

NAD⁺, Sirtuins, and Cardiovascular Disease

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Abstract: Cardiovascular disease (CVD) is the most prevalent disease worldwide and there is intense interest in pharmaceutical approaches to reduce the burden of this chronic, aging-related condition. The sirtuin (SIRT) family of NAD⁺-dependent protein deacetylases and ADP-ribosyltransferases have emerged as exciting targets for CVD management that can impact the cardiovascular system both directly and indirectly, the latter by modulating whole body metabolism. SIRT1-4 regulate the activities of a variety of transcription factors, coregulators, and enzymes that improve metabolic control in adipose tissue, liver, skeletal muscle, and pancreas, particularly during obesity and aging. SIRT1 and 7 can control myocardial development and resist stress- and aging-associated myocardial dysfunction through the deacetylation of p53 and forkhead box O1 (FoxO1). By modulating the activity of endothelial nitric oxide synthase (eNOS), FoxO1, and p53, and the expression of angiotensin II type 1 receptor (AT1R), SIRT1 also promotes vasodilatory and regenerative functions in endothelial and smooth muscle cells of the vascular wall. Given the array of potentially beneficial effects of SIRT activation on cardiovascular health, interest in developing specific SIRT agonists is well-substantiated. Because SIRT activity depends on cellular NAD⁺ availability, enzymes involved in NAD⁺ biosynthesis, including nicotinamide phosphoribosyltransferase (Nampt), may also be valuable pharmaceutical targets for managing CVD. Herein we review the actions of the SIRT proteins on the cardiovascular system and consider the potential of modulating SIRT activity and NAD⁺ availability to control CVD.

Key Words: Cardiovascular disease, aging, sirtuin, SIRT1, NAD⁺, nicotinamide phosphoribosyltransferase.

1. INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide. Although generally thought to be a condition of the developed, westernized world, more than 60% of the current global burden of CVD is now carried by developing countries [1]. Given that age is the most powerful risk factor for CVD, it is not surprising that as longevity in developing nations is increasing, so is the global occurrence of CVD. Thus, targeting the root of this aging-related disease has become the focus of intense pharmaceutical research interest.

Among the most promising molecular targets for impacting aging events are the sirtuin family of NAD⁺-dependent protein deacetylases and ADP-ribosyltransferases. Extensive studies in yeast, worms, and flies have established important roles for the “silent information regulators” (Sir2, Sir-2.1, and dSir2, respectively) in controlling longevity (reviewed in [2, 3]). However, it is becoming increasingly apparent that sirtuin biology in mammals is much more complex, with the existence of 7 unique family members (SIRT1-7) having distinct tissue and subcellular distributions (reviewed in [2, 3]). In recent years, important new insights into the relationship between sirtuins and CVD have made the development of selective pharmaceuticals aimed at one or more of the SIRT proteins an attractive goal. In this review we discuss the current literature regarding the roles of SIRT proteins in

the cardiovascular system and we examine the potential of modulating SIRT activity, both directly and through the manipulation of cellular NAD⁺ levels, as a strategy for managing CVD.

2. SIRTUINS AND THE REGULATION OF METABOLISM

The mammalian sirtuins, particularly SIRT1-4, have been implicated in the control of metabolism in multiple cell types. The roles of sirtuins in metabolism have been the focus of a number of excellent recent reviews [2-7]. Here we briefly consider the “indirect” benefits of modulating sirtuin activity, i.e. improving whole body metabolism, on cardiovascular health.

SIRT1 is a ubiquitous, nuclear and cytoplasmic protein deacetylase, the activity of which is controlled by cellular NAD⁺ availability [8-11]. In white adipose tissue, SIRT1 functions to mobilize fatty acids and prevent preadipocyte differentiation. These “fat-loss” events occur *via* SIRT1-mediated repression of peroxisome proliferator activated receptor- γ (PPAR- γ) interactions with its cofactors, nuclear receptor corepressor (NCoR) and silencing mediator of retinoid and thyroid hormone receptor (SMRT) [12]. In skeletal muscle and liver, SIRT1 activates PPAR- γ coactivator-1 α (PGC-1 α) by deacetylation. In muscle, this increases fatty acid utilization and aerobic capacity [13, 14] whereas the consequence for liver is increased gluconeogenesis [15]. Additional control of hepatic gluconeogenesis by SIRT1 is conferred by deacetylation and nuclear retention of the forkhead box transcription factor, FoxO1 [16]. As would be

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expected, the SIRT1-mediated reduction in white adipose tissue mass and enhancement of skeletal muscle aerobic capacity enhance insulin sensitivity [13]. In addition however, SIRT1 has also been found to directly increase insulin action in these tissues through repression of protein tyrosine phosphatase 1B (PTP1B) expression [17], a critical negative regulator of insulin signaling. Furthermore, in the pancreas, SIRT1 enhances glucose-stimulated insulin secretion through repression of uncoupling protein 2 (Ucp2) expression [18], and protects β -cells from glucose toxicity during hyperglycemia, through deacetylation of FoxO1 [19].

Like SIRT1, SIRT2 is a ubiquitous, nuclear and cytoplasmic protein deacetylase. Originally characterized as an NAD⁺-dependent tubulin deacetylase involved in preventing chromosomal instability during mitosis [10, 20, 21], SIRT2 has recently been shown to inhibit adipocyte differentiation by regulating FoxO1 acetylation [22]. Whether SIRT2 also controls metabolism in skeletal muscle, liver or pancreas is unknown.

Taken together, the actions of SIRT1 in adipose tissue, skeletal muscle, liver, and pancreas, and the actions of SIRT2 in adipose tissue, have the capacity to counter obesity and the metabolic syndrome (abdominal obesity, hyperlipidemia, and insulin resistance) - widely prevalent conditions that are associated with cardiovascular complications including endothelial dysfunction, atherosclerosis, and cardiomyopathy (Fig. 1A). In support of this concept, recent studies in aged, obese mice treated with resveratrol, an activator of SIRT1 known to improve metabolic control [13, 23], showed improved aortic vascular reactivity and elasticity, and reduced vessel wall oxidative stress [24]. These improvements in vascular health were associated with changes in gene expression in adipose tissue, skeletal muscle, and liver consistent with improved metabolism [24].

In contrast to SIRT1 and 2, SIRT3 and 4 are predominantly mitochondrial proteins and thus are highly expressed in tissues rich in mitochondria, including brown adipose tissue, liver, heart, and brain [25, 26]. SIRT3 is an NAD⁺-dependent protein deacetylase [27] that may control adaptive thermogenesis, as it increases mitochondrial function in brown adipocytes [28]. Although SIRT3-deficient mice have normal metabolic parameters and adaptive thermogenesis under basal conditions [25], SIRT3 transcript abundance is reduced in brown adipose tissue in several strains of obese mice [28]. Further investigation thus appears to be required to determine whether there is a role for SIRT3 in controlling metabolism.

SIRT4 is highly expressed in pancreatic β -cells, in addition to the tissues noted above, and has well-defined roles in the control of amino acid [26] and glucose [29] stimulated insulin secretion. Unlike SIRT1-3, SIRT4 is an NAD⁺-dependent ADP-ribosyltransferase [26, 29]. SIRT4-mediated ADP-ribosylation represses glutamate dehydrogenase (GDH) activity [26] and may alter the activity of insulin degrading enzyme (IDE) and the ANT2/3 subunit of ATP/ADP translocase [29] to diminish insulin secretion. Whether SIRT4 expression is induced in β -cells during obesity and the metabolic syndrome is unknown. However, based on current findings, it is reasonable to speculate that modulating the activities of SIRT3 and 4 in brown adipose and pancreatic β -cells,

respectively, could improve metabolic control during obesity and aging, potentially diminishing subsequent cardiovascular complications (Fig. 1A). Further investigation *in vivo* and the development of small molecule agonists and antagonists are nonetheless still required to provide a body of evidence as potentially compelling as that which has been reported for SIRT1.

3. SIRTUINS IN THE HEART

3.1. Cardiac Development

Genetic loss-of-function mouse models have revealed important, direct roles for SIRT1 and 7 in the heart during development [30] and aging [31]. The actions of these sirtuins in the heart appear to be largely related to their functions as NAD⁺-dependent p53 deacetylases [8, 31, 32]. SIRT1 deficiency, due to either complete knockout or expression of a mutant protein in which exon 4 is deleted, resulted in early postnatal lethality, partly as a result of defects in cardiac septation [30]. It was found that p53 was hyperacetylated in response to genotoxic stress in MEFs from these animals [30], and since hyperacetylation potentiates p53 action [8, 32], it is likely that the apoptotic processes required for proper cardiac development are deregulated in these strains of SIRT1-deficient mice. However, defects in cardiac development and altered p53 activation may not be universal to all strains of SIRT1-deficient mice [33, 34]. Recent studies have also revealed that SIRT1 positively regulates autophagy [35], a process particularly important in for neonatal survival immediately prior to nursing. Disruption of autophagy was evident in the hearts of SIRT1-deficient mice and likely contributes to the postnatal lethality observed in these animals [35]. Interestingly, the subcellular localization of SIRT1 shifts from being exclusively nuclear in myoblasts and embryonic mouse heart, to being both nuclear and cytoplasmic in adult mouse heart and differentiated myocytes [36], further suggesting a role for this sirtuin in cardiac development.

Like SIRT1, SIRT7 deficiency (SIRT7^{-/-}) also results in cardiac defects as a result of p53 hyperacetylation [31]. Although originally identified as an activator of RNA polymerase I transcription [37], SIRT7 has recently been shown to deacetylate p53 *in vitro*, consistent with the hyperacetylation of p53 and increased apoptosis in the myocardium of SIRT7^{-/-} mice [31]. However, unlike most SIRT1-deficient mice, SIRT7^{-/-} mice are viable but develop cardiac hypertrophy and inflammatory cardiomyopathy between 7 and 11 months of age, resulting in shortened lifespan. Taken together, studies of SIRT1- and SIRT7-deficient mice indicate important but different roles for these sirtuins, largely *via* their NAD⁺-dependent p53 deacetylase activity. This likely reflects the importance of SIRT1 and SIRT7 activity at different stages of myocardial development and during aging (Fig. 1B).

3.2. Response to Myocardial Stress

In addition to its role in cardiac development, SIRT1 has recently been shown to participate in myocardial stress response mechanisms in adult animals. In mice, rats, and dogs, SIRT1 protein expression is induced during pressure overload, left ventricular hypertrophy, and heart failure [38-40]. Interestingly, aging, diabetes, and exposure to paraquat, all

of which are associated with myocardial oxidative stress, also substantially induce SIRT1 protein in the hearts of mice, rats and monkeys [38, 39]. Studies in embryonic fibroblasts from SIRT1-deficient mice suggest that SIRT1 promotes senescence, i.e. permanent withdrawal from the cell cycle, in response to the chronic, sublethal oxidative stress associated with replicative aging [41]. This is presumably a protective effect in order to prevent the propagation of cells with DNA damage. However, the purpose of induction of SIRT1 expression in response to stress in post-mitotic tissue, such as myocardium, is not well understood.

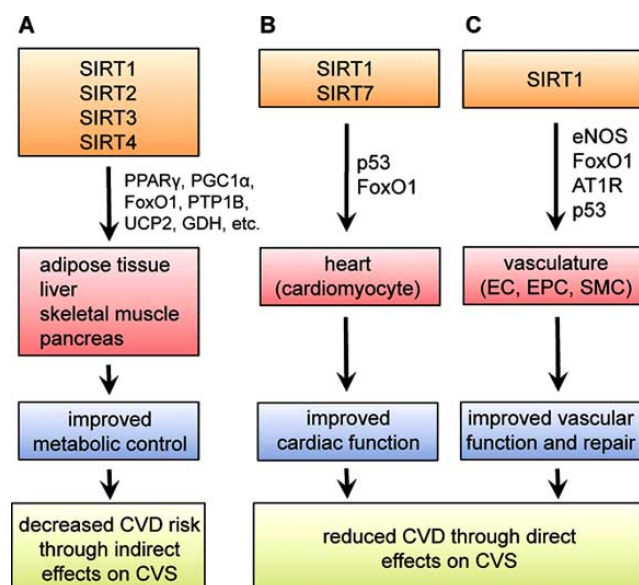


Fig. (1). Sirtuins have both indirect and direct effects on the cardiovascular system (CVS). (A) Sirtuins 1 through 4 (SIRT1-4), by altering the activities or expression of factors including, but not limited to, peroxisome proliferator activated receptor γ (PPAR γ), PPAR γ coactivator 1 α (PGC1 α), forkhead box O1 (FoxO1), protein tyrosine phosphatase 1B (PTP1B), uncoupling protein 2 (UCP2) and glutamate dehydrogenase (GDH), improve metabolic control in adipose tissue, liver, skeletal muscle, and pancreas, particularly during obesity and aging. These SIRT-mediated metabolic changes have significant potential to decrease the risk of cardiovascular disease (CVD). (B) SIRT1 and 7, through the deacetylation of p53 and FoxO1, control myocardial development and response to stress during aging, resulting in improved cardiac function and, potentially, reduced CVD. (C) SIRT1, by modulating endothelial nitric oxide synthase (eNOS), FoxO1, and p53 activity and angiotensin II type 1 receptor (AT1R) expression, promotes vasodilatory and vascular regenerative functions in endothelial cells (EC), endothelial progenitor cells (EPC), and smooth muscle cells (SMC). These changes may improve vascular function and repair.

It has been suggested that SIRT1-mediated deactivation of proapoptotic p53 and FoxO3a, and activation of FoxO1-dependent antioxidant enzyme expression, limit cardiomyocyte death in response to stress [38, 39]. However, these responses do not appear to translate to enhanced cardiomyocyte survival in scenarios of induction of endogenous SIRT1 [39] or high levels of transgenic SIRT1 expression [38]. It is possible that any benefit of upregulating SIRT1 expression during stress relies on an adequate supply of NAD⁺ to sus-

tain the imminent increase in SIRT1 activity (Fig. 2). This concept is supported by data from two independent lines of investigation. First, studies of poly(ADP-ribose) polymerase-1 (PARP) activation in cardiomyocytes during hypertrophy and heart failure implicate this NAD⁺-dependent ADP-ribosyltransferase in the depletion of cardiomyocyte NAD⁺ levels and concomitant reduction of SIRT1 activity in response to severe stress [42, 43]. Replenishing cardiomyocyte NAD⁺, either by supplementing cell culture media or by increasing endogenous synthesis by overexpressing nicotinamide phosphoribosyltransferase (Nampt) or nicotinamide mononucleotide adenylyltransferase (Nmnat) (Fig. 2), prevented the loss of SIRT1 activity resulting from PARP activation and improved cell survival [42]. Second, observations in mice and rats indicate that induction of SIRT1 expression in the heart during exercise training and fructose feeding, scenarios in which NAD⁺ is plentiful, enhances antioxidant enzyme expression and limits stress-induced hypertrophy [44, 45]. Given current interest in the development of specific SIRT agonists, further investigation is warranted to determine the extent to which pharmacological enhancement of SIRT activity is affected by cellular NAD⁺ availability.

4. SIRTUINS IN THE VASCULATURE

4.1. Endothelial Cells

Endothelial dysfunction is a hallmark of aging and aging-related cardiovascular diseases including hypertension and atherosclerosis, and is worsened in the setting of type 2 diabetes mellitus [46-49]. Recent clinical studies suggest that the development of endothelial dysfunction during aging is associated with oxidative stress in endothelial cells (EC) [50]. Oxidative damage to EC can impair vital endothelium-dependent functions, including vasodilation, vascular repair, and angiogenesis. Given that all seven sirtuins are expressed in human vascular EC [51], and that SIRT1, in particular, is important for oxidative stress responses in other cell types, it is likely that one or more SIRT proteins control several aspects of EC function and survival. To date, only SIRT1 has been shown to regulate endothelial cell physiology.

Mattagajasingh *et al.* recently identified an important, direct role for SIRT1 in controlling endothelium-dependent vasodilation [52]. *In vitro*, SIRT1 deacetylates lysines 496 and 506 of endothelial nitric oxide synthase (eNOS), thereby stimulating its activity and EC production of NO. Corresponding studies in mice subjected to caloric restriction, the beneficial metabolic effects of which are at least partially mediated by SIRT1, have confirmed enhanced deacetylation of eNOS in response to SIRT1 activation *in vivo* [52]. Recent studies in mice given low doses of red wine, the predominant source of the activator of SIRT1, resveratrol, show coordinate increases in SIRT1 and eNOS expression [53]. The combined effects of SIRT1 activation in improving metabolic control (Fig. 1A) and increasing eNOS expression and activity (Fig. 1C) suggest that both indirect and direct mechanisms could mitigate age- and type 2 diabetes-related impairments of endothelium-dependent vasodilation.

Premature senescence of EC is another likely determinant of cardiovascular disease. Oxidative stress can promote premature senescence in cultured vascular EC [54]. Also, recent studies of arterial EC isolated from patients with severe athe-

rosclerosis suggest that cellular senescence is accelerated by oxidative stress associated with CVD risk factors [55]. Senescent EC, although in permanent mitotic arrest, are neither metabolically inert nor committed to immediate death. Rather, they are characterized by decreased NO and prostacyclin production, increased PAI-1 expression, and enhanced monocyte adhesion properties [54], traits which contribute substantially to the pathogenesis of cardiovascular disease. Inhibition of SIRT1 expression and activity in human umbilical vein EC has recently been shown to induce premature senescence, characterized by increased PAI-1 expression and decreased expression and activity of eNOS [56]. Consistent with this, over-expression of SIRT1 [56, 57], activation of SIRT1 with resveratrol [57], or induction of SIRT1 expression by cilostazol (a PDE3 inhibitor) [58] prevents oxidative stress-induced premature senescence and the associated inflammatory phenotype in endothelial cells. Interestingly, cilostazol appears to induce SIRT1 expression through an eNOS-dependent pathway [58], a mechanism previously identified in white adipose tissue of mice subjected to caloric restriction [59]. In light of the SIRT1-eNOS link observed by Mattagajasingh *et al.* [52], these data raise the possibility of a vasodilatory feed-forward loop between SIRT1 and eNOS.

In addition to vasodilation, maintenance of EC capacity for vascular repair and angiogenesis during aging is increasingly recognized as vital to the control of CVD. FoxO1 and 3a are negative regulators of postnatal angiogenesis and restrain EC proliferation, migration, and neovessel formation [60-62]. Potente *et al.* recently demonstrated that NAD⁺-dependent deacetylation of FoxO1 by SIRT1 inhibits its anti-angiogenic activity in human vascular EC, and that neovascularization after acute hind-limb ischemic injury was blunted in EC-specific SIRT1-deficient mice [51]. Moreover, studies of putative circulating human endothelial progenitor cells (EPC) exposed to high glucose suggest that the resulting oxidative stress diminishes SIRT1 expression and activity, with concomitant increases in FoxO1 acetylation [63]. Thus, SIRT1 may promote vascular repair and regeneration by deacetylation pathways in both mature and progenitor EC types (Fig. 1C).

4.2. Smooth Muscle Cells

Like EC, vascular smooth muscle cells (SMC) are critical for both the control of blood pressure and the repair and remodeling of aging and diseased arteries. The roles of SMC in the initiation and progression of atherosclerosis and arterial remodeling during aging have been recently reviewed [46, 64]. However, to date and in comparison to EC, the potential roles of sirtuins in regulating SMC function, including vasoreactivity and vascular repair, remain relatively unexplored.

Miyazaki *et al.* recently identified a role for SIRT1 in the control of blood pressure *via* SMC and the renin-angiotensin system [65]. In rat aortic SMC, SIRT1 was found to inhibit expression of the angiotensin II (Ang II) type 1 receptor (AT1R), which is responsible for mediating Ang II-dependent vasoconstriction and sodium retention. *In vivo*, administration of resveratrol to mice diminished aortic AT1R expression and blunted Ang II-induced hypertension [65]. Combined with the ability of SIRT1 to activate eNOS

and enhance endothelium-dependent vasodilation [52], these observations suggest that SIRT1 plays a significant, direct role in controlling hypertension at the level of the vessel wall (Fig. 1C).

Senescent SMC accumulate in the arteries of aged animals [46] and are a recently identified characteristic of human atherosclerotic lesions [66]. In addition to their inability to contribute to lesion repair through proliferation, senescent SMC exhibit increased expression of genes that contribute to lesion instability, including adhesion molecules, PAI-1, and matrix metalloproteinases [67]. As observed for EC, oxidative stress appears to contribute to SMC senescence during atherosclerosis [66]. We recently showed that in cultured human vascular SMC, overexpression of nicotinamide phosphoribosyltransferase (Namt), the rate-limiting enzyme for NAD⁺ salvage from nicotinamide (Fig. 2), conferred resistance to acute oxidative stress, delayed senescence, and increased replicative lifespan [68]. These effects were mediated through increased activation of SIRT1, resulting in enhanced SIRT1-mediated degradation of p53 (Fig. 1C). In addition to extending replicative lifespan, we found that over-expression of Nampt promotes SMC maturation to a contractile phenotype [69], a necessity for the reacquisition of vasomotor function during the repair and stabilization of atherosclerotic lesions. This effect also appears to require SIRT activity, since sirtinol, an inhibitor of SIRT1 and 2, blocks SMC maturation [69].

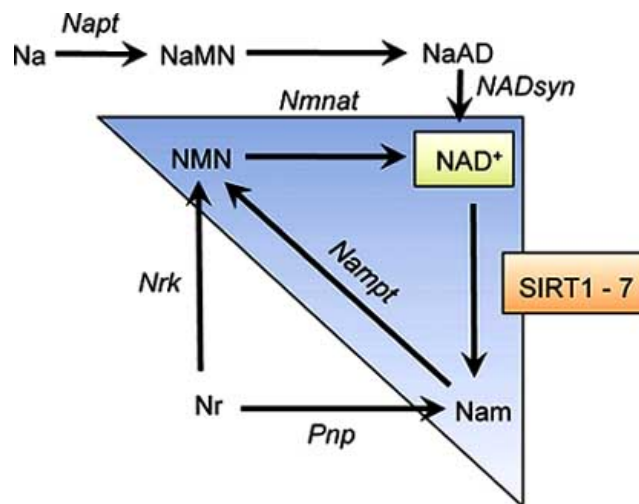


Fig. (2). Mammalian NAD⁺ biosynthesis system. Nicotinamide phosphoribosyltransferase (Namt) is the rate-limiting enzyme for mammalian NAD⁺ salvage synthesis (outlined by blue triangle) from nicotinamide (Nam). Sirtuins 1 through 7 (SIRT1-7) generate Nam from NAD⁺ as a byproduct of protein deacetylase and ADP-ribosyltransferase reactions. Pathways to NAD⁺ generation from nicotinic acid (Na) and nicotinamide riboside (Nr) are peripheral to the Nam salvage pathway, resulting in the generation of nicotinic acid mononucleotide (NaMN) by nicotinic acid phosphoribosyltransferase (Napt) and nicotinamide mononucleotide (NMN) by nicotinamide riboside kinase (NrK), respectively. Both NaMN and NMN are substrates for nicotinamide/nicotinic acid mononucleotide adenylyltransferase (Nmnat). Purine nucleoside phosphorylase (Pnp) may also contribute to NAD⁺ synthesis through the conversion of Nr to Nam. NaAD, nicotinic acid adenine dinucleotide. NADsyn, NAD⁺ synthase.

In an atherogenic vessel wall environment, vascular SMC can contribute to occlusive lesion development through both inappropriate proliferation and foam cell formation (reviewed in [64]). Over the past decade, liver X receptors (LXR) have been identified as important negative regulators of SMC proliferation, by preventing the G1 to S phase transition, and cholesterol accumulation, by promoting cholesterol efflux (reviewed in [70]). Interestingly, Li *et al.* recently showed that SIRT1 deacetylates LXR α and β , leading to receptor ubiquitination and degradation [71]. Although counterintuitive, enhanced clearance of activated nuclear receptors from promoter regions actually facilitates subsequent rounds of transcription, increasing LXR transcriptional activity. Li *et al.* further showed that, in macrophages from SIRT1^{-/-} mice, the induction of genes involved in cholesterol efflux by LXR agonists was blunted [71]. Thus, in SMC, SIRT1 activation might be expected to enhance both LXR-mediated inhibition of proliferation and LXR-mediated cholesterol efflux. The intriguing possibility that SIRT1 may prevent excessive SMC proliferation and foam cell formation, combined with the beneficial effects of SIRT1 on SMC vasoreactivity and lesion stabilization, suggests that activation of SIRT1 either by small molecule activators or through augmentation of NAD⁺ regeneration (Fig. 2), could have therapeutic potential during both the early progression and latter stages of atherosclerosis.

5. REGULATION OF SIRTUIN ACTIVITY

5.1. Small Molecule Activators

Considering the array of potential benefits of sirtuin activation on cardiovascular health (Fig. 1), interest in the development of pharmacological SIRT agonists is well-substantiated. Initial screens have identified six small molecule activators of Sir2 and its homologs [72, 73]. Interestingly, the compounds identified were all plant polyphenols. Resveratrol appears to be particularly potent in the activation of Sir2 homologs, including SIRT1 [72], and has received considerable attention for its ability to improve metabolism and overall health during obesity and aging in mice [13, 23, 24]. It is noteworthy that the vasculoprotective effects of resveratrol have long been recognized and have largely been attributed to its antioxidant and anti-inflammatory properties (recently reviewed in [74, 75]). In fact, whether the pro-survival effects of resveratrol and other polyphenolic SIRT1 activators can be attributed solely to direct sirtuin activation is controversial [76-78]. A recently proposed alternate hypothesis that could account for the effects of these compounds on mammalian health, particularly during aging, is based on the concept that sublethal exposure to stressors induces a response that confers stress resistance, i.e. "stress-response hormesis" [79]. Plant-derived foods contain a wide variety of reactive polyphenolic molecules that can both induce oxidative stress and stimulate endogenous cellular antioxidant pathways (reviewed in [80, 81]). Consistent with this, isoflavones and silibinin have been shown to induce SIRT1 expression in cardiomyocytes [82] and renal proximal tubular cells [78], an effect that has also been observed in response to sublethal oxidative stress in cardiomyocytes [38] and neural progenitor cells [83]. As suggested in sections 3. and 4.2., upregulating SIRT1 expression during cellular stress is likely to be beneficial, provided an adequate supply

of NAD⁺ is available to sustain the imminent increase in SIRT1 activity. However, regardless of whether the mechanism of sirtuin activation by polyphenols is through direct activation or stress-response hormesis, plant polyphenols may be useful pharmacologic agents for stimulating SIRT-mediated cardio- and vasculoprotective responses.

Recent structural studies of bacterial and yeast sirtuins have elucidated pockets that participate in NAD⁺ binding and catalysis [84] and nicotinamide inhibition of catalytic activity [85]. Although there is controversy regarding overlap between the NAD⁺ and nicotinamide binding sites [84-86], these studies provide insight for the development of specific small molecule regulators of sirtuin activity. In fact, a recent high-throughput screen conducted by Sirtris Pharmaceuticals Inc. identified several compounds that are structurally distinct from previously identified plant polyphenol sirtuin activators, 1000-fold more potent than resveratrol, and bind SIRT1 at an allosteric site amino-terminal to the catalytic domain [87]. Studies in rodent models of obesity and diabetes suggest that, like resveratrol, this new generation of SIRT1 activators may be useful for the treatment of type 2 diabetes [87] and stand to improve cardiovascular outcomes through both indirect and direct mechanisms (Fig. 1).

5.2. Cellular NAD⁺ Availability

The discovery that SIRT1 activity is NAD⁺-dependent [88, 89] and sensitive to the cellular [NAD⁺]/[NADH] ratio [90] led to speculation that enzymes involved in NAD⁺ biosynthesis could regulate SIRT activity. Revollo *et al.* found that an NAD⁺ biosynthetic pathway could regulate SIRT1 activity in mouse fibroblasts and established that nicotinamide phosphoribosyltransferase (Nampt), but not nicotinamide/nicotinic acid mononucleotide adenylyltransferase (Nmnat), enhances SIRT1 transcriptional regulatory activity [11] (Fig. 2). Nampt, also described as the cytokine, pre-B-cell colony enhancing factor and, more controversially, as the adipokine, visfatin, has been shown to increase resistance to oxidative stress through SIRT1 activation in human SMC (see section 4.2.) [68] and genotoxic stress in HEK293 cells through mitochondrial SIRT3 and 4 [91]. Interestingly, there may also be a role for extracellular Nampt as a systemic NAD⁺ biosynthetic enzyme in the regulation of SIRT1. Mature white adipocytes appear to secrete active Nampt through a non-classical pathway and mouse plasma contains micromolar concentrations (80 – 90 μ M) of nicotinamide mononucleotide (NMN), the product of Nampt [92] (Fig. 2). Studies of Nampt^{+/-} and β -cell-specific SIRT1-overexpressing mice have shown that plasma NMN augments glucose-stimulated insulin secretion [92], a metabolic parameter known to be linked to SIRT1 activity [18, 93]. Thus, enhancing intracellular or possibly systemic Nampt activity, or directly supplementing circulating NMN, may increase sirtuin activation. Currently, there are no known small molecule activators of Nampt. However, structural studies of Nampt, in its free form, bound to NMN, or in the presence of the specific inhibitor, FK-866, have identified the active site and revealed that Nampt is a dimeric type II phosphoribosyltransferase [94-96]. Advances in small molecule regulators may thus be forthcoming.

Recently two additional enzymes have been shown to augment cellular NAD⁺ content, providing resistance to oxidative stress and extending replicative lifespan in yeast and human cell cultures [97, 98]. Nicotinic acid phosphoribosyltransferase (Napt) and nicotinamide riboside kinase (Nrk) are peripheral to the NAD⁺ salvage synthesis pathway but augment NAD⁺ production through conversion of nicotinic acid to nicotinic acid mononucleotide (NaMN) and nicotinamide riboside to NMN, respectively (Fig. 2). Both NaMN and NMN are substrates for Nmnat. Although Nmnat activity is not rate-limiting for NAD⁺ synthesis in mammalian cells [11], studies in yeast and human cells indicate that exogenous nicotinic acid and nicotinamide riboside can substantially increase NAD⁺ content and increase SIRT1-associated functions [97, 98]. In yeast, nicotinamide riboside can also contribute to NAD⁺ synthesis through its conversion to nicotinamide by purine nucleoside phosphorylase (Pnp) [97]. Despite the existence of Pnp activity in human cells, its role in mammalian NAD⁺ metabolism is not firmly established. In any case, product inhibition of sirtuin activity through the generation of nicotinamide [99] may not be a desirable intermediate step (Fig. 2). Fortunately, recent structural studies of human Nrk have identified its active site residues, providing insight for the development of specific pharmacological agonists [100].

6. SUMMARY

There is a pressing need to better manage CVD, particularly as the population ages. Recent data have established that sirtuins play diverse roles in the cardiovascular system. This functional diversity arises through the existence of 7 mammalian SIRT1s and a growing list of molecular targets for its deacetylation and ADP ribosyltransfer reactions. Furthermore, the potential benefits of sirtuin activity on cardiovascular health lie not only in their direct actions on cells of the cardiovascular system, but in the favorable metabolic profile created through their actions on non-cardiovascular tissues. Accordingly, there is exciting potential to improve cardiovascular health by modulating SIRT activity. This could be accomplished by drugs or biologicals that activate one or more sirtuins, or one or more steps in the NAD⁺ biosynthetic pathway.

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ABBREVIATIONS

CVD	= Cardiovascular disease
EC	= Endothelial cells
eNOS	= Endothelial nitric oxide synthase
EPC	= Endothelial progenitor cells
GDH	= Glutamate dehydrogenase

Nam	= Nicotinamide
Nampt	= Nicotinamide phosphoribosyltransferase
NCoR	= Nuclear receptor corepressor
PPAR- γ	= Peroxisome proliferator activated receptor- γ
Pnp	= Purine nucleoside phosphorylase
PTP1B	= Protein tyrosine phosphatase 1B
SMRT	= Silencing mediator of retinoid and thyroid hormone receptor
SMC	= Smooth muscle cells

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