNOs), oxidative stress (MnSOD, FOXOs), cellular energy status (UCP, AMPK), cytoskeletal remodeling (Ang II), and deacetylation of histone H3K14 and H4K16. AMPK, AMP-activated protein kinase; Ang II, angiotensin II; eNOs, endothelial nitric oxide synthase; FOXO, Forkhead box O; IL-6, Interleukin 6; MMP-14, metalloproteinase14; MnSOD, manganese superoxide dismutase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TNF-α, tumor necrosis factor; PAI, plasminogen activator inhibitor, p53, tumor protein; UCP, uncoupling protein; (+), positively regulated; (−), negatively regulated; (?), unknown.

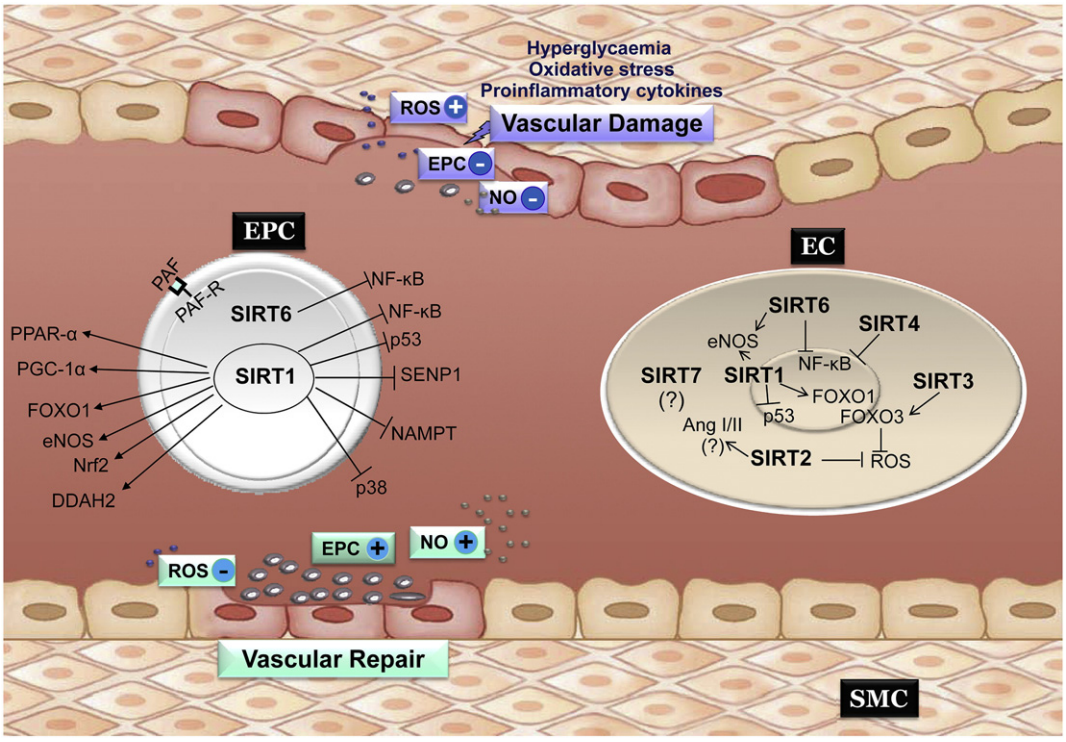

Fig. 3. Roles of sirtuins in normal and altered endothelial function. SIRT1 is involved in endothelial vascular repair by enhancing FOXO1 levels and counteracting cellular senescence through the regulation of p53 levels. SIRT1 protects EC against ROS production by decreasing the levels of H3K14Ac and H4K16Ac. Its epigenetic inhibition of PAI-1 expression exerts a protective effect against vascular endothelial senescence by decreasing the acetylation of H4K16Ac on the PAI-1 promoter region. SIRT2 and SIRT3 regulate ROS production. In addition, SIRT2 promotes Ang II-induced EC cytoskeletal remodeling. Moreover, SIRT3 acts *via* FOXO3 activation. The endothelial NO bioavailability *via* eNOS activation is controlled by both SIRT1 and SIRT6. In human pulmonary microvascular EC, SIRT4 is involved in the inflammatory response *via* a mechanism that probably entails the NF-κB pathway. In EPC, hyperglycemia, oxidative stress, and proinflammatory cytokines downregulate SIRT1 levels by affecting cell survival through the acetylation/deacetylation status of a wide range of protein targets (SENP1, p53, NAMPT, and RelA/p65). SIRT1 downregulation is responsible for high p53 acetylation and JNK activation. In EPC, hyperglycemia and oxidative stress also determine an increased biosynthesis of PAF and expression of its receptor (PAF-R). SIRT1 expression and upregulation is consistent with the DDAH2 expression in the course of EPC differentiation. Ang II, angiotensin II; EC, endothelial cells; EPC, endothelial progenitor cells; eNOs: endothelial nitric oxide synthase; DDAH2, dimethylarginine dimethylaminohydrolase; FOXO1: forkheadbox O transcription factor1; NAMPT, nicotinamide phosphoribosyltransferase; NF-κB: nuclear factor-kappaB; PAI-1;plasminogen activator inhibitor-1; PAF platelet-activating factor; PAFR, platelet-activating factor receptor; PGI2, prostaglandin I2; PGC-1α: peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPAR-α, peroxisome proliferator-activated receptor alfa; ROS, reactive oxygen species; SENP1, sentrin/SUMO-specific protease 1; VEGF: vascular endothelial growth factor; SMC, smooth muscle cells; JNK, Jun NH2-terminal kinase; (?), unknown.

In vitro studies showed that SIRT1 stimulates the EC production of NO through the deacetylation of lysine 496 and 506 of eNOS [46]. Similarly, in mice subjected to calorie restriction, the beneficial metabolic effects mediated by SIRT1 activation enhanced the deacetylation of eNOS (Fig. 3) [46]. A significant upregulation of aortic eNOS and SIRT1 expression induced by physical training has been observed in hypercholesterolemic mice following oral administration of low doses of red wine, as a source of resveratrol, a well-known SIRT1 activator [50], suggesting that modulation of SIRT1 may have implications for the prevention of atherosclerotic lesion progression. Moreover, SIRT1 levels have been found to be significantly higher in EC exposed to physiologically pulsatile flow than pathophysiologically relevant oscillatory flow [51]. Indeed, the marked increase in SIRT1 levels caused by laminar flow synergistically increased the eNOS-derived NO bioavailability in EC *via* AMP-activated protein kinase (AMPK) [51]. In response to atheroprotective pulsatile shear stress, SIRT1 is phosphorylated by Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ) at Ser-27 and Ser-47, whereas ablation of either CaMKKβ or endothelial SIRT1 increases atherosclerosis in mice [52].

The vasoprotective effect of SIRT1 overexpression has been demonstrated through an elegant *in vitro* and *in vivo* approach by Zhang et al. [53]. Overexpression of SIRT1 prevented the oxidized low density lipoproteins (oxLDL)-induced apoptosis of human umbilical EC *via* a marked increase of eNOS expression. Accordingly, endothelial-specific overexpression of SIRT1 in $ApoE^{-/-}$ mice induced eNOS and significantly blunted the high-fat diet-induced attenuation of endothelium-dependent relaxation in isolated aortic rings [53]. Most importantly, in $ApoE^{-/-}$ mice, endothelial specific overexpression of SIRT1 also attenuated the aortic plaque development in response to the high-fat diet [53]. A further evidence of the protective role played by SIRT1 against atherosclerosis comes from the observation that atherosclerotic plaques from subjects with impaired glucose metabolism are characterized by increased metalloproteases activity and decreased tissue inhibitor of metalloproteinase 3 (TIMP3) expression, which is strictly regulated by SIRT1 [54]. It has also been proposed that the endothelial protective effects of SIRT1 include factors other than eNOS-dependent NO production [55]. Indeed, no changes in endothelial function or aortic eNOS activity have been observed between hypercholesterolemic $ApoE^{-/-}$ $SIRT1^{+/-}$ and $ApoE^{-/-}$ $SIRT1^{+/+}$ mice [55].

SIRT1-mediated inhibition of plasminogen activator inhibitor-1 (PAI-1) expression exerts a protective effect against vascular endothelial senescence [56]. Indeed, SIRT1 overexpression reverses the increased PAI-1 expression in senescent EC and aortas of old mice, accompanied by improved endothelial function and reduced arterial stiffness [56]. In particular, the antisenescence effect of SIRT1-mediated inhibition of PAI-1 expression occurs through an epigenetic regulatory mechanism involving the reduction of H4K16Ac level on the PAI-1 promoter [56].

On the whole, in EC and macrophages the atheroprotective effects of SIRT1 are exerted through its anti-thrombotic and anti-inflammatory function by interfering with the NF-κB signaling pathway and by suppressing the expression of endothelial tissue factor (coagulation factor III) [57–59]. Post-transcriptional stabilization of SIRT1 by HuR, an ELAV (embryonic lethal, abnormal vision, Drosophila) family of RNA-binding proteins, represses inflammation- and -hyperglycemia-induced

E-selectin release, suggesting that increasing SIRT1 expression might represent a strategy to counteract the accelerated vascular disease in metabolic disorders [60].

The p38-SIRT1 axis has been found markedly relevant in the modulation of the cardiovascular benefits deriving from angiotensin-converting-enzyme (ACE)-inhibitors [61]. Indeed, the characterization of the downstream effector of zofenoprilat, an ACE-inhibitor, revealed that this compound reverts SIRT1 downregulation induced by Ang II *via* activation of p38, which directly bounds SIRT1 sequestering it in the cytoplasm [61]. Under conditions of energy stress, SIRT1 activity controls the expression of the ACE-2 transcript, whose enhanced expression is protective in diabetes and CVD, as it counterbalances the actions of ACE by metabolizing the vasoactive and fibrogenic peptide Ang II into angiotensin-(1–7) [62]. The epigenetic regulation of ACE-2 by SIRT1 occurs through the binding of SIRT1 to the ACE-2 promoter which is increased after treatment with the AMP mimic AICAR (5-amino-4-imidazolecarboxamide riboside) and decreased after IL-1β treatment [62].

SIRT1 expression levels also influence cerebral artery relaxation *via* endothelial-derived NO and mediate regeneration of blood vessels in ischemic neuronal tissue, in part through the deacetylation of hypoxia-induced factor (HIF) 1α and 2α [63]. However, although endogenous SIRT1 showed an important function as a stress-induced protector in a mouse model of oxygen-induced proliferative retinopathy [63], recent data indicate that overexpression of SIRT1 or treatment with small activator molecules did not provide additional protection against retinopathy in mice [64].

2.1.2. Endothelial progenitor cells

Maintenance of EC functionality for vascular repair and angiogenesis is vital to the control of CVD and vascular complications of type 1 or type 2 diabetes. These processes are strictly related to the level and functionality of endothelial progenitor cells (EPC), a population of circulating progenitors displaying the capacity to repair and regenerate vascular EC [65–70]. SIRT1 deacetylation of FOXO1, a negative regulator of postnatal angiogenesis, inhibits its antiangiogenic activity in human vascular EC, as also confirmed in EC-specific SIRT1-deficient mice model after acute hind-limb ischemic injury (Fig. 3) [9,33]. EPC exposure to high-glucose reduces SIRT1 expression levels, blocks deacetylation of FOXO1 by SIRT1, and reduces eNOS phosphorylation levels (Fig. 3) [71,72]. Moreover, changes in the acetyl-FOX1 levels are related to treatment of EPC with SIRT1 inhibitor or activator such as nicotinamide and resveratrol, respectively [71,73]. These data suggest that, in EPC, SIRT1 activity is affected by high-glucose-induced oxidative stress. Consistent with these findings, *in-vitro* and *ex-vivo* studies demonstrate that the reduced EPC functionality in the presence of hyperglycemia or inflammatory stimuli relates to lower SIRT1 protein expression levels and activity [8]. In fact, levels of SIRT1 protein were found to be decreased in EPC from individuals with type 2 diabetes and, specifically, to a higher extent in patients with poor glycemic control than in those with good glycemic control [8]. Moreover, a randomized, prospective, open label study on 194 patients with ST-elevation myocardial infarction (STEMI) undergoing percutaneous coronary intervention (PCI) showed that the ECP number, differentiation capability, and SIRT1 protein level are affected by peri-procedural intensive glycemic control [74]. Among these patients, 88 normoglycemic patients (glucose < 140 mg/dl) served as the control group and the hyperglycemic patients (glucose ≥ 140 mg/dl) were randomized to intensive glycemic control (IGC) for almost 24 h after PCI (n = 54; 80–140 mg/dl) or conventional glycemic control (CGC, n = 52; 180–200 mg/dl). The EPC from hyperglycemic patients showed lower number and differentiation and lower SIRT1 levels than normoglycemic patients. Interestingly, after insulin infusion, the ICG group, which showed a mean plasma glucose during peri-procedural period lower than in CGC group, showed higher levels of EPC with higher capability to differentiate and SIRT1 expression [74]. Tight glycemic control, for at least 24 h, was associated with a doubled increase in myocardial salvage of the IGC patients compared to the CGC group, despite both the intensive and conventionally treated groups returned to their usual glucose lowering management at the end of the active phase of the study. This study, which supports the proposition that stress-hyperglycemia is associated with impaired myocardial salvage in patients on admission for acute myocardial infarction, shows the first evidence of the critical role of SIRT1 in the maintenance of EPC functionality, thus, contributing to an increased myocardial salvage [74].

The EPC senescence negatively correlates with the expression and activity of SIRT1 [75,76]. In heterozygous methylenetetrahydrofolate reductase (*Mthfr*)-deficient ($Mthfr^{+/-}$) mice the generation of ROS *via* uncoupling of eNOS leads to downregulation of SIRT1 and to increased EPC senescence [75]. MTHFR counteracts the endothelial dysfunction linked to hyperhomocysteinemia by converting homocysteine to methionine. In EPC obtained from $Mthfr^{+/-}$ mice, the main source of ROS was eNOS, as demonstrated by the reduced production of ROS following treatment with sepiapterin, an analog of BH4.

In patients with chronic obstructive pulmonary disease (COPD), the epigenetic alterations linked to cigarette smoke-oxidative stress are associated with a reduced SIRT1 expression in EPC, *via* the ataxia telangiectasia mutated (ATM) kinase mediated DNA damage response (DDR) pathway [76]. Inhibition of SIRT1 expression in circulating EPC isolated form peripheral blood of COPD patients caused an increased senescence and also an increased acetylation of p53 at Lys-382, suggesting that the protective effect of SIRT1 against senescence in EPC may be in part mediated by deacetylation of p53 (Fig. 3) [76].

SIRT1 expression has been shown to increase with differentiation of EPC into EC and its interruption inhibits dimethylarginine dimethylaminohydrolase (DDAH)2, VEGF, and KDR expression, but shows no effect on ADMA levels [77]. Induction of EPC senescence *via* inhibiting SIRT1 is also caused by microRNA-34a (miR-34a) [78]. Overexpression of miR-34a increased the level of acetylated FOXO1, an effect mimicked in EPC following SIRT1 knockdown [78]. Shear stress, a mechanical force generated by blood flow, has been recognized as an important modulator of EPC differentiation. Specifically, EPC differentiation into EC is dependent on shear stress imposed by blood flow, known to increase SIRT1 level and activity through a PI3k/Akt–SIRT1–histone H3 acetylation pathway [79]. In particular, the shear stress significantly augmented SIRT1 expression in EPC, whereas SIRT1 siRNA diminished the expression of EC markers and increased the expression of SMC markers [79].

Post-ischaemic neovascularization has been elegantly demonstrated to be improved by intracellular NAMPT–NAD(+)–SIRT1 cascade with Notch signaling involved in the enhanced post-ischaemic neovascularization (Fig. 3) [80]. Proliferation, migration, and tube formation of cultured BM-derived EPC is suppressed by inhibition of NAMPT whereas overexpression of NAMPT induced opposite effects through a SIRT1-dependent enhancement of Notch-1 intracellular domain deacetylation [80].

2.1.3. Smooth muscle cells

Age-related loss of SIRT1 protein expression in human SMC correlates with a loss of capacity for vascular repair, diminished stress response, and increased senescence [81]. In SMC, SIRT1 acts as a modulator of neointima formation and protects against DNA damage, medial degeneration, atherosclerosis, and hypertension (Fig. 4) [82–84]. As demonstrated by Li et al. SIRT1 expression decreases in the process of neointima formation and its overexpression in SMC-specific human SIRT1 transgenic mice inhibits neointima formation following vascular injury [82]. More in details, the reduced neointima formation is linked to the repression of activator protein-1(AP-1) activity by SIRT1 and the decreased expression of cyclin D1 and MMP-9 (Fig. 4) [82].

Pro-atherosclerotic effects of SIRT1 in SMC are linked to its reduced expression due to oxidant stress and LDL [83]. As a consequence, the inhibition of DNA repair, through the defective deacetylation and

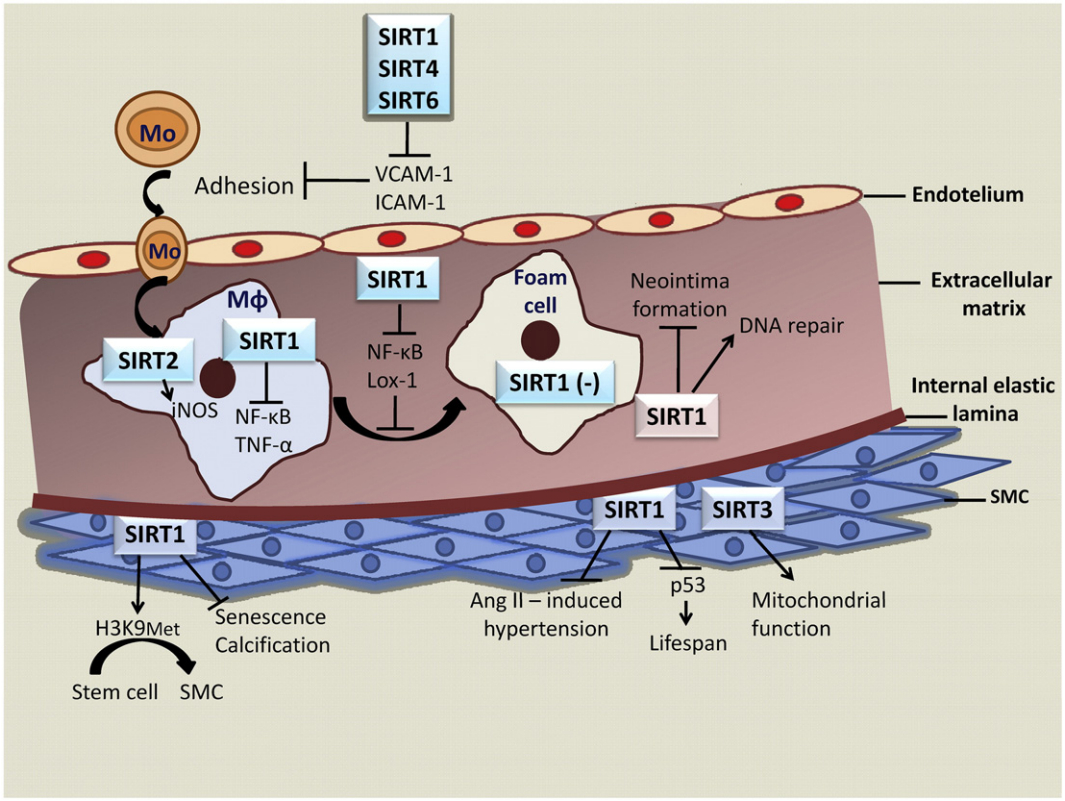

Fig. 4. Role of sirtuins in SMC and immune cells. Section of the blood vessel showing the intima (EC layer, extracellular matrix, and internal elastic lamina) and the SMC layer. SIRT1 takes part to the mechanisms regulating SMC differentiation, proliferation, and neointima formation though several mechanisms including modulation of stem cell differentiation *via* enrichment of H3K9-tri-methylation around the SMC gene-promoter regions. SIRT1 overexpression inhibits Ang II-induced vascular remodeling. SIRT1-mediated p53 degradation regulates SMC lifespan *via* NAMPT. In addition, SIRT1 inhibits NF-κB/Lox-1 signaling, preventing macrophage foam cell formation. Along with SIRT4 and SIRT6, SIRT1, inhibits monocyte adhesion and foam cell formation, acting on VCAM-1/ICAM-1 expression. In macrophages, SIRT2 acts by increasing iNOS expression. Ang II, angiotensin II; EC, endothelial cells; ICAM-1, intercellular adhesion molecule 1; iNOS, inducible nitric oxide synthase; Lox-1, lectin-like oxidized low-density lipoprotein (LDL) receptor-1; Mo, monocyte; M , macrophage; NAMPT, nicotinamide phosphoribosyltransferase; NF- B: nuclear factor-kappaB; p53, tumor protein; SMC, smooth muscle cell; TNF-α, tumor necrosis factor alpha; VCAM-1, vascular cell adhesion protein 1.

activation of Nijmegen Breakage Syndrome-1 (NBS1) but not p53, contributes to the persistence of DNA damage and the promotion of SMC growth arrest, senescence, and apoptosis (Fig. 4) [83]. These pro-atherosclerotic effects may be augmented by the reduced SIRT1 expression in EC or macrophages, with increased foam cell formation.

At atherosclerotic plaque level, SIRT1 activation has been proposed as a possible means of prevention of complications associated with plaque rupture. Indeed, in SMC, SIRT1 deacetylates the regulatory factor for X-box (RFX5) and antagonizes RFX5-mediated collagen type I gene repression by IFN-γ, thus preventing major events leading plaque to become unstable and more prone to rupture [85].

In mouse aortas, SIRT1 expression is decreased by Ang II infusion-induced hypertension (Fig. 4) [84]. SIRT1 overexpression in SMC-specific SIRT1 transgenic (SV-Tg) mice prevents the increase in systolic blood pressure caused by Ang II infusion, alleviates vascular remodeling in thoracic and renal aortas, reduces the expression of transforming growth factor-β 1 and significantly inhibits ROS generation, vascular inflammation, and collagen synthesis in arterial walls [84]. SIRT1 plays also an essential role in preventing hyperphosphatemia-induced arterial calcification which can be associated with both premature and replicative senescence (Fig. 4) [86]. SMC replicative senescence is preceded by a marked decline in the expression and activity of NAMPT [87]. Indeed, overexpression of NAMPT gene into aging human SMC enhanced resistance to oxidative stress and reduced fraction of acetylated p53, along with a lengthened cell life span (Fig. 4) [87]. Importantly, SIRT1 has been identified as a transcriptional activator in the regulation of SMC gene program [88]. Specifically, during SMC differentiation from murine and human embryonic stem cells, SIRT1 is positively regulated by miR-34a and modulates the enrichment of H3K9 tri-methylation around the SMC gene-promoter regions [88].

2.1.4. Immune cells in vascular system

In vitro results on human mononuclear U937 cell line indicate that SIRT1 expression is downregulated during foam cell formation (Fig. 4) [89]. Activation of SIRT1 by SRT1720 blocks the formation of foam cells which is paralleled by the increased expression of liver X receptor (LXR)–ATP-binding cassette (ABC) A1/ABCG1/C-C chemokine receptor type 7 (CCR7) and a decreased expression of NF-κB and its targets [89]. Recently, SIRT1 has been shown to take part to the mechanism regulating monocyte adhesion to EC *via* miR-34a [90]. Endogenous miR-34a is critically involved in the flow-dependent regulation of endothelial inflammation, as its expression in EC is downregulated by atheroprotective physiological high shear stress and upregulated by atheroprone oscillatory shear stress. Overexpression of miR-34a increases the protein levels of VCAM-1 and ICAM-1, consequently promoting monocyte adhesion to EC, whereas its blockade decreases basal VCAM-1 and ICAM-1 protein expression levels. Interestingly, SIRT1 overexpression has been shown to partially prevent miR-34a-induced VCAM-1 and ICAM-1 expression on EC (Fig. 4) [90]. In macrophages, deacetylation of RelA/p65-NF-κB by SIRT1 suppresses the expression of Lox-1, a scavenger receptor for oxLDL, thereby, preventing macrophage foam cell formation (Fig. 4) [57,58]. Moreover, the diabetic vascular inflammation and macrophage infiltration in *db*/*db* mice has been shown to be ameliorated by treatment with resveratrol which reduces ICAM-1, VCAM-1 and monocyte chemoattractant protein-1

(MCP-1) expression in aortic tissue along with an increased expression of SIRT1 [91].

2.2. SIRT2

Genome-wide characterization of the gene expression profile in response to SIRT2 knockdown indicates that SIRT2-sensitive genes are involved in multiple cellular functions, such as actin binding ferrous iron transport, cell aminoacid metabolism, transmembrane receptor protein serine/threonine kinase signaling, morphogenesis, functions associated with the trans-Golgi network [92]. In particular, pharmacological inhibition of SIRT2 attenuated the hydrogen peroxide-induced EC death (Fig. 3) [92]. However, the specific gene products involved in mediating this SIRT2 effect during oxidative stress are still unknown. Moreover, pretreatment with sirtinol and SIRT2 depletion has been shown to block the Ang II-induced EC migration, which also blocked the mechanical stretch-induced changes of microtubules, suggesting an emerging role of SIRT2 in the hypertension-induced vascular remodeling (Figs. 2, 3) [93]. In macrophages, deficiency of SIRT2 ameliorates iNOS, NO expression, and ROS levels with suppression of LPS-induced activation of NF-κB, suggesting that this sirtuin can serve as promising candidate for treating LPS-induced inflammatory diseases (Fig. 4) [94].

2.3. SIRT3

SIRT3 is a key enzyme in the metabolic regulation and ROS homeostasis during vascular disease [95–97] (Fig. 2). SIRT3 increases EC survival in response to hypoxia [95]. Specifically, hypoxia elicits an increase of SIRT3 mRNA and protein expression and an increased deacetylation of FOXO3 [95]. Furthermore, manganese superoxide dismutase (MnSOD), a FOXO3-dependent protein, is upregulated in EC to facilitate ROS detoxification in response to hypoxia [95]. In addition, enhancement of SIRT3 activity by resveratrol increases MnSOD/SOD2 enzyme activity and expression by upregulation of FOXO3 binding and transcriptional activity at the MnSOD/SOD2 promoter [96]. Pretreatment with resveratrol or SIRT3 overexpression enhanced autophagy and retarded EC apoptosis under long-term oxLDL treatment, whereas SIRT3 knockdown by siRNAs dramatically increased apoptosis of oxLDL-treated EC [96]. The control of systemic levels of oxidative stress exerted by SIRT3 contributes to delaying cardiovascular risk factor development [98]. Indeed, low-density lipoprotein receptor (LDLR)/SIRT3 double-knockout (LDLR$^{-/-}$/SIRT3$^{-/-}$) mice fed on high-cholesterol diet showed no effects on advanced atherosclerotic lesions, even though levels of systemic oxidative stress were increased [98]. SIRT3 has been proposed to retard cardiovascular risk factor development by controlling systemic levels of oxidative stress, limiting expedited weight gain, and allowing rapid metabolic adaptation [98]. Finally, SIRT3 is a critical regulator of the mitochondrial function in pulmonary artery SMC. Mice lacking SIRT3 have increased acetylation and inhibition of mitochondrial enzymes and develop spontaneous pulmonary arterial hypertension (Fig. 4) [99].

2.4. SIRT4

SIRT4 has been shown to be involved in endothelial dysfunction associated with chronic obstructive pulmonary disease (COPD) [100]. In particular, the SIRT4 expression is downregulated in human pulmonary microvascular EC treated *in vitro* with cigarette smoke extract (CSE) (Figs. 2, 3) [100]. SIRT4 overexpression inhibited CSE-induced mononuclear cell adhesion to pulmonary microvascular EC, mitigating the induction of VCAM-1 and E-selectin (Fig. 4) [100]. Furthermore, SIRT4 overexpression prevented the CSE-induced NF- B activation and its downstream proinflammatory target genes, *i.e.*, interleukin (IL)-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-6 (Fig. 3), suggesting that SIRT4 protects EC exposed to CSE stress *via* a mechanism that may involve the NF-κB pathway [100].

2.5. SIRT5

Variants in SIRT5 gene has been found to be associated with the risk of carotid plaques (Fig. 2) [101]. The associations of 85 single nucleotide polymorphisms (SNP) in the 11 SIRT and UCP genes with the presence and number of carotid plaques were investigated in a group of 1018 stroke-free subjects from the Northern Manhattan Study with high-definition carotid ultrasonography and genotyping. Interestingly, a significant association between UCP5 variant rs5977238 and the risk of carotid plaque was observed, as well as an interaction between smoking, SIRT5, and UCP4 variants for a decreased risk of plaque presence and plaque number (Fig. 2) [101]. Despite several limitations of this study, exploring the impact of these genes on vascular aging and premature atherosclerosis may be helpful for detecting asymptomatic individuals at increased risk for vascular disease [101].

2.6. SIRT6

SIRT6 is a H3K9 and H3K56 deacetylase that specifically represses the activities of several transcription factors involved in aging and inflammation, including NF-κB, c-JUN, and HIF-1α [102,103]. It prevents the development of cardiac hypertrophy and heart failure, modulates glucose metabolism, inhibits EC senescence and inflammation, and represses tumor growth. SIRT6 may play a critical role in the regulation of SMC differentiation in response to the cyclic strain and associations of SIRT6 and UCP5 genes with carotid plaque may interfere with NF-κB or HIF-1α and accelerate vascular aging [102,104].

SIRT6 is highly expressed in EC where it possesses a functional role in protecting from DNA damage and telomere dysfunction [105]. In fact, SIRT6 protein silencing by siRNA causes the inhibition of EC replication and increase of EC senescence [105]. An increase of PAI-1 and ICAM-1 mRNA levels has been observed following SIRT6 depletion which also causes the lowering of eNOS expression and the EC ability to form *in vitro* vessels [105] (Figs. 2, 4). SIRT6 has been shown to be reduced in EC following treatment with hydrogen peroxide, while its overexpression partially reverses the hydrogen peroxide-induced EC dysfunction and senescence [106] (Fig. 2). To date, the molecular events through which SIRT6 exerts a protective role at vascular level, regulating the EC response to stress, ROS production, and hyperglycemia are still unclear. The involvement of SIRT6 in atherosclerotic progression of diabetic patients has been recently described [107]. Indeed, evaluation of the effect of incretin-based therapies on the phenotype of atherosclerotic carotid plaque from asymptomatic type 2 diabetic patients ($n = 52$) and non-diabetic patients ($n = 30$) undergoing carotid endarterectomy revealed a modulation SIRT6 expression levels [107]. Interestingly, diabetic plaques had more inflammation and oxidative stress, along with a lower SIRT6 expression and collagen content, whereas plaques from glucagon-like-peptide-1 (GLP-1)-treated diabetic patients presented less inflammation and oxidative stress along with increased SIRT6 expression and collagen content, thus indicating a more stable plaque phenotype [107]. *In vitro* experiments on EPC and EC showed that the loss of SIRT6 during short-term exposure to high-glucose is paralleled by the increased expression of NF- B whereas overexpression of SIRT6 in the presence of cotreatment with GLP-1 receptor agonists relates to a decreased NF- B expression [107].

It has been hypothesized that SIRT6 activity can be regulated *via* reactive nitrogen species-mediated posttranslational modification under oxidative and nitrosative stress [108]. Indeed, incubation of purified recombinant SIRT6 protein with 3-morpholinosydnonimine (SIN-1), a peroxynitrite donor that generates NO and superoxide simultaneously, increased its tyrosine nitration and decreased its intrinsic catalytic activity [108]. Interestingly, mass spectrometry analysis allowed the identification of the tyrosine 257 which is nitrated after SIN-1 treatment and mutated to phenylalanine, causing loss of SIRT6 activity [108].

2.7. SIRT7

This sirtuin has been identified as a deacetylase of H3K18Ac [109]. As for SIRT1, SIRT7 can control myocardial development and resist stress- and aging-associated myocardial dysfunction through the deacetylation of p53 and FOXO1 [110]. SIRT7 regulates HIF-1α and HIF-2α protein levels *via* direct physical interactions and its overexpression decreases HIF-1α and HIF-2α protein levels, HIF transcriptional activity, and target gene expression [111]. To date, at endothelial level, it has been only reported a reduced SIRT7 mRNA expression in EC during high-glucose exposure [112].

3. Sirtuin modulators under clinical evaluation for vascular disease therapy

In the last years, pharmacological modulation of sirtuins has been widely studied and the observation that SIRT1 activation by resveratrol shows beneficial effects in CVD increased the interest in developing more potent SIRT1 activators. *In vitro* and *in vivo* experimental models, along with a consistent number of clinical trials, have focused on the pharmacokinetics and metabolism of resveratrol and other sirtuin modulators, including SRT2183, SRT1460, SRT1720, SRT2379, SRT501, SRT2104, and SRT3025 [6,28,29,113,114] (Fig. 5). Few patents describing SIRT inhibitors have been found in 2012–2014 period [115]. Despite reports on the efficacy of SIRT1 activators/inhibitors are often contradictory, probably due to the limited standardization of the disease models and limited cross-validation of the findings, the ability of sirtuin-activating compounds to treat vascular system injuries, metabolic disorders, inflammation, wound healing, and endothelial dysfunctions is under intensive research.

As for the *in vitro* and *in vivo* experimental models, activation of SIRT1 by resveratrol has been shown to be beneficial for the regulation of oxidative stress, inflammation, cellular senescence, and endothelial dysfunction [73,116]. Resveratrol, by activating SIRT1, impairs synthesis and increases catabolism of ADMA in young and senescent EC, with a more accentuated effect in senescent DDAH2 activity [117]. Moreover, treatment with resveratrol inhibits the TNF-α reduced SIRT1 expression in EC [59] and protects EC from oxLDL-induced oxidative damage [118]. Activation of SIRT1 by the compound BTM-0512, a resveratrol derivative, showed a beneficial role on high glucose-induced EC dysfunction [119]. In mice model, the compound SRT1720, a potent SIRT1 activator [6], reduces glucose levels and liver triglyceride content and recover mitochondrial functions after acute oxidant injury [120–122]. Furthermore, an extension in lifespan and an improved general health has been observed in mice fed on standard diet with SRT1720 supplementation [123]. Among the sirtuin inhibitors, the sirtinol and its analog *m*- and *p*-sirtinol, which act on SIRT1 and SIRT2, show a protective role against inflammation in microvascular EC [124–126]. Activation of SIRT1 by SRT3025, a novel SIRT1 activator, has been demonstrated to reduce hepatic proprotein convertase subtilisin/kexin type 9 (Pcsk9) secretion and to enhance LDL receptor expression in atherosclerosis-prone ApoE$^{-/-}$ mice, leading to a decreased plaque formation [127]. These data suggest a novel mechanism that links SIRT1 to the metabolism of LDL-cholesterol *in vitro* and *in vivo*.

Among natural compounds able to modulate SIRT1, stachydrine, a proline betaine present in considerable quantities in juices from fruits of the *Citrus* genus, inhibits the deleterious effect of high-glucose

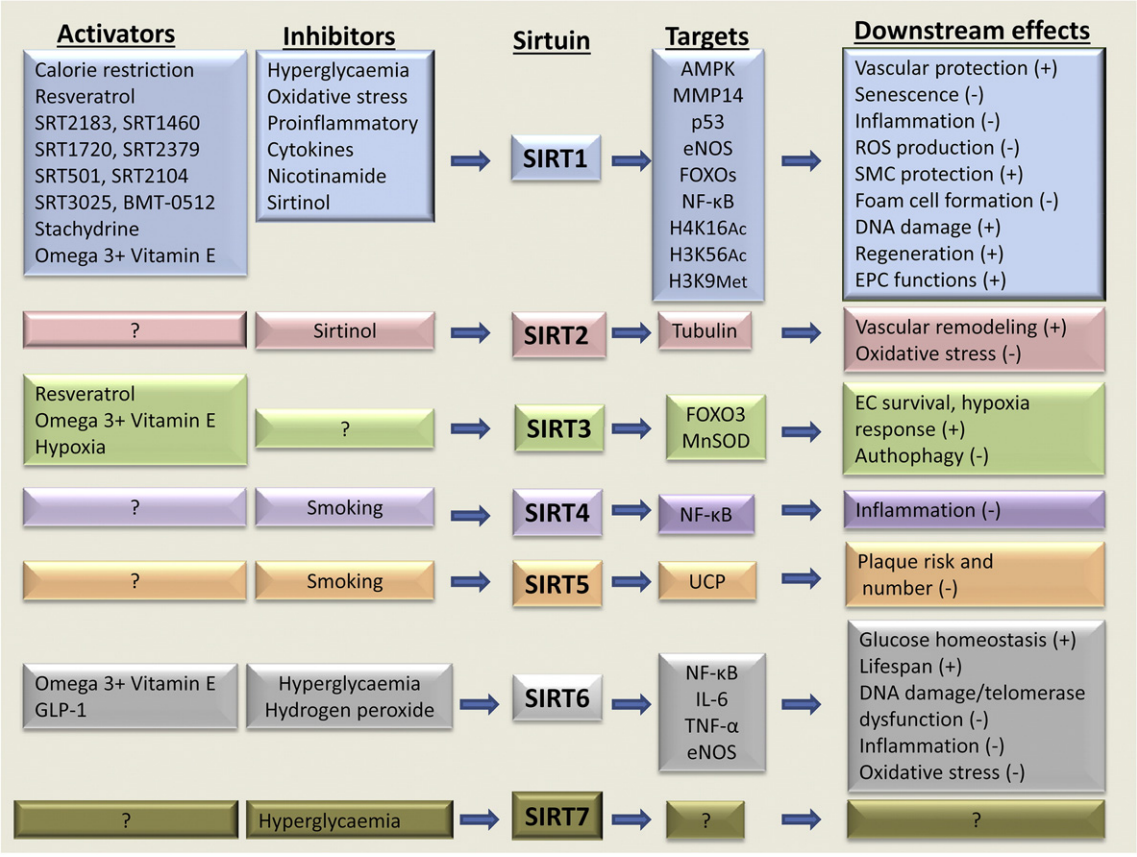

Fig. 5. Sirtuin regulation in vascular homeostasis and diseases. Overview of sirtuin activators/inhibitors that modulate different cellular targets involved in vascular homeostasis and diseases. AMPK, AMP-activated protein kinase; eNOS, endothelial nitric oxide synthase; FOXO, Forkhead box O; IL-6, Interleukin 6; MMP-14, metalloproteinase14; MnSOD, manganese superoxide dismutase; NF- B, nuclear factor-kappaB; TNF-α, tumor necrosis factor; p53, tumor protein; UCP, uncoupling proteins; (+), positively regulated; (−), negatively regulated; (?), unknown.

on EC by acting through the modulation of SIRT1 pathway [128]. In particular, stachydrine counteracts the detrimental effects of high-glucose by downregulating $p16^{INK4A}$ protein levels and by preventing the inhibition of SIRT1 activity and expression [128]. However, to date, the mechanism by which stachydrine acts on $p16^{INK4A}$ and SIRT1 expression is not fully elucidated. Multiple regulatory mechanisms are known to take part to the signal transduction pathway responsible for SIRT1 regulation in the endothelial dysfunction during altered glucose homeostasis [129]. Indeed, modulation of SIRT1 can be achieved by regulating the enzymatic activity through substrate availability or post-translational modifications and by acting at expression levels through transcription factors, RNA binding proteins, miRNAs, or ubiquitin–proteasome system [129].

As for the ongoing clinical trials aimed at evaluating the role of sirtuin modulators in the prevention or treatment of endothelial dysfunction and reduced new blood vessel growth, the current trials (http://clinicaltrials.gov/) are reported in Table 1. A randomized, completed placebo-controlled study investigates different doses of SRT3025, a novel SIRT1 activator, in normal healthy volunteers (NCT01340911). A phase I trial is evaluating the effect of oral trans-resveratrol extract from *Polygonum cuspidatum* on the SIRT1 expression in skeletal muscle of 10 patients with type 2 diabetes (NCT01677611). Another study, aimed at examining the effect of chronic administration of polyphenols contained in Red Grape Cells powder on SIRT1 mRNA expression in patients with type 2 diabetes, is currently recruiting participants (NCT01938521). Moreover, another study, still recruiting participants (NCT01668836), will evaluate SIRT1 influence in vascular reactivity, antioxidant capacity, and markers of inflammation before and after the interventions with caloric restriction or resveratrol administration.

An advanced clinical trial (Phase 4) is actually investigating the effects of omega 3 supplementation and its concurrent supplementation with vitamin E on the serum levels of antioxidant enzymes and the expressions of the PGC-1a, hTERT, FOXO1, FOXO3a, SIRT1, SIRT3, SIRT6 genes in peripheral blood mononuclear cells from patients with coronary artery disease (NCT02011906).

A clinical trial that evaluated the safety of oral SRT2104, an SIRT1 activator, and its effects on vascular dysfunction in otherwise healthy cigarette smokers and subjects with type 2 diabetes mellitus has been recently completed and results are expected (NCT01031108). This study was set up to explore the effects of SRT2104 on potential biomarkers of activity for glucose control (HbA1c, glycated albumin and fructosamine) and/or SIRT1 activation. Venkatasubramanian et al. [130] reported that in a randomized double-blind, placebo-controlled crossover trial, the treatment of twenty-four healthy cigarette smokers with SRT2104 was associated with an 11% mean reduction in serum LDL cholesterol concentrations, but without demonstrable differences in vasomotor function, endothelial function, or platelet activation assessments compared with placebo. The favorable effects on lipid profile suggested that SIRT1 activation may have a beneficial role in patients at risk of developing or with established CVD.

The safety and tolerability of SRT2104 (0.25, 0.5, 1.0, and 2.0 g/day) when administered once daily for 28 consecutive days in type 2 diabetic subjects is under Phase 2 trial evaluation (NCT00937326). Another clinical study on the pharmacokinetic profile of SRT2104 (0.03, 0.1, 0.25, 0.5, 1.0, 2.0, and 3.0 g/day) after a single dose and multiple administrations in healthy male volunteers has been completed and results are expected (NCT00933530). Moreover, three completed Phase 1 clinical studies, with results not yet posted, evaluated the bioavailability of and the pharmacokinetics of SRT2104 as a 250 mg suspension (NCT00937872), as a single 500 mg dose oral suspension (NCT00938275), or 2.0 g/day (NCT01018017) in type 2 diabetic patients and defined the intravenous pharmacokinetics of this compound. Likewise, SRT2379, another selective small-molecule activator of SIRT1, has been investigated in a Phase 1 clinical trial (NCT01018628) aimed at evaluating its effect on plasma concentrations of fibroblast growth factor 21 (FGF21) and at identifying other possible biomarkers suitable for future clinical assessment of oral SIRT1 activators for the treatment of type 2 diabetes.

4. Conclusions and perspectives

Mammalian sirtuins, critical modulators of the signaling pathways controlling age-related diseases, have emerged as promising therapeutic targets for CVD management. Sirtuins enhance stress resistance, improve the ability to counteract oxidative stress, and modulate cell metabolism and inflammatory responses, thus, offering the possibility to attenuate or prevent CVD through appropriate modulation with specific activators/inhibitors. To date, the most effective class of sirtuin activators has been reported for SIRT1, whose activation has emerged as a promising therapeutic approach to treat vascular disorders and to retard aging related CVD. However, future investigations are needed to clarify the complete mechanism of action of SIRT1 and other sirtuins at endothelial level. Indeed, many questions remain to be addressed, with particular regard to those concerning the potential role of the other sirtuins, such as SIRT6, in vascular diseases. In the light of the consistent number of the ongoing clinical trials with either SIRT1 inhibitors or activators, it is conceivable that in the next future one or more of them will enter in the clinical practice. However, the development of novel compounds, as well as more isoform-specific activators and/or inhibitors for SIRT1 should be pursued without neglecting further research to evaluate possible negative effects associated with chronic sirtuin activation.

Table 1
SIRT1 modulators under clinical evaluation. NIH ongoing clinical trials evaluating the safety of SIRT1 modulators in subjects with endothelial dysfunction in CVD and vascular complications of type 2 diabetes (http://clinicaltrials.gov/).

Compound	Sponsor	Status	Phase	Condition	NIH code
Trans-resveratrol from *Polygonum cuspidatum*	Khoo Teck Puat Hospital	Completed	1	Type 2 diabetes	NCT01677611
Resveratrol	InCor Heart Institute	Recruiting	–	Vascular system injuries, endothelial dysfunction	NCT01668836
Resveratrol	University of Aarhus	Completed	–	Metabolic syndrome, obesity	NCT01150955
Omega 3 and vitamin E supplementation	Tehran University of Medical Sciences	Enrolling by invitation	4	Coronary artery disease	NCT02011906
Polyphenols contained in red grape cells	Tel Aviv University	Recruiting	0	Type 2 diabetes	NCT01938521
SRT2104	Sirtris, GSK Company	Completed	1	Type 2 diabetes	NCT01031108
SRT2104	GlaxoSmithKline	Completed	2	Type 2 diabetes	NCT01018017
SRT2104	Sirtris, GSK Company	Completed	1	Type 2 diabetes	NCT00937872
SRT2104	Sirtris, GSK Company	Completed	2	Type 2 diabetes	NCT00937326
SRT2104	Sirtris, GSK Company	Completed	1	Type 2 diabetes	NCT00938275
SRT2104	GlaxoSmithKline	Completed	1	Type 2 diabetes	NCT00933062
SRT2104	Sirtris, GSK Company	Completed	1	Type 2 diabetes	NCT00933530
SRT2379	GlaxoSmithKline	Completed	1	Type 2 diabetes	NCT01018628
SRT3025	Sirtris, GSK Company	Completed	1	Type 2 diabetes	NCT01340911

Transparency document

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

References

[1] M. El Assar, J. Angulo, S. Vallejo, C. Peiro, C.F. Sanchez-Ferrer, L. Rodriguez-Manas, Mechanisms involved in the aging-induced vascular dysfunction, Front. Physiol. 3 (2012) 132.
[2] L.J. Ignarro, M.L. Balestrieri, C. Napoli, Nutrition, physical activity, and cardiovascular disease: an update, Cardiovasc. Res. 73 (2007) 326–340.
[3] D. Versari, E. Daghini, A. Virdis, L. Ghiadoni, S. Taddei, Endothelial dysfunction as a target for prevention of cardiovascular disease, Diabetes Care 32 (Suppl. 2) (2009) S314–S321.
[4] G.K. Kolluru, S.C. Bir, C.G. Kevil, Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing, Int. J. Vasc. Med. 2012 (2012) 918267.
[5] P.R. Evora, C.F. Baldo, A.C. Celotto, V.K. Capellini, Endothelium dysfunction classification: why is it still an open discussion? Int. J. Cardiol. 137 (2009) 175–176.
[6] J.C. Milne, P.D. Lambert, S. Schenk, D.P. Carney, J.J. Smith, D.J. Gagne, L. Jin, O. Boss, R.B. Perni, C.B. Vu, J.E. Bemis, R. Xie, J.S. Disch, P.Y. Ng, J.J. Nunes, A.V. Lynch, H. Yang, H. Galonek, K. Israelian, W. Choy, A. Iffland, S. Lavu, O. Medvedik, D.A. Sinclair, J.M. Olefsky, M.R. Jirousek, P.J. Elliott, C.H. Westphal, Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes, Nature 450 (2007) 712–716.
[7] S. Lavu, O. Boss, P.J. Elliott, P.D. Lambert, Sirtuins—novel therapeutic targets to treat age-associated diseases, Nat. Rev. Drug Discov. 7 (2008) 841–853.
[8] M.L. Balestrieri, L. Servillo, A. Esposito, N. D'Onofrio, A. Giovane, R. Casale, M. Barbieri, P. Paolisso, M.R. Rizzo, G. Paolisso, R. Marfella, Poor glycaemic control in type 2 diabetes patients reduces endothelial progenitor cell number by influencing SIRT1 signalling via platelet-activating factor receptor activation, Diabetologia 56 (2013) 162–172.
[9] M.F. Oellerich, M. Potente, FOXOs and sirtuins in vascular growth, maintenance, and aging, Circ. Res. 110 (2012) 1238–1251.
[10] M. Viswanathan, L. Guarente, Regulation of *Caenorhabditis elegans* lifespan by sir-2.1 transgenes, Nature 477 (2011) E1–E2.
[11] D.B. Lombard, S.D. Pletcher, C. Canto, J. Auwerx, Ageing: longevity hits a roadblock, Nature 477 (2011) 410–411.
[12] M. Kaeberlein, R.W. Powers 3rd, Sir2 and calorie restriction in yeast: a skeptical perspective, Ageing Res. Rev. 6 (2007) 128–140.
[13] L. Guarente, H. Franklin, Epstein lecture: sirtuins, aging, and medicine, N. Engl. J. Med. 364 (2011) 2235–2244.
[14] D. Herranz, M. Munoz-Martin, M. Canamero, F. Mulero, B. Martinez-Pastor, O. Fernandez-Capetillo, M. Serrano, Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer, Nat. Commun. 1 (2010) 3.
[15] M.C. Haigis, D.A. Sinclair, Mammalian sirtuins: biological insights and disease relevance, Annu. Rev. Pathol. 5 (2010) 253–295.
[16] R.A. Frye, Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins, Biochem. Biophys. Res. Commun. 273 (2000) 793–798.
[17] M. Tanno, J. Sakamoto, T. Miura, K. Shimamoto, Y. Horio, Nucleocytoplasmic shuttling of the NAD$^+$-dependent histone deacetylase SIRT1, J. Biol. Chem. 282 (2007) 6823–6832.
[18] A. Vaquero, M.B. Scher, D.H. Lee, A. Sutton, H.L. Cheng, F.W. Alt, L. Serrano, R. Sternglanz, D. Reinberg, SirT2 is a histone deacetylase with preference for histone H4 Lys 16 during mitosis, Genes Dev. 20 (2006) 1256–1261.
[19] J.Y. Huang, M.D. Hirschey, T. Shimazu, L. Ho, E. Verdin, Mitochondrial sirtuins, Biochim. Biophys. Acta 1804 (2010) 1645–1651.
[20] G. Liszt, E. Ford, M. Kurtev, L. Guarente, Mouse Sir2 homolog SIRT6 is a nuclear ADP-ribosyltransferase, J. Biol. Chem. 280 (2005) 21313–21320.
[21] E. Ford, R. Voit, G. Liszt, C. Magin, I. Grummt, L. Guarente, Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription, Genes Dev. 20 (2006) 1075–1080.
[22] D. Bordo, Structure and evolution of human sirtuins, Curr. Drug Targets 14 (2013) 662–665.
[23] S. Kugel, R. Mostoslavsky, Chromatin and beyond: the multitasking roles for SIRT6, Trends Biochem. Sci. 39 (2014) 72–81.
[24] R.A. Mathias, T.M. Greco, A. Oberstein, H.G. Budayeva, R. Chakrabarti, E.A. Rowland, Y. Kang, T. Shenk, I.M. Cristea, Sirtuin 4 is a lipoamidase regulating pyruvate dehydrogenase complex activity, Cell 1597 (2014) 1615–1625.
[25] H. Jiang, S. Khan, Y. Wang, G. Charron, B. He, C. Sebastian, J. Du, R. Kim, E. Ge, R. Mostoslavsky, H.C. Hang, Q. Hao, H. Lin, SIRT6 regulates TNF-α secretion through hydrolysis of long-chain fatty acyl lysine, Nature 496 (2013) 110–113.
[26] J. Du, Y. Zhou, X. Su, J.J. Yu, S. Khan, H. Jiang, J. Kim, J. Woo, J.H. Kim, B.H. Choi, B. He, W. Chen, S. Zhang, R.A. Cerione, J. Auwerx, Q. Hao, H. Lin, Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase, Science 334 (2011) 806–809.
[27] P. Martínez-Redondo, A. Vaquero, The diversity of histone versus nonhistone sirtuin substrates, Genes Cancer 4 (2013) 148–163.
[28] S. Sanchez-Fidalgo, I. Villegas, M. Sanchez-Hidalgo, C.A. de la Lastra, Sirtuin modulators: mechanisms and potential clinical implications, Curr. Med. Chem. 19 (2012) 2414–2441.
[29] A. Camins, F.X. Sureda, F. Junyent, E. Verdaguer, J. Folch, C. Pelegri, J. Vilaplana, C. Beas-Zarate, M. Pallas, Sirtuin activators: designing molecules to extend life span, Biochim. Biophys. Acta 1799 (2010) 740–749.
[30] B.P. Hubbard, D.A. Sinclair, Small molecule SIRT1 activators for the treatment of aging and age-related diseases, Trends Pharmacol. Sci. 35 (2014) 146–154.
[31] J.A. Baur, Z. Ungvari, R.K. Minor, D.G. Le Couteur, R. de Cabo, Are sirtuins viable targets for improving healthspan and lifespan? Nat. Rev. Drug Discov. 11 (2012) 443–461.
[32] N.M. Borradaile, J.G. Pickering, NAD(+), sirtuins, and cardiovascular disease, Curr. Pharm. Des. 15 (2009) 110–117.
[33] M. Potente, L. Ghaeni, D. Baldessari, R. Mostoslavsky, L. Rossig, F. Dequiedt, J. Haendeler, M. Mione, E. Dejana, F.W. Alt, A.M. Zeiher, S. Dimmeler, SIRT1 controls endothelial angiogenic functions during vascular growth, Genes Dev. 21 (2007) 2644–2658.
[34] M.D. Hirschey, T. Shimazu, E. Jing, C.A. Grueter, A.M. Collins, B. Aouizerat, A. Stancakova, E. Goetzman, M.M. Lam, B. Schwer, R.D. Stevens, M.J. Muehlbauer, S. Kakar, N.M. Bass, J. Kuusisto, M. Laakso, F.W. Alt, C.B. Newgard, R.V. Farese Jr., C.R. Kahn, E. Verdin, SIRT3 deficiency and mitochondrial protein hyperacetylation accelerate the development of the metabolic syndrome, Mol. Cell 44 (2011) 177–190.
[35] J. Hou, Z.Z. Chong, Y.C. Shang, K. Maiese, Early apoptotic vascular signaling is determined by Sirt1 through nuclear shuttling, forkhead trafficking, bad, and mitochondrial caspase activation, Curr. Neurovasc. Res. 7 (2010) 95–112.
[36] S. Zhou, H.Z. Chen, Y.Z. Wan, Q.J. Zhang, Y.S. Wei, S. Huang, J.J. Liu, Y.B. Lu, Z.Q. Zhang, R.F. Yang, R. Zhang, H. Cai, D.P. Liu, C.C. Liang, Repression of P66Shc expression by SIRT1 contributes to the prevention of hyperglycemia-induced endothelial dysfunction, Circ. Res. 109 (2011) 639–648.
[37] M. Orimo, T. Minamino, H. Miyauchi, K. Tateno, S. Okada, J. Moriya, I. Komuro, Protective role of SIRT1 in diabetic vascular dysfunction, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 889–894.
[38] G. Togliatto, A. Trombetta, P. Dentelli, S. Gallo, A. Rosso, P. Cotogni, R. Granata, R. Falcioni, T. Delale, E. Ghigo, M.F. Brizzi, Unacylated ghrelin (UnAG) induces oxidative stress resistance in a glucose intolerance mouse model and peripheral artery disease by restoring endothelial cell miR-126 expression, Diabetes 64 (2015) 1370–1382.
[39] Z. Song, Y. Liu, B. Hao, S. Yu, H. Zhang, D. Liu, B. Zhou, L. Wu, M. Wang, Z. Xiong, C. Wu, J. Zhu, X. Qian, Ginsenoside Rb1 prevents H2O2-induced HUVEC senescence by stimulating sirtuin-1 pathway, PLoS One 9 (2014) e112699.
[40] J. Jamal, M.R. Mustafa, P.F. Wong, Paeonol protects against premature senescence in endothelial cells by modulating Sirtuin 1 pathway, J. Ethnopharmacol. 154 (2014) 428–436.
[41] H. Ota, M. Akishita, M. Eto, K. Iijima, M. Kaneki, Y. Ouchi, Sirt1 modulates premature senescence-like phenotype in human endothelial cells, J. Mol. Cell. Cardiol. 43 (2007) 571–579.
[42] H. Ota, M. Eto, S. Ogawa, K. Iijima, M. Akishita, Y. Ouchi, SIRT1/eNOS axis as a potential target against vascular senescence, dysfunction and atherosclerosis, J. Atheroscler. Thromb. 17 (2010) 431–435.
[43] H. Ota, M. Akishita, H. Tani, T. Tatefuji, S. Ogawa, K. Iijima, M. Eto, T. Shirasawa, Y. Ouchi, trans-Resveratrol in *Gnetum gnemon* protects against oxidative-stress-induced endothelial senescence, J. Nat. Prod. 76 (2013) 1242–1247.
[44] C.L. Kao, L.K. Chen, Y.L. Chang, M.C. Yung, C.C. Hsu, Y.C. Chen, W.L. Lo, S.J. Chen, H.H. Ku, S.J. Hwang, Resveratrol protects human endothelium from H(2)O(2)-induced oxidative stress and senescence via SirT1 activation, J. Atheroscler. Thromb. 17 (2010) 970–979.
[45] Y. Zu, L. Liu, M.Y. Lee, C. Xu, Y. Liang, R.Y. Man, P.M. Vanhoutte, Y. Wang, SIRT1 promotes proliferation and prevents senescence through targeting LKB1 in primary porcine aortic endothelial cells, Circ. Res. 106 (2010) 1384–1393.
[46] I. Mattagajasingh, C.S. Kim, A. Naqvi, T. Yamamori, T.A. Hoffman, S.B. Jung, J. DeRicco, K. Kasuno, K. Irani, SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 14855–14860.
[47] P. Storz, Forkhead homeobox type O transcription factors in the responses to oxidative stress, Antioxid. Redox Signal. 14 (2011) 593–605.
[48] G. Voghel, N. Thorin-Trescases, N. Farhat, A. Nguyen, L. Villeneuve, A.M. Mamarbachi, A. Fortier, L.P. Perrault, M. Carrier, E. Thorin, Cellular senescence in endothelial cells from atherosclerotic patients is accelerated by oxidative stress associated with cardiovascular risk factors, Mech. Ageing Dev. 128 (2007) 662–671.
[49] R. Vasko, S. Xavier, J. Chen, C.H. Lin, B. Ratliff, M. Rabadi, J. Maizel, R. Tanokuchi, F. Zhang, J. Cao, M.S. Goligorsky, Endothelial sirtuin 1 deficiency perpetrates nephrosclerosis through downregulation of matrix metalloproteinase-14: relevance to fibrosis of vascular senescence, J. Am. Soc. Nephrol. 25 (2014) 276–291.
[50] C. Napoli, M.L. Balestrieri, V. Sica, L.O. Lerman, E. Crimi, G. De Rosa, C. Schiano, L. Servillo, F.P. D'Armiento, Beneficial effects of low doses of red wine consumption on perturbed shear stress-induced atherogenesis, Heart Vessels 23 (2008) 124–133.
[51] Z. Chen, I.C. Peng, X. Cui, Y.S. Li, S. Chien, J.Y. Shyy, Shear stress, SIRT1, and vascular homeostasis, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 10268–10273.
[52] L. Wen, Z. Chen, F. Zhang, X. Cui, W. Sun, G.G. Geary, Y. Wang, D.A. Johnson, Y. Zhu, S. Chien, J.Y. Shyy, Ca2 +/calmodulin-dependent protein kinase kinase beta phosphorylation of Sirtuin 1 in endothelium is atheroprotective, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) E2420–E2427.
[53] Q.J. Zhang, Z. Wang, H.Z. Chen, S. Zhou, W. Zheng, G. Liu, Y.S. Wei, H. Cai, D.P. Liu, C.C. Liang, Endothelium-specific overexpression of class III deacetylase SIRT1 decreases atherosclerosis in apolipoprotein E-deficient mice, Cardiovasc. Res. 80 (2008) 191–199.
[54] M. Cardellini, R. Menghini, E. Martelli, V. Casagrande, A. Marino, S. Rizza, O. Porzio, A. Mauriello, A. Solini, A. Ippoliti, R. Lauro, F. Folli, M. Federici, TIMP3 is reduced in atherosclerotic plaques from subjects with type 2 diabetes and increased by SirT1, Diabetes 58 (2009) 2396–2401.

[55] S. Stein, N. Schafer, A. Breitenstein, C. Besler, S. Winnik, C. Lohmann, K. Heinrich, C.E. Brokopp, C. Handschin, U. Landmesser, F.C. Tanner, T.F. Luscher, C.M. Matter, SIRT1 reduces endothelial activation without affecting vascular function in ApoE^{-}/$^{-}$ mice, Aging (Albany NY) 2 (2010) 353–360.

[56] Y.Z. Wan, P. Gao, S. Zhou, Z.Q. Zhang, D.L. Hao, L.S. Lian, Y.J. Li, H.Z. Chen, D.P. Liu, SIRT1-mediated epigenetic downregulation of plasminogen activator inhibitor-1 prevents vascular endothelial replicative senescence, Aging Cell 13 (2014) 890–899.

[57] S. Stein, C.M. Matter, Protective roles of SIRT1 in atherosclerosis, Cell Cycle 10 (2011) 640–647.

[58] J. Xie, X. Zhang, L. Zhang, Negative regulation of inflammation by SIRT1, Pharmacol. Res. 67 (2013) 60–67.

[59] L. Yang, J. Zhang, C. Yan, J. Zhou, R. Lin, Q. Lin, W. Wang, K. Zhang, G. Yang, X. Bian, A. Zeng, SIRT1 regulates CD40 expression induced by TNF-alpha via NF-kB pathway in endothelial cells, Cell. Physiol. Biochem. 30 (2012) 1287–1298.

[60] G. Ceolotto, S.V. De Kreutzenberg, A. Cattelan, A.S. Fabricio, E. Squarcina, M. Gion, A. Semplicini, G.P. Fadini, A. Avogaro, Sirtuin 1 stabilization by HuR represses TNF-alpha- and glucose-induced E-selectin release and endothelial cell adhesiveness in vitro: relevance to human metabolic syndrome, Clin. Sci. (Lond.) 127 (2014) 449–461.

[61] F. Marampon, G.L. Gravina, L. Scarsella, C. Festuccia, F. Lovat, C. Ciccarelli, B.M. Zani, L. Polidoro, D. Grassi, G. Desideri, S. Evangelista, C. Ferri, Angiotensin-converting-enzyme inhibition counteracts angiotensin II-mediated endothelial cell dysfunction by modulating the p38/SirT1 axis, J. Hypertens. 31 (2013) 1972–1983.

[62] N.E. Clarke, N.D. Belyaev, D.W. Lambert, A.J. Turner, Epigenetic regulation of angiotensin-converting enzyme 2 (ACE2) by SIRT1 under conditions of cell energy stress, Clin. Sci. (Lond.) 126 (2014) 507–516.

[63] J. Chen, S. Michan, A.M. Juan, C.G. Hurst, C.J. Hatton, D.T. Pei, J.S. Joyal, L.P. Evans, Z. Cui, A. Stahl, P. Sapieha, D.A. Sinclair, L.E. Smith, Neuronal sirtuin1 mediates retinal vascular regeneration in oxygen-induced ischemic retinopathy, Angiogenesis 16 (2013) 985–992.

[64] S. Michan, A.M. Juan, C.G. Hurst, Z. Cui, L.P. Evans, C.J. Hatton, D.T. Pei, M. Ju, D.A. Sinclair, L.E. Smith, J. Chen, Sirtuin1 over-expression does not impact retinal vascular and neuronal degeneration in a mouse model of oxygen-induced retinopathy, PLoS One 9 (2014) e85031.

[65] C.J. Loomans, E.J. de Koning, F.J. Staal, M.B. Rookmaaker, C. Verseyden, H.C. de Boer, M.C. Verhaar, B. Braam, T.J. Rabelink, A.J. van Zonneveld, Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes, Diabetes 53 (2004) 195–199.

[66] G.P. Fadini, M. Miorin, M. Facco, S. Bonamico, I. Baesso, F. Grego, M. Menegolo, S.V. de Kreutzenberg, A. Tiengo, C. Agostini, A. Avogaro, Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus, J. Am. Coll. Cardiol. 45 (2005) 1449–1457.

[67] K.K. Hirschi, D.A. Ingram, M.C. Yoder, Assessing identity, phenotype, and fate of endothelial progenitor cells, Arterioscler. Thromb. Vasc. Biol. 28 (2008) 1584–1595.

[68] G.P. Fadini, E. Boscaro, S. de Kreutzenberg, C. Agostini, F. Seeger, S. Dimmeler, A. Zeiher, A. Tiengo, A. Avogaro, Time course and mechanisms of circulating progenitor cell reduction in the natural history of type 2 diabetes, Diabetes Care 33 (2010) 1097–1102.

[69] M.L. Balestrieri, C. Fiorito, E. Crimi, F. Felice, C. Schiano, L. Milone, A. Casamassimi, A. Giovane, V. Grimaldi, V. del Giudice, P.B. Minucci, F.P. Mancini, L. Servillo, F.P. D'Armiento, B. Farzati, C. Napoli, Effect of red wine antioxidants and minor polyphenolic constituents on endothelial progenitor cells after physical training in mice, Int. J. Cardiol. 126 (2008) 295–729.

[70] A.H. Kumar, N.M. Caplice, Clinical potential of adult vascular progenitor cells, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 1080–1087.

[71] M.L. Balestrieri, M. Rienzo, F. Felice, R. Rossiello, V. Grimaldi, L. Milone, A. Casamassimi, L. Servillo, B. Farzati, A. Giovane, C. Napoli, High glucose downregulates endothelial progenitor cell number via SIRT1, Biochim. Biophys. Acta 1784 (2008) 936–945.

[72] L.R. Zhao, Y.J. Du, L. Chen, Z.G. Liu, Y.H. Pan, J.F. Liu, B. Liu, Quercetin protects against high glucose-induced damage in bone marrow-derived endothelial progenitor cells, Int. J. Mol. Med. 34 (2014) 1025–1031.

[73] M.L. Balestrieri, C. Schiano, F. Felice, A. Casamassimi, A. Balestrieri, L. Milone, L. Servillo, C. Napoli, Effect of low doses of red wine and pure resveratrol on circulating endothelial progenitor cells, J. Biochem. 143 (2008) 179–186.

[74] R. Marfella, M.R. Rizzo, M. Siniscalchi, P. Paolisso, M. Barbieri, C. Sardu, A. Savinelli, N. Angelico, S. Del Gaudio, N. Esposito, P.F. Rambaldi, N. D'Onofrio, L. Mansi, C. Mauro, G. Paolisso, M.L. Balestrieri, Peri-procedural tight glycemic control during early percutaneous coronary intervention up-regulates endothelial progenitor cell level and differentiation during acute ST-elevation myocardial infarction: effects on myocardial salvage, Int. J. Cardiol. 168 (2013) 3954–3962.

[75] C.A. Lemarie, L. Shbat, C. Marchesi, O.J. Angulo, M.E. Deschenes, M.D. Blostein, P. Paradis, E.L. Schiffrin, Mthfr deficiency induces endothelial progenitor cell senescence via uncoupling of eNOS and downregulation of SIRT1, Am. J. Physiol. Heart Circ. Physiol. 300 (2011) H745–H753.

[76] K.E. Paschalaki, R.D. Starke, Y. Hu, N. Mercado, A. Margariti, V.G. Gorgoulis, A.M. Randi, P.J. Barnes, Dysfunction of endothelial progenitor cells from smokers and chronic obstructive pulmonary disease patients due to increased DNA damage and senescence, Stem Cells 31 (2013) 2813–2826.

[77] Q. Yuan, Y.P. Bai, R.Z. Shi, S.Y. Liu, X.M. Chen, L. Chen, Y.J. Li, C.P. Hu, Regulation of endothelial progenitor cell differentiation and function by dimethylarginine dimethylaminohydrolase 2 in an asymmetric dimethylarginine-independent manner, Cell Biol. Int. 38 (2014) 1013–1022.

[78] T. Zhao, J. Li, A.F. Chen, MicroRNA-34a induces endothelial progenitor cell senescence and impedes its angiogenesis via suppressing silent information regulator 1, Am. J. Physiol. Endocrinol. Metab. 299 (2010) E110–E116.

[79] B.B. Cheng, Z.Q. Yan, Q.P. Yao, B.R. Shen, J.Y. Wang, L.Z. Gao, Y.Q. Li, H.T. Yuan, Y.X. Qi, Z.L. Jiang, Association of SIRT1 expression with shear stress induced endothelial progenitor cell differentiation, J. Cell. Biochem. 113 (2012) 3663–3671.

[80] P. Wang, H. Du, C.C. Zhou, J. Song, X. Liu, X. Cao, J.L. Mehta, Y. Shi, D.F. Su, C.Y. Miao, Intracellular NAMPT–NAD^{+}–SIRT1 cascade improves post-ischaemic vascular repair by modulating Notch signalling in endothelial progenitors, Cardiovasc. Res. 104 (2014) 477–488.

[81] A.M. Thompson, R. Wagner, E.M. Rzucidlo, Age-related loss of SirT1 expression results in dysregulated human vascular smooth muscle cell function, Am. J. Physiol. Heart Circ. Physiol. 307 (2014) H533–H541.

[82] L. Li, H.N. Zhang, H.Z. Chen, P. Gao, L.H. Zhu, H.L. Li, X. Lv, Q.J. Zhang, R. Zhang, Z. Wang, Z.G. She, R. Zhang, Y.S. Wei, G.H. Du, D.P. Liu, C.C. Liang, SIRT1 acts as a modulator of neointima formation following vascular injury in mice, Circ. Res. 108 (2011) 1180–1189.

[83] I. Gorenne, S. Kumar, K. Gray, N. Figg, H. Yu, J. Mercer, M. Bennett, Vascular smooth muscle cell sirtuin 1 protects against DNA damage and inhibits atherosclerosis, Circulation 127 (2013) 386–396.

[84] P. Gao, T.T. Xu, J. Lu, L. Li, J. Xu, D.L. Hao, H.Z. Chen, D.P. Liu, Overexpression of SIRT1 in vascular smooth muscle cells attenuates angiotensin II-induced vascular remodeling and hypertension in mice, J. Mol. Med. 92 (2014) 347–357.

[85] J. Xia, X. Wu, Y. Yang, Y. Zhao, M. Fang, W. Xie, H. Wang, Y. Xu, SIRT1 deacetylates RFX5 and antagonizes repression of collagen type I (COL1A2) transcription in smooth muscle cells, Biochem. Biophys. Res. Commun. 428 (2012) 264–270.

[86] A. Takemura, K. Iijima, H. Ota, B.K. Son, Y. Ito, S. Ogawa, M. Eto, M. Akishita, Y. Ouchi, Sirtuin 1 retards hyperphosphatemia-induced calcification of vascular smooth muscle cells, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 2054–2062.

[87] E. van der Veer, C. Ho, C. O'Neil, N. Barbosa, R. Scott, S.P. Cregan, J.G. Pickering, Extension of human cell lifespan by nicotinamide phosphoribosyltransferase, J. Biol. Chem. 282 (2007) 10841–10845.

[88] X. Yu, L. Zhang, G. Wen, H. Zhao, L.A. Luong, Q. Chen, Y. Huang, J. Zhu, S. Ye, Q. Xu, W. Wang, Q. Xiao, Upregulated sirtuin 1 by miRNA-34a is required for smooth muscle cell differentiation from pluripotent stem cells, Cell Death Differ. (2014)http://dx.doi.org/10.1038/cdd.2014.206 ([Epub ahead of print]).

[89] H.T. Zeng, Y.C. Fu, W. Yu, J.M. Lin, L. Zhou, L. Liu, W. Wang, SIRT1 prevents atherosclerosis via liver-X-receptor and NF-κB signaling in a U937 cell model, Mol. Med. Rep. 8 (2013) 23–28.

[90] W. Fan, R. Fang, X. Wu, J. Liu, M. Feng, G. Dai, G. Chen, G. Wu, Shear-sensitive microRNA-34a modulates flow-dependent regulation of endothelial inflammation, J. Cell Sci. 128 (2015) 70–80.

[91] R. Guo, B. Liu, K. Wang, S. Zhou, W. Li, Y. Xu, Resveratrol ameliorates diabetic vascular inflammation and macrophage infiltration in db/db mice by inhibiting the NF-κB pathway, Diab. Vasc. Dis. Res. 11 (2014) 92–102.

[92] J. Liu, X. Wu, X. Wang, Y. Zhang, P. Bu, Q. Zhang, F. Jiang, Global gene expression profiling reveals functional importance of Sirt2 in endothelial cells under oxidative stress, Int. J. Mol. Sci. 14 (2013) 5633–5649.

[93] A. Hashimoto-Komatsu, T. Hirase, M. Asaka, K. Node, Angiotensin II induces microtubule reorganization mediated by a deacetylase SIRT2 in endothelial cells, Hypertens. Res. 34 (2011) 949–956.

[94] A.S. Lee, Y.J. Jung, D. Kim, T. Nguyen-Thanh, K.P. Kang, S. Lee, S.K. Park, W. Kim, SIRT2 ameliorates lipopolysaccharide-induced inflammation in macrophages, Biochem. Biophys. Res. Commun. 450 (2014) 1363–1369.

[95] A.H. Tseng, L.H. Wu, S.S. Shieh, D.L. Wang, SIRT3 interactions with FOXO3 acetylation, phosphorylation and ubiquitinylation mediate endothelial cell responses to hypoxia, Biochem. J. 464 (2014) 157–168.

[96] X. Luo, Z. Yang, S. Zheng, Y. Cao, Y. Wu, Sirt3 activation attenuated oxidized low-density lipoprotein-induced human umbilical vein endothelial cells' apoptosis by sustaining autophagy, Cell Biol. Int. (2014)http://dx.doi.org/10.1002/cbin.10291 (May 5).

[97] H. Zeng, X. He, X. Hou, L. Li, J.X. Chen, Apelin gene therapy increases myocardial vascular density and ameliorates diabetic cardiomyopathy via upregulation of sirtuin 3, Am. J. Physiol. Heart Circ. Physiol. 306 (2014) H585–H597.

[98] S. Winnik, D.S. Gaul, F. Preitner, C. Lohmann, J. Weber, M.X. Miranda, Y. Liu, L.J. van Tits, J.M. Mateos, C.E. Brokopp, J. Auwerx, B. Thorens, T.F. Luscher, C.M. Matter, Deletion of Sirt3 does not affect atherosclerosis but accelerates weight gain and impairs rapid metabolic adaptation in LDL receptor knockout mice: implications for cardiovascular risk factor development, Basic Res. Cardiol. 109 (2014) 399.

[99] R. Paulin, P. Dromparis, G. Sutendra, V. Gurtu, S. Zervopoulos, L. Bowers, A. Haromy, L. Webster, S. Provencher, S. Bonnet, E.D. Michelaki, Sirtuin 3 deficiency is associated with inhibited mitochondrial function and pulmonary arterial hypertension in rodents and humans, Cell Metab. 20 (2014) 827–839.

[100] Y. Chen, H. Wang, G. Luo, X. Dai, SIRT4 inhibits cigarette smoke extracts-induced mononuclear cell adhesion to human pulmonary microvascular endothelial cells via regulating NF-kappaB activity, Toxicol. Lett. 226 (2014) 320–327.

[101] C. Dong, D. Della-Morte, L. Wang, D. Cabral, A. Beecham, M.S. McClendon, C.C. Luca, S.H. Blanton, R.L. Sacco, T. Rundek, Association of the sirtuin and mitochondrial uncoupling protein genes with carotid plaque, PLoS One 6 (2011) e27157.

[102] R. Mostoslavsky, K.F. Chua, D.B. Lombard, W.W. Pang, M.R. Fischer, L. Gellon, P. Liu, G. Mostoslavsky, S. Franco, M.M. Murphy, K.D. Mills, P. Patel, J.T. Hsu, A.L. Hong, E. Ford, H.L. Cheng, C. Kennedy, N. Nunez, R. Bronson, D. Frendewey, W. Auerbach, D. Valenzuela, M. Karow, M.O. Hottiger, S. Hursting, J.C. Barrett, L. Guarente, R. Mulligan, B. Demple, G.D. Yancopoulos, F.W. Alt, Genomic instability and aging-like phenotype in the absence of mammalian SIRT6, Cell 124 (2006) 315–329.

[103] L. Zhong, R. Mostoslavsky, SIRT6: a master epigenetic gatekeeper of glucose metabolism, Transcription 1 (2010) 17–21.

[104] Q.P. Yao, P. Zhang, Y.X. Qi, S.G. Chen, B.R. Shen, Y. Han, Z.Q. Yan, Z.L. Jiang, The role of SIRT6 in the differentiation of vascular smooth muscle cells in response to cyclic strain, Int. J. Biochem. Cell Biol. 49 (2014) 98–104.
[105] A. Cardus, A.K. Uryga, G. Walters, J.D. Erusalimsky, SIRT6 protects human endothelial cells from DNA damage, telomere dysfunction, and senescence, Cardiovasc. Res. 97 (2013) 571–579.
[106] R. Liu, H. Liu, Y. Ha, R.G. Tilton, W. Zhang, Oxidative stress induces endothelial cell senescence via downregulation of Sirt6, Biomed. Res. Int. 2014 (2014) 902842.
[107] M.L. Balestrieri, M.R. Rizzo, M. Barbieri, P. Paolisso, N. D'Onofrio, A. Giovane, M. Siniscalchi, F. Minicucci, C. Sardu, D. D'Andrea, C. Mauro, F. Ferraraccio, L. Servillo, F. Chirico, P. Caiazzo, G. Paolisso, R. Marfella, Sirtuin 6 expression and inflammatory activity in diabetic atherosclerotic plaques: effects of incretin treatment, Diabetes (2014)http://dx.doi.org/10.2337/db14-1149.
[108] S. Hu, H. Liu, Y. Ha, X. Luo, M. Motamedi, M.P. Gupta, J.X. Ma, R.G. Tilton, W. Zhang, Posttranslational modification of Sirt6 activity by peroxynitrite, Free Radic. Biol. Med. (2014)http://dx.doi.org/10.1016/j.freeradbiomed.2014.11.011 (Dec 1. pii: S0891-5849(14)01374-4).
[109] O. Vakhrusheva, C. Smolka, P. Gajawada, S. Kostin, T. Boettger, T. Kubin, T. Braun, E. Bober, Sirt7 increases stress resistance of cardiomyocytes and prevents apoptosis and inflammatory cardiomyopathy in mice, Circ. Res. 102 (2008) 703–710.
[110] N.M. Borradaile, J.G. Pickering, Nicotinamide phosphoribosyltransferase imparts human endothelial cells with extended replicative lifespan and enhanced angiogenic capacity in a high glucose environment, Aging Cell 8 (2009) 100–112.
[111] M.E. Hubbi, H. Hu, D.M. Gilkes Kshitiz, G.L. Semenza, Sirtuin-7 inhibits the activity of hypoxia-inducible factors, J. Biol. Chem. 288 (2013) 20768–20775.
[112] R. Mortuza, S. Chen, B. Feng, S. Sen, S. Chakrabarti, High glucose induced alteration of SIRTs in endothelial cells causes rapid aging in a p300 and FOXO regulated pathway, PLoS One 8 (2013) e54514.
[113] K.R. Patel, E. Scott, V.A. Brown, A.J. Gescher, W.P. Steward, K. Brown, Clinical trials of resveratrol, Ann. NY Acad. Sci. 1215 (2011) 161–169.
[114] L.B. Gano, A.J. Donato, H.M. Pasha, C.M. Jr Hearon, A.L. Sindler, D.R. Seals, The SIRT1 activator SRT1720 reverses vascular endothelial dysfunction, excessive superoxide production and inflammation with aging in mice, Am. J. Physiol. Heart Circ. Physiol. 307 (2014) H1754–H1763.
[115] P. Mellini, S. Valente, A. Mai, Sirtuin modulators: an updated patent review (2012–2014), Expert Opin. Ther. Pat. 25 (2015) 5–15.
[116] S. Chung, H. Yao, S. Caito, J.W. Hwang, G. Arunachalam, I. Rahman, Regulation of SIRT1 in cellular functions: role of polyphenols, Arch. Biochem. Biophys. 501 (2010) 79–90.
[117] F. Scalera, B. Fulge, J. Martens-Lobenhoffer, A. Heimburg, S.M. Bode-Boger, Red wine decreases asymmetric dimethylarginine via SIRT1 induction in human endothelial cells, Biochem. Biophys. Res. Commun. 390 (2009) 703–709.
[118] H. Guo, Y. Chen, L. Liao, W. Wu, Resveratrol protects HUVECs from oxidized-LDL induced oxidative damage by autophagy upregulation via the AMPK/SIRT1 pathway, Cardiovasc. Drugs Ther. 27 (2013) 189–198.
[119] Q. Yuan, L. Chen, D.X. Xiang, Y.J. Li, C.P. Hu, Effect of resveratrol derivative BTM-0512 on high glucose-induced dysfunction of endothelial cells: role of SIRT1, Can. J. Physiol. Pharmacol. 89 (2011) 713–722.
[120] J.J. Smith, R.D. Kenney, D.J. Gagne, B.P. Frushour, W. Ladd, H.L. Galonek, K. Israelian, J. Song, G. Razvadauskaite, A.V. Lynch, D.P. Carney, R.J. Johnson, S. Lavu, A. Iffland, P.J. Elliott, P.D. Lambert, K.O. Elliston, M.R. Jirousek, J.C. Milne, O. Boss, Small molecule activators of SIRT1 replicate signaling pathways triggered by calorie restriction in vivo, BMC Syst. Biol. 3 (2009) 31.
[121] J.A. Funk, S. Odejinmi, R.G. Schnellmann, SRT1720 induces mitochondrial biogenesis and rescues mitochondrial function after oxidant injury in renal proximal tubule cells, J. Pharmacol. Exp. Ther. 333 (2010) 593–601.
[122] Y. Yamazaki, I. Usui, Y. Kanatani, Y. Matsuya, K. Tsuneyama, S. Fujisaka, A. Bukhari, H. Suzuki, S. Senda, S. Imanishi, K. Hirata, M. Ishiki, R. Hayashi, M. Urakaze, H. Nemoto, M. Kobayashi, K. Tobe, Treatment with SRT1720, a SIRT1 activator, ameliorates fatty liver with reduced expression of lipogenic enzymes in MSG mice, Am. J. Physiol. Endocrinol. Metab. 297 (2009) E1179–E1186.
[123] S.J. Mitchell, A. Martin-Montalvo, E.M. Mercken, H.H. Palacios, T.M. Ward, G. Abulwerdi, R.K. Minor, G.P. Vlasuk, J.L. Ellis, D.A. Sinclair, J. Dawson, D.B. Allison, Y. Zhang, K.G. Becker, M. Bernier, R. de Cabo, The SIRT1 activator SRT1720 extends lifespan and improves health of mice fed a standard diet, Cell. Rep. 6 (2014) 836–843.
[124] C.M. Grozinger, E.D. Chao, H.E. Blackwell, D. Moazed, S.L. Schreiber, Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening, J. Biol. Chem. 276 (2001) 38837–38843.
[125] A. Mai, S. Massa, S. Lavu, R. Pezzi, S. Simeoni, R. Ragno, F.R. Mariotti, F. Chiani, G. Camilloni, D.A. Sinclair, Design, synthesis, and biological evaluation of sirtinol analogues as class III histone/protein deacetylase (Sirtuin) inhibitors, J. Med. Chem. 48 (2005) 7789–7795.
[126] A. Orecchia, C. Scarponi, F. Di Felice, E. Cesarini, S. Avitabile, A. Mai, M.L. Mauro, V. Sirri, G. Zambruno, C. Albanesi, G. Camilloni, C.M. Failla, Sirtinol treatment reduces inflammation in human dermal microvascular endothelial cells, PLoS One 6 (2011) e24307.
[127] M.X. Miranda, L.J. van Tits, C. Lohmann, T. Arsiwala, S. Winnik, A. Tailleux, S. Stein, A.P. Gomes, V. Suri, J.L. Ellis, T.A. Lutz, M.O. Hottiger, D.A. Sinclair, J. Auwerx, K. Schoonjans, B. Staels, T.F. Luscher, C.M. Matter, The Sirt1 activator SRT3025 provides atheroprotection in Apoe$^{-/-}$ mice by reducing hepatic Pcsk9 secretion and enhancing Ldlr expression, Eur. Heart J. 36 (2015) 51–59.
[128] L. Servillo, N. D'Onofrio, L. Longobardi, I. Sirangelo, A. Giovane, D. Cautela, D. Castaldo, A. Giordano, M.L. Balestrieri, Stachydrine ameliorates high-glucose induced endothelial cell senescence and SIRT1 downregulation, J. Cell. Biochem. 114 (2013) 2522–2530.
[129] J.R. Revollo, X. Li, The ways and means that fine tune Sirt1 activity, Trends Biochem. Sci. 38 (2013) 160–167.
[130] S. Venkatasubramanian, R.M. Noh, S. Daga, J.P. Langrish, N.V. Joshi, N.L. Mills, E. Hoffmann, E.W. Jacobson, G.P. Vlasuk, B.R. Waterhouse, N.N. Lang, D.E. Newby, Cardiovascular effects of a novel SIRT1 activator, SRT2104, in otherwise healthy cigarette smokers, J. Am. Heart Assoc. 2 (2013) e000042.