The role of sirtuins in cardiac disease

Shouji Matsushima1,2 and Junichi Sadoshima1
1Department of Cell Biology and Molecular Medicine, Cardiovascular Research Institute, Rutgers New Jersey Medical School, Newark, New Jersey; and 2Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan

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Matsushima S, Sadoshima J. The role of sirtuins in cardiac disease. Am J Physiol Heart Circ Physiol 309: H1375–H1389, 2015. First published July 31, 2015; doi:10.1152/ajpheart.00053.2015.—Modification of histones is one of the important mechanisms of epigenetics, in which genetic control is determined by factors other than an individual’s DNA sequence. Sirtuin family proteins, which are class III histone deacetylases, were originally identified as gene silencers that affect the mating type of yeast, leading to the name “silent mating-type information regulation 2” (SIR2). They are characterized by their requirement of nicotinamide adenine dinucleotide for their enzyme activity, unlike other classes of histone deacetylases. Sirtuins have been traditionally linked to longevity and the beneficial effects of calorie restriction and DNA damage repair. Recently, sirtuins have been shown to be involved in a wide range of physiological and pathological processes, including aging, energy responses to low calorie availability, and stress resistance, as well as apoptosis and inflammation. Sirtuins can also regulate mitochondrial biogenesis and circadian clocks. Seven sirtuin family proteins (Sirt1-7) have been identified as mammalian SIR2 orthologs, localized in different subcellular compartments, namely, the cytoplasm (Sirt1, 2), the mitochondria (Sirt3, 4, 5), and the nucleus (Sirt1, 2, 6, 7). Sirt1 is evolutionarily close to yeast SIR2 and has been the most intensively investigated in the cardiovascular system. Endogenous Sirt1 plays a pivotal role in mediating the cell death/survival process and has been implicated in the pathogenesis of cardiovascular disease. Downregulation of Sirt2 is protective against ischemia-reperfusion injury. Increased Sirt3 expression has been shown to correlate with longevity in humans. In addition, Sirt3 protects cardiomyocytes from aging and oxidative stress and suppresses cardiac hypertrophy. Sirt6 has also recently been demonstrated to attenuate cardiac hypertrophy, and Sirt7 is known to regulate apoptosis and stress responses in the heart. On the other hand, the roles of Sirt4 and Sirt5 in the heart remain largely uncharacterized.

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Increased Sirt3 expression has been shown to correlate with longevity in humans. In addition, Sirt3 protects cardiomyocytes from aging and oxidative stress and suppresses cardiac hypertrophy (126). Sirt6 was also recently demonstrated to attenuate cardiac hypertrophy (127), and Sirt7 regulates apoptosis and stress responses in the heart (137). The functions of Sirt4 and Sirt5, however, have been poorly investigated in the heart in vivo. In this review, we summarize the most recent findings regarding the physiological and pathological roles of sirtuins in the heart and discuss how sirtuins can be targets of treatment for cardiovascular disease.

**Regulation and Targets of Sirtuins**

Sirt1 is widely expressed in mammalian cells. Although Sirt1 was originally identified as a nuclear-localizing protein, recently, its subcellular localization has been shown to depend on cell type. In embryonic mouse hearts, Sirt1 is highly expressed in the nucleus but declines with further organogenesis. The expression level of Sirt1 in adult hearts is about 20% of that in embryonic hearts (132). Sirt3 was originally identified as a mitochondria-localizing protein. However, it is also found in the nucleus and cytoplasm (119, 126).

The expression and activity of Sirt1 are controlled by several pathophysiological stresses, transcription factors and cofactors, and post-translational modification. Several regulatory factors of Sirt1 have been identified in non-cardiac cells. Sirt1 is upregulated by E2F1, Forkhead box class O (FoxO) 1, and FoxO3 (92, 140) in mammalian cells (PC cells) and human non-small cell lung carcinoma cells (H1299 cells), whereas it is repressed by hypermethylated in cancer and COOH-terminal binding protein (16, 154) in fibroblasts. Hu antigen R stabilizes Sirt1 mRNA in HeLa cells, and several microRNAs, such as miR-34a, miR-134, miR-199a, and miR-217, regulate Sirt1 expression in neuronal cells and HeLa cells (30, 146). Cyclin B/cyclin-dependent kinase 1 and c-Jun NH2-terminal kinase 1 (JNK1) phosphorylate Sirt1, thereby enhancing its deacetylase activity in C2C12 cells and HEK293 cells (91). In addition, sumoylation represents another important regulatory mechanism. Sirt1 deacetylase activity is increased by sumoylation of Sirt1 at K734, which is repressed by sentrin-specific peptidase 1 (148), in cancer cell lines. Sumoylation enhances Sirt1 activity during ischemic preconditioning (IPC) in the mouse heart (87). Active regulator of Sirt1 and deleted in breast cancer 1 are known as positive and negative regulators in cancer cell lines, respectively (51, 155). Furthermore, some caspases suppress the activity of Sirt1 through cleavage and degradation in HeLa cells (96).

The deacetylase activity of Sirt1 is regulated by the availability of NAD+. Nicotinamide phosphoribosyltransferase (Nampt) catalyzes the transfer of a phosphoribosyl pyrophosphate to NAM to produce NAM mononucleotide, thereby serving as a rate-limiting enzyme in the mammalian NAD+ salvage pathway. Recent studies have shown that Nampt is regulated according to the circadian rhythm by clock genes, including BMAL1 and PER2, via Sirt1-mediated deacetylation, which, in turn, positively regulates Sirt1 activity in NIH3T3 cells, mouse embryonic fibroblasts (MEFs), and hepatocytes (8, 89, 113). Pressure overload, nutrient starvation, exercise, and acute IPC upregulate Sirt1 (5, 39) in the heart. Nucleocyttoplasmic shuttling may also regulate the function of Sirt1, since Sirt1 has both nuclear localization and nuclear export signals, which are regulated via phosphorylation by phosphatidylinositol 3-kinase (PI3K) in C2C12 cells (132). Sirt1 is thought to localize in both the cytoplasm and the nucleus at baseline and move to the nucleus in response to stress in the heart (132).

Only a few proteins have been demonstrated to be directly deacetylated and functionally regulated by Sirt1. These include FoxOs, p53, and poly-adenosine 5′-diphosphate (ADP) ‐ribose polymerase (PARP) 1 (5, 102, 111, 131). FoxO, a mammalian homolog of Daf16, which is involved in DNA repair, cell cycle, cell death/survival, and regulation of reactive oxygen species (ROS), is both positively and negatively regulated by Sirt1. Sirt1 deacetylates and activates FoxOs to upregulate expression of antioxidants, including Mn-superoxide dismutase (MnSOD), catalase, and thioredoxin-1 (Trx1), and anti‐apoptotic factors, like B-cell lymphoma-extra large (Bcl-XL), in cardiomyocytes (14, 20, 39). Sirt1 also activates Akt/PI3K signaling, leading to enhancement of the cardiac hypertrophic response (125).

As described above, the regulatory mechanisms and targets of Sirt1 have been well investigated. However, less is known regarding those of other sirtuin family proteins. Several studies have raised the possibility that sirtuin proteins are regulated by a common pathway and that the sirtuin proteins regulate one another. AMP-activated protein kinase (AMPK) increased expression of Sirt1, Sirt2, Sirt3, and Sirt6, whereas Sirt5 mRNA was downregulated, in mouse hepatocytes (15). Sirt1 can bind and activate the Sirt6 promoter, which may amplify the response to stress in hepatocytes (50). Recently, fatty acyl modification of TNF-α was also found to be regulated by Sirt6 in MEF cells (46). Sirt6 interacts with CLOCK/BMAL1 and governs their chromatin recruitment to circadian gene promoters in hepatocytes (75). The roles of Sirt proteins and their signaling mechanisms in the cardiovascular system are discussed below.

**Regulation of Energy Metabolism by Sirtuins**

The heart is characterized by high energy demand because its main function is to pump against blood pressure. Cardiomyocytes play a central role in contraction of the heart and contain a substantial number of mitochondria, which mediate energy production. Mitochondria are ATP-producing organelles that play a critical role in energy metabolism in the heart. Among the sirtuin family proteins, Sirt1 regulates mitochondrial function by deacetylating nuclear proteins, whereas Sirt3 does so by deacetylating mitochondrial proteins (130). The heart predominantly uses free fatty acid as a substrate for adenosine triphosphate (ATP) production under physiological conditions. In failing hearts, the preferred substrate switches from free fatty acid to glucose to produce more ATP per molecule of oxygen consumed. However, the advanced failing heart develops insulin resistance in the myocardium and undergoes a decline in glucose utilization, limiting ATP production (94).

Peroxisome proliferator-activated receptor (PPAR)-γ coactivator 1α (PGC-1α), a transcriptional coactivator of nuclear receptors, including PPAR-α and estrogen-related receptor (ERR)-α, is a master regulator of metabolism and mitochondrial biogenesis. Sirt1 directly binds to PGC-1α and deacetyl-
lates it in 293T cells (116) and PC12 cells (93). Sirt1 stimulates the ability of PGC-1α to coactivate hepatocyte nuclear factor 4α, thereby positively regulating gluconeogenic genes in response to pyruvate in hepatic cells (116). In the same cell type, Sirt1 also enhances the ability of PGC-1α to inhibit glycolytic genes in response to pyruvate (116). In PC12 cells, wild-type Sirt1, but not a Sirt1 mutant that cannot interact with PGC-1α, negatively regulates the transcriptional activity of PGC-1α (93). Thus the functional consequences of PGC-1α deacetylation appear diverse and cell type or transcription factor dependent. Furthermore, Krishnan et al. (54) demonstrated that Sirt2, but not Sirt1, affects fatty acid oxidation in adipocytes and that knockdown of PGC-1α cancels the effect of overexpression of Sirt2 upon fatty acid oxidation. Thus the availability of other family members also contributes to the net effect of sirtuins upon PGC-1α. Systemic deletion of Sirt1 in mice induces the development of dilated cardiomyopathy, which is accompanied by mitochondrial dysfunction (104). However, Sirt1 is upregulated in the failing heart and contributes to downregulation of genes involved in mitochondrial function that are regulated by ERRs (97). Thus the effect of Sirt1 upon mitochondrial function and metabolism in the heart is also complex and possibly dose dependent.

Overexpression of Sirt1 in pancreatic β-cells enhances insulin secretion in response to glucose and improves glucose metabolism by increasing ATP production via suppression of uncoupling protein-2 expression (84). Resveratrol, a Sirt1 activator, inhibits vacuolization, degeneration, and inflammation in the heart in an insulin resistance model (11). Akt is also a target of Sirt1 in insulin signaling. Akt is acetylated in various tissues, including the heart, under basal conditions, and acetylation of Akt at K14 and K20 suppresses Akt activity. Deacetylation of Akt by Sirt1 is necessary for the binding of Akt to phosphatidylinositol 3,4,5-trisphosphate (PIP3) in the heart (125). It should be noted, however, that one group reported that they were unable to detect acetylation of Akt in HEK 293T cells (112). Thus it is possible that under some experimental conditions, the effect of sirtuins upon Akt may not be through direct deacetylation. In cardiomyocytes, overexpression of Sirt1 inhibited phenylephrine-induced down-regulation of fatty acid oxidation genes by enhancing PPAR-α binding to the p65 subunit of NF-κB (105) (Fig. 1).

Sirt3 localizes in mitochondria and regulates a diverse array of mitochondrial functions, suggesting that Sirt3 is a mitochondrial fidelity protein. Although Sirt3 knockout mice show an almost normal phenotype at birth, they exhibit hyperacetylation of mitochondrial proteins (64). Target proteins of Sirt3 in mitochondria in the liver include MnSOD, NADH dehydrogenase 1α subcomplex 9, and succinate dehydrogenase complex subunit A (4, 133), suggesting that Sirt3 is closely tied to energy metabolism. Sirt3 also deacetylates and activates acetyl-CoA synthetase 2, a key enzyme in acetyl-CoA production, and long-chain acyl-CoA dehydrogenase, a key enzyme of fatty acid oxidation, in the liver (36). Knockout of Sirt3 exhibited a 50% decrease in basal ATP content in the heart (4). Together, these data indicate that Sirt3 increases energy production by activating the mitochondrial electron transport chain (ETC) and upregulating fatty acid oxidation.

Reduced Sirt2 function directly diminishes deacetylation of PGC-1α and expression of β-oxidation and mitochondrial genes in adipocytes (54). Ramakrishnan et al. (112) reported that although Sirt2 interacted with Akt and activated it in insulin-responsive cell lines, they could not detect acetylation of Akt. So far, the role of Sirt2 in mitochondrial function and energy metabolism in the heart remains unclear. Inhibition of Sirt4 increases fatty acid oxidation in liver and mitochondrial...
function in muscle. Sirt4 also inhibits glutamate dehydrogenase and impairs insulin secretion in pancreatic β-cells (32). Overexpression of Sirt5 increases oxygen consumption and ATP synthesis in human hepatocellular carcinoma cells but is not involved in mitochondrial biogenesis. Importantly, however, knockout of either Sirt4 or Sirt5 does not induce mitochondrial protein hyperacetylation (32). Sirt6 is involved in hepatic glucose production, inducing PGC-1α acetylation in a general control of amino-acid synthesis 5 (acetyltransferase)-dependent manner and suppressing hepatic glucose production (23). Sirt6 also controls circadian chromatin recruitment of sterol regulatory element-binding protein 1, resulting in the cyclic regulation of genes implicated in the metabolism of cholesterol and fatty acids in the liver (75). In the liver, Sirt7 positively regulates the protein level of nuclear receptor testosterone receptor 4(transforming growth factor-β-activated kinase 1, involved in lipid metabolism, thereby activating testicular receptor-4-target genes to increase uptake of fatty acids and synthesis/storage of triglyceride (149).

Role of Sirtuins in Cardiac Hypertrophy

In general, cardiac hypertrophy is the primary response of the heart to hemodynamic overload, such as pressure or volume overload, and is characterized by enhanced protein synthesis and sarcomeric reorganization, leading to increased cardiomyocyte size. Hypertrophic growth initially reduces wall stress, maintains cardiac function, and serves as a compensatory response to hypertrophic stimuli, but it eventually initiates the development of cardiac remodeling and heart failure. Pathological hypertrophy is recognized as a strong independent factor in heart failure. Reversible modification of the acetylation status of proteins by histone acetyltransferases and HDACs critically regulates the activity of signaling molecules mediating cardiac hypertrophy (34). Class I HDACs, including HDAC1, 2, 3, and 8, positively regulate cardiac hypertrophy, whereas class II HDACs, including HDAC4, 5, 7, and 9, negatively regulate cardiac hypertrophy (152). Class II HDACs are expressed only in nonproliferative cells, including cardiomyocytes. The export of class II HDACs from the nucleus to the cytosol in the heart directly induces transcription of nuclear factor of activated T cell and MEF2, master positive regulators of cardiac hypertrophy (9). Class II HDACs are regulated by several forms of post-translational modification, including phosphorylation, ubiquitination, and sumoylation (9, 52, 108). Recently, it was shown that Trx1 regulates the nucleocytoplasmic shuttling of HDAC4 through a redox-dependent mechanism (3). By forming a multiprotein complex with Trx1-binding protein-2 and Dnajb5, Trx1 reduces HDAC4 at C667 and C669, which are easily oxidized to form a disulfide bond in response to hypertrophic stimuli. The redox status of these cysteines plays a critical role in the localization of HDAC4, thereby regulating cardiac hypertrophy (3).

Sirtuins have been demonstrated to be involved in cardiac hypertrophy, although their effects vary depending upon experimental conditions. Resveratrol attenuates the phenylephrine-induced hypertrophic response in cardiomyocytes and pressure overload-induced cardiac hypertrophy. Pharmacological inhibition of Sirt1 (with NAM or sirtinol) decreases, whereas overexpression of Sirt1 increases the size of cardiomyocytes (6). Overexpression of Sirt1 attenuates endothelin-1-induced cardiomyocyte hypertrophy in vitro via degradation of H2A.z. Upregulation and activation of Sirt1 induced by phenylephrine (an α-adrenergic agonist) are blocked by inhibition or downregulation of AMPK, leading to attenuated cardiac hypertrophy. Phenylephrine-induced hypertrophy in cardiomyocytes is attenuated by resveratrol and overexpression of Sirt1. This antihypertrophic effect is canceled by downregulation of PPAR-α. On the other hand, pressure overload upregulates Sirt1 and PPAR-α in the heart, which coordinately suppress genes that are regulated by ERRs, thereby leading to the development of cardiac hypertrophy and heart failure (Fig. 1) (97). Importantly, haploinsufficiency of PPAR-α and Sirt1 attenuates cardiac hypertrophy in response to pressure overload (97). Interestingly, although low (2.5-fold) to moderate (7.5-fold) levels of overexpression of Sirt1 in the heart attenuate age-dependent cardiac hypertrophy, a high level (12.5-fold) of Sirt1 increases it (5). Thus the effect of Sirt1 upon cardiac hypertrophy may be affected by a balance of interaction with other factors, such as PPAR-α, or severity and type of stress. In addition, cardiac hypertrophy is a multifactorial disease and may also be affected by angiogenesis, apoptosis, and fibrosis. Akt is one of the key molecules regulating cardiac hypertrophy. A recent study demonstrated that Sirt1 critically regulates Akt activation by deacetylation at lysine residues in the pleckstrin homology domains of Akt. Acetylation of Akt and phosphoinositide-dependent protein kinase 1 (PDK1) blocks binding of Akt and PDK1 to PIP3. Deacetylation of Akt by Sirt1 enhances binding of Akt and PDK1 to PIP3 and promotes their activation, thereby leading to cardiac hypertrophy (Fig. 1) (125). On the other hand, Sirt1 knockout mice have smaller hearts than wild-type mice and exhibit resistance to the development of cardiac hypertrophy in response to hypertrophic stimuli. Overall, these findings lead to speculation that the role of Sirt1 as a progrowth or antigrowth factor for cardiomyocytes is tightly controlled by the magnitude of Sirt1 expression.

Sirt3 has also been shown to confer resistance to cardiac hypertrophy. Sirt3 is upregulated during mild cardiac hypertrophy, whereas it is decreased in severe cardiac hypertrophy (126). Overexpression of Sirt3 causes suppression of agonist-induced cardiac hypertrophy. Conversely, Sirt3 knockout mice exhibit exacerbation of the hypertrophic response. The beneficial effects of Sirt3 are mediated by the activation of FoxO3-dependent transcription of catalase and MnSOD, suppression of ROS-induced Ras activity, MAPK/ERF, and Akt/PI3K signaling (Fig. 2) (124). Exogenous NAD+ treatment blocks antihypertrophic liver kinase B1-AMPK signaling, which is accompanied by activation of Sirt3 but not Sirt1(103), indicating the critical role of Sirt3 in cardiac hypertrophy and redundancy in Sirt1 targets.

The role of Sirt6 in cardiac hypertrophy has been investigated using loss- and gain-of-function models (127). Sirt6 knockout mice exhibited cardiac hypertrophy and heart failure, whereas overexpression of Sirt6 attenuated cardiac hypertrophy, indicating that Sirt6 negatively regulates cardiac hypertrophy. Unlike Sirt1 and Sirt3, Sirt6 functions to directly attenuate insulin-like growth factor (IGF)-Akt signaling at the chromatin level (Fig. 3) (127).

Whereas a recent study demonstrated that Sirt2 is required for optimal Akt activation in insulin- and growth factor-responsive cells, the role of Sirt2 in cardiac hypertrophy is still unclear. Likewise, although Sirt4, Sirt5, and Sirt7, like Sirt1
and Sirt3, appear to be protective against stress in the heart, there has been no report thus far regarding the role of these proteins in cardiac hypertrophy.

**Role of Sirtuins in Cardiac Function and Heart Failure**

Heart failure represents a condition in which cardiac output is insufficient to meet the needs of peripheral organs. Heart failure is caused by almost all cardiac diseases, including myocardial infarction, hypertension, valvular heart disease, cardiomyopathy, and arrhythmia, and is one of the leading causes of death worldwide. These cardiac diseases frequently provoke left ventricular dilatation and hypertrophy, which are collectively referred to as ventricular remodeling and contribute to the development of depressed cardiac performance (101). Pathophysiological left ventricular remodeling is characterized by cardiomyocyte hypertrophy and death and interstitial fibrosis. The mechanisms mediating these processes are triggered by hemodynamic stress, as well as by the activation...
of neurohumoral factors, including the autonomic nervous system and the renin-angiotensin system, and ROS. Given the effects of sirtuins in cardiac hypertrophy, cell death/survival, and oxidative stress, sirtuins appear to be closely involved in cardiac remodeling and heart failure.

Sirt1-deficient mice exhibit notable developmental defects of the heart and only infrequently survive postnatally (17). Adult hearts from Sirt1 heterozygous knockout mice show dilated cardiomyopathy, a phenotype characterized by an absence of fibrosis and reduction of cardiomyocyte size, indicating that Sirt1 primarily affects cardiomyocyte hypertrophy. Expression of Sirt1 is upregulated in dog hearts undergoing failure induced by rapid pacing (6). Several studies examining the effects of resveratrol indicate that Sirt1 may have a beneficial role in failing hearts and that endogenous Sirt1 upregulation is a protective mechanism in the early stage of heart failure. Administration of resveratrol (2.5 mg·kg⁻¹·day⁻¹, 10 wk) prevents the development of concentric hypertrophy and cardiac dysfunction in the spontaneously hypertensive rat without affecting blood pressure, which may be partially mediated by suppression of ROS (135). In Dahl salt-sensitive rats fed with a high-salt diet, resveratrol (18 mg·kg⁻¹·day⁻¹) improves survival and counteracts the development of cardiac dysfunction without regression of hypertension or cardiac hypertrophy (114). Resveratrol also preserves mitochondrial fatty acid oxidation and PPAR-α expression in Dahl salt-sensitive rats (114). Administration of resveratrol (~145 mg/kg, 24 wk) to the TO-2 hamster, a model of dilated cardiomyopathy, suppresses cardiac fibrosis, preserves cardiac function, and improves survival by increasing MnSOD expression and reducing oxidative stress (131). Although effects of resveratrol on cardiac remodeling such as cardiac hypertrophy and fibrosis may depend on the dose of resveratrol, period of administration of resveratrol, or model of heart failure, Sirt1 is thought to be a potential target for treatment of heart failure by reducing oxidative stress and preserving mitochondrial fatty acid oxidation.

Recent studies, however, including ours, demonstrated that upregulation of Sirt1 is not necessarily protective in the heart in the presence of pressure overload. In the mouse model of heart failure, endogenous Sirt1 is upregulated by pressure overload and, together with simultaneous upregulation of PPAR-α in the nucleus, Sirt1 exacerbates cardiac dysfunction (5, 49, 95). In this situation, Sirt1 interacts with PPAR-α, thereby inhibiting expression of genes related to the mitochondrial ETC and tricarboxylic acid cycle that are normally regulated by ERR-α, through their binding to the ERR element. Haploinsufficiency of either Sirt1 or PPAR-α attenuates not only cardiac hypertrophy but also cardiac dysfunction induced by pressure overload (95). Further investigations are needed to clarify the function of Sirt1 in heart failure caused by different types of stress and the underlying molecular mechanisms mediating the effect of Sirt1 in the heart.

Little is known regarding the role of other sirtuins in heart failure. Sirt3-deficient mice exhibit signs of cardiac hypertrophy and interstitial fibrosis at baseline and severe cardiac hypertrophy in response to hypertrophic stimuli (116). In addition, overexpression of Sirt3 prevented isoproterenol-induced cardiac hypertrophy and dysfunction by decreasing FoxO3a-induced expression of MnSOD and catalase and reducing ROS levels. Reduction of ROS inhibited Ras activation and its downstream targets, such as MAPK/ERK and the PI3K/Akt pathway, thereby repressing the activity of GATA4, nuclear factor of activated T cell, eukaryotic initiation factor 4E, and S6 ribosomal protein (116).

The expression level of Sirt6 is decreased in the failing heart in humans, and a recent study demonstrated the beneficial role of Sirt6 in heart failure (127). Deletion of Sirt6 induces cardiac hypertrophy and heart failure in mice, whereas overexpression of Sirt6 attenuates development of cardiac hypertrophy in response to hypertrophic stimuli. Sirt6 binds to and suppresses the promoters of genes involved in IGF-signaling by interacting with c-Jun and deacetylating histone H3 at K9, thereby inhibiting the abnormal activation of IGF-Akt signaling implicated in the progression of heart failure.

Another important aspect of cardiac remodeling is angiogenesis. Dissociation between angiogenesis and cardiac muscle growth triggers the development of pathological hypertrophy and eventually causes heart failure (123). Thus the effect of sirtuins on vascular phenotype is also implicated in the pathogenesis of heart failure. Sirt1 plays a critical role in sprouting angiogenesis during vascular development, and Sirt1 in endothelial cells impairs the formation of new blood vessels in ischemic tissues (107). Sirt1 interacts with and deacetylates FoxO1, which plays an essential role in the negative regulation of blood vessel development, in human umbilical vein endothelial cells (HUVECs) (107). Inhibition of Sirt1 blocks nitric oxide (NO) production and endothelium-dependent vasodilation (79), and resveratrol activates endothelial NO synthase and increases NO production, thereby protecting the heart from I/R injury (42). In addition, resveratrol induces angiogenesis in a myocardial infarction model (28). The induction of angiogenesis by Sirt1 may be mediated by p53. p53 acts as an antiangiogenic factor because it inhibits the activity of hypoxia-inducible factor-1, which regulates hypoxic adaptation genes such as VEGF, and Sirt1 deacetylates p53 and reduces its transcriptional activity in cardiomyocytes (6). Supporting this speculation, endothelial-cell-specific ablation of Sirt1 induces impairment of angiogenesis and diastolic dysfunction in the heart through downregulation of VEGF receptors FLT 1 and FLK 1 (70). Sirt3 also appears to be involved in angiogenesis in the heart. In fact, Sirt3 induces bone marrow cell-induced VEGF expression and angiogenesis post-myocardial infarction (151). However, less is known regarding the involvement of Sirt3 in angiogenesis. In addition, thus far, there have been no reports of a role for other sirtuin family proteins.

Ca²⁺ homeostasis is also closely involved in the pathogenesis of heart failure. Sarco(endoplasmic reticulum Ca²⁺-AT-Pase 2a-regulated Ca²⁺ uptake into the sarcoplasmic reticulum is decreased in the failing heart (7). Resveratrol improves sarco(endoplasmic reticulum Ca²⁺-ATPase 2a promoter activity under high-glucose conditions in a Sirt1-dependent manner. However, the role of sirtuins in Ca²⁺ regulation in cardiomyocytes remains unclear.

Sirtuins and I/R Injury

I/R is a major cause of myocardial injury and a life-threatening event in the clinical setting. Ischemia or hypoxia in the heart induces a rapid decrease in ATP content in cardiomyocytes. To survive under these conditions, cardiomyocytes...
switch their metabolism from fatty acid oxidation (aerobic mechanism) to glycolysis (anaerobic). Prolonged ischemia induces cytochrome-c release from mitochondria and triggers apoptosis in cardiomyocytes. Mitochondrial permeability transition pore (mPTP) opening is followed by increases in Ca^{2+} overload, depletion of ATP, and decreases in the intracellular pH, eventually causing necrosis in cardiomyocytes (71). Although rapid reperfusion supplies oxygen and fuel, including glucose and fatty acids, and washes out toxic substances derived from necrotic cells, the rapid recovery of the oxygen supply and extracellular pH causes further ROS production and Ca^{2+} overload, leading to reperfusion injury such as necrosis and apoptosis (24). If cardiomyocytes survive after reperfusion, both oxidative stress and endoplasmic reticulum stress can induce cellular malfunction. Thus I/R induces irreversible damage to cardiac muscle and leads to cardiac remodeling and heart failure characterized by dilation of the ventricle and reduced contractility.

In the consideration of the favorable effects of Sirt1 in energy metabolism and stress resistance in the heart, Sirt1 can be expected to have a beneficial effect on I/R injury. Indeed, alteration of the expression level of Sirt1 in cardiomyocytes has been demonstrated to affect myocardial injury and cardiac function after I/R. After I/R, expression of Sirt1 is decreased in the heart, and cardiac-specific deletion of Sirt1 increases apoptosis and myocardial injury during I/R (39). Conversely, overexpression of cardiac-specific Sirt1 decreases apoptosis and infarct size and improves functional recovery after I/R by upregulating cardioprotective molecules, including MnSOD, Trx1, and Bcl-xL, and downregulating proapoptotic molecules, including Bax (Fig. 1) (39). NF-κB, isocitrate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and endothelial NO synthase have been proposed as functional targets of IPC-induced Sirt1 (88). On the other hand, resveratrol-induced Sirt1 activation attenuates cardiac I/R injury via a reduction in p38 and JNK phosphorylation and an increase in ERK phosphorylation, which are regulated by Akt/apoptosis signal-regulating kinase 1 signaling (13).

Whereas Sirt1 is upregulated by aging, oxidative stress, and heart failure, it is downregulated by I/R (39), suggesting that regulation of Sirt1 is stimulus specific. The precise mechanism by which a given stress upregulates/downregulates Sirt1 and controls the function of specific substrates is not well understood. Sirt1 deacetylase activity is regulated in an NAD^{+}-dependent manner. Administration of NAM mononucleotide increases NAD^{+} in the heart and attenuates myocardial I/R injury, accompanied by a decrease in acetylation of FoxO1. However, this effect is not observed in Sirt1 knockout mice (147). The protective effects of IPC and CR, which upregulate Nampt, the rate-limiting enzyme for NAD^{+} synthesis, are abolished by Sirt1 knockout, suggesting that the Nampt-Sirt1 axis plays a pivotal role in mediating the protective effects of IPC and CR in I/R injury (147). Prolonged CR enhances myocardial ischemic tolerance by increasing nuclear Sirt1 in a nitric oxide-dependent manner in middle-aged mice (122). In addition, several compounds, such as Sildenafil (phosphodiesterase-5 inhibitor) and Curcumin (a strong natural antioxidant), exhibit cardioprotective effects against I/R injury via activation of Sirt1 (120).

Except for those examining Sirt1, there are presently only a few studies regarding the role of sirtuins in I/R. Sirt2 has been shown to be upregulated by anoxia-reoxygenation injury. On the other hand, downregulation of Sirt2 upregulates 14-3-3-ζ and changes the subcellular localization of Bel-2-associated death promoter from mitochondrial to cytosolic, leading to a cardioprotective phenotype (67). A recent study demonstrated that Sirt3 plays a protective role in I/R. In an isolated perfused heart model, adult Sirt3 heterozygous knockout mice exhibit less cardiac functional recovery and a larger infarct size than wild-type mice during I/R, accompanied by an increase in mitochondrial protein acetylation and a decrease in the activity of mitochondrial complex I and MnSOD (Fig. 2) (106). Unlike the other sirtuins, Sirt4 does not have NAD^{+-}dependent deacetylase activity but rather functions as a histone ADP-ribosyltransferase (69). Sirt4 has been shown to decrease apoptosis in H9C2 cells under hypoxic conditions by suppressing mitochondrial Bax translocation (60), but it has not been investigated in the context of I/R. Sirt6 has also been investigated in the context of hypoxia. Cardiomyocytes from transgenic mice with overexpression of Sirt6 exhibit resistance to hypoxia, accompanied by activation of AMPK-α, an increase in B-cell lymphoma 2 (Bcl2), inhibition of NF-κB, and decreases in ROS production and phospho-Akt (p-Akt) (Fig. 3) (72). Although Sirt5 and Sirt7 also have protective roles in the heart (137), their roles during I/R remain to be elucidated.

Involvement of Sirtuins and Oxidative Stress

Oxidative stress, which is the presence of ROS in excess of the antioxidant capacity, induces myocardial damage. The mitochondrial ETC is the one of the major sources of ROS in the heart because cardiomyocytes have a large number of mitochondria and electrons continuously leak from the ETC. Endogenous antioxidants, such as SOD, catalase, and glutathione peroxidase (GPx), cooperatively detoxify ROS to H_{2}O. Superoxide anion (O_{2}^{-}) is dismutated by SOD to produce hydrogen peroxide (H_{2}O_{2}), which is detoxified to H_{2}O by catalase or GPx. However, O_{2}^{-} can also be a source of hydroxyl radical and peroxynitrite, highly reactive ROS (121). Under pathological conditions such as heart failure, mitochondrial ROS production in cardiomyocytes increases to a level that exceeds the antioxidant capacity, leading to oxidative stress. An increase in ROS production causes oxidation of mitochondrial proteins and induces mitochondrial dysfunction, further exacerbating oxidative stress in a process known as ROS-induced ROS production (43).

Increasing lines of evidence suggest that oxidative stress plays a crucial role in the pathogenesis of cardiovascular disease. MnSOD-deficient mice exhibit dilated ventricles with endocardial fibrosis and die 10 days after birth (57). Heterozygous deletion of MnSOD in mice causes a decrease in ejection fraction and progression of cardiac remodeling (63). Overexpression of catalase suppresses doxorubicin-induced cardiotoxicity in mice (49), and other antioxidants, such as GPx and peroxiredoxin-3, prevent the progression of cardiac remodeling and heart failure after myocardial infarction by suppressing mitochondrial oxidative stress (78).

Sirt1 deacetylates FoxO1 at K242, K245, and K262 and induces its nuclear translocation, thereby activating transcription of MnSOD in HEK293 cells (20). In addition, Sirt1 was reported to upregulate MnSOD via hypoxia-inducible factor-2α and FoxO4 (22). Several lines of evidence suggest that...
Sirt1 plays a protective role against oxidative stress in cardiomyocytes and the heart (5). Cardiac-specific overexpression of Sirt1 induces an increase in protein expression of catalase after exposure to paraquat (5). Overexpression of Sirt1 also upregulates MnSOD and Trx1 and attenuates oxidative stress during cardiac I/R (39). However, it should be noted that a high level of Sirt1 expression in the heart conversely induces oxidative stress through dysregulation of mitochondrial biogenesis and function at baseline in mice (5). Thus it appears that the cardiac effect of Sirt1 against oxidative stress is dose dependent. Furthermore, the activity of Sirt1 is controlled by the ratio of NAD\(^+\) to NADH, an intracellular redox-sensitive mechanism, in skeletal muscle (29). An intimate relationship between Sirt1 and oxidative stress may affect cell survival in some pathological situations.

Sirt3 also increases antioxidant capacity by upregulating MnSOD through FoxO3a in the heart (124) (Fig. 2). In addition, Sirt3 increases the ROS-scavenging activity of MnSOD by deacetylating it at K53/K68 and K122 in mouse embryonic fibroblasts (110, 133). Other antioxidants are regulated by Sirt3 as well, probably in an indirect manner. Sirt3 is thought to increase NADPH production by deacetylating and activating isocitrate dehydrogenase 2 and glutamate dehydrogenase, which in turn enhances the conversion of oxidized glutathione to reduced glutathione (64). Sirt3 inhibits ROS-induced Ras/PI3K-Akt signaling by suppressing mitochondrial ROS production (124).

Recently, the biological function of Sirt2 in oxidative stress was reported. Oxidative stress, such as due to increased hydrogen peroxide levels, increases expression of Sirt2 in NIH3T3 cells, and Sirt2 lowers the ROS level by upregulating MnSOD through deacetylation and activation of FoxO3. A cell-permeable peptide-1-SIRT2 protein attenuates lipopolysaccharide- and H\(_2\)O\(_2\)-induced cell death in a murine macrophage-like cell line. Likewise, peptide-1-SIRT2 decreases cellular levels of ROS, accompanied by elevation of MnSOD, catalase, and GPx expression. The Sirt2-mediated deacetylation and activation of glucose-6-phosphate dehydrogenase stimulates the pentose phosphate pathway to supply cytosolic NADPH to counteract oxidative damage and protect mouse erythrocytes (142). On the other hand, a study using a cDNA microarray demonstrated that Sirt2 knockdown in primary HUVECs causes global gene expression changes in response to oxidative stress, most of which are not altered by Sirt1 knockdown. Importantly, pharmacological blockade of Sirt2 attenuates oxidative stress-induced endothelial cell injury (62). Thus the effects of Sirt2 are thought to depend on the type of cell and stimulation. Thus far, no intensive study regarding Sirt2 and oxidative stress in the heart has been reported.

Sirt5 is known to be downregulated by oxidative stress in cardiomyocytes and to act as a safeguard against ROS by reducing ROS-induced cell death (61). However, it is still unclear whether and how Sirt5 regulates the ROS level in the heart. Sirt6 physically interacts with PARP1 and increases PARP1 poly-ADP-ribosylase activity via mono-ADP-ribosylation of PARP1 at K521, thereby enhancing DNA double-strand break repair under conditions of oxidative stress (74). Sirt7-deficient cardiomyocytes exhibit increases in apoptosis under basal conditions and a significant increase in sensitivity to oxidative stress (137). These findings suggest that Sirt5, Sirt6, and Sirt7 play protective roles against ROS-induced injury, consistent with their beneficial effects under other stress conditions. Less is known, however, regarding the role of Sirt4 in oxidative stress.

NADPH oxidase (Nox) proteins, which are dedicated producers of O\(_2^*\) and/or H\(_2\)O\(_2\), were recently recognized to be major sources of ROS in cells. Thus far, seven members of the Nox family of proteins (Nox1-5 and dual oxidases 1 and 2) have been identified. Nox isoforms are thought to be widely involved in myocardial damage/death, cardiac hypertrophy, cell proliferation/differentiation, and fibrosis (68). Recent studies suggest an intimate link between sirtuins and Nox. Nox1 inhibits acetylation of p53 at K382. This same lysine is deacetylated by Sirt1, resulting in impairment of the transcriptional activity of p53 and inhibition of apoptosis in cancer cells, suggesting that inhibition of p53 transcriptional activity by Nox1 is Sirt1 dependent (109). Resveratrol protects against high glucose-induced increases in expression of p47\(^{phox}\), a cytosolic component of Nox, and sirtinol, a Sirt1 inhibitor, aggravates the increase in p47\(^{phox}\) protein expression induced by high glucose in rat aortas and cultured bovine aortic endothelial cells. Inhibition of Sirt1 significantly increases Nox activity, mRNA expression of the Nox subunits, p22\(^{phox}\) and Nox4, and vascular superoxide production, all of which are attenuated by resveratrol. In addition, Sirt1 inhibition-induced endothelial dysfunction is inhibited by treatment with apocynin or SOD in rat aortas (150). These data suggest that the protective effect of Sirt1 against oxidative stress in the heart may be partly due to suppression of Nox4 activity, since Nox4 is a major Nox isoform in cardiomyocytes (2). Furthermore, the activities of sirtuins are NAD\(^+\) dependent and Nox isoforms use NADH or NADPH as a substrate for the generation of ROS. This raises the exciting possibility that Nox isoforms may regulate sirtuin activity by changing the balance of NAD\(^+\)/NADH and NADP\(^+\)/NADPH or vice versa. However, whether sirtuins and Nox isoforms affect each other in an NAD\(^+\)/NADH or NADP\(^+\)/NADPH-dependent manner in the heart has not yet been elucidated.

**Role of Sirtuins in Cell Survival and Aging in the Heart**

Several lines of evidence suggest that the balance between survival/growth and death in cardiomyocytes plays a pivotal role in cardiac function in cardiovascular disease (86). Sirt1 knockout mice (Sirt1\(^{-/-}\)) exhibit abnormal development of the heart and prenatal lethality, suggesting that Sirt1 performs important functions in cell growth and death during development (17). Sirt1 is predominantly expressed in cardiomyocytes. Inhibition of Sirt1 by NAM, sirtinol, or expression of dominant-negative Sirt1 induces nuclear fragmentation and cleavage of caspase-3 (6). Sirt1 has been reported to deacetylate p53 and FoxO, thereby regulating transcription and apoptosis in mouse and human cells (17, 65, 83, 138). In the heart, Sirt1 positively regulates expression of the antiapoptotic protein Bcl-xl, whereas it negatively regulates proapoptotic proteins Bax and cleaved caspase-3 through activation of FoxO (39). Importantly, inhibition of the activity of Sirt1, but not other HDACs, increases the acetylation and transcriptional activity of p53 in cardiomyocytes, suggesting that Sirt1 plays a more critical role in regulating p53 than other classes of HDACs in cardiomyocytes. These data strongly suggest that Sirt1 exerts protective
effects in the heart. The deacetylase activity of Sirt1 critically depends on the nuclear NAD$^+$ content, which is consumed when DNA strand breaks activate PARP. Thus decreases in Sirt1 activity induce cardiomyocyte death in some physiological and pathological conditions, such as aging, myocardial I/R, or exposure to genotoxic reagents that cause DNA damage and reduce the nuclear ratio of NAD$^+$ to NAM (153).

Sirt3 deacetylates Ku70 to sequester Bcl2-associated X protein away from mitochondria, thus inhibiting apoptosis (Fig. 2) (126). Likewise, Sirt4, Sirt5, Sirt6, and Sirt7 also exhibit antiapoptotic effects in the heart (60, 61, 72, 137).

The mPTP is found in the mitochondrial inner membrane and passes small molecules nonselectively in response to stimuli. mPTP opening is a hallmark of mitochondrial dysfunction and causes depletion of ATP and release of proapoptotic factors, eventually leading to apoptosis and necrosis of cardiomyocytes (117). mPTP opening is closely related to apoptosis/necrosis and the aging process. A recent study showed that Sirt3, a mitochondrial sirtuin protein, deacetylates cyclophilin D, a component of the mPTP, and inhibits mPTP opening, thereby reducing oxidative stress and slowing down cardiomyocyte aging in the heart (Fig. 2). Thus regulation of the mPTP by Sirt3 is an important pathway for the cardioprotective effect of Sirt3.

Aging phenotypes are determined by cooperation of diverse intrinsic and extrinsic factors that are closely related to the function of organs and the risk of disease. Generally, aging affects the expression of INK4/ARF family senescence factors (53). Aging hearts are characterized by increased myocyte volume, proliferation of myocyte nuclei, increases in apoptosis and necrosis, and interstitial fibrosis (48). In addition, cellular protective mechanisms, such as induction of antioxidants and heat shock proteins, are attenuated in aging hearts (128). Importantly, the heart is one of the most prominent organs that exhibit aging-induced mitochondrial DNA deletions and mitochondrial dysfunction (47). Therapeutic intervention to retard aging should decrease accumulation of senescent myocytes and cell death, leading to prevention of adult heart diseases.

A Sir2-dependent mechanism is thought to be involved in regulating the longevity of organisms, from yeast to primates (18, 59). However, some studies have demonstrated that the molecular mechanisms mediating longevity in lower organisms may be different from those in higher organisms. Sir2-deficient nonreplicating yeast cells exhibit greater stress resistance in extremely long-lived yeast mutants, indicating that Sir2 increases replicative life span but inhibits chronological life span under extreme stress conditions in yeast (25).

In mice on high-calorie diets, stimulation of Sirt1 by the sirtuin-activating compound resveratrol is capable of prolonging life span (11). Moderate upregulation of Sirt1 attenuates age-dependent cardiac phenotypes, whereas animals with a high level of Sirt1 (12.5-fold overexpression of Sirt1) develop heart failure spontaneously (5). This may be due to the consumption of NAD$^+$ by Sirt1. Because NAD$^+$ is necessary for the mitochondrial ETC and ATP production, a decrease in NAD$^+$ could cause a deficiency in ATP content and cellular malfunction, thereby leading to cell death. PGC-1α, a key regulator of fatty acid oxidation, is decreased in aging hearts, and Sirt1 regulates PGC-1α expression (118), indicating that Sirt1 may exert an antiaging effect by improving energy metabolism.

As a mechanism mediating life span extension, the hormesis hypothesis, in which accumulated stress resistance conferred by chronic nonlethal stress leads to life span extension, is currently well accepted. This hypothesis allows for speculation that stimulation of Sirt1 by low-grade stress, such as CR or repetitive ischemia, may partly mediate the beneficial effect of preconditioning.

Sirtuins and Autophagy

Autophagy is a mechanism of degradation of cytosolic proteins and organelles involving sequestration into double-membrane vesicles (autophagosomes) and subsequent fusion of the autophagosomes with lysosomes for degradation. Autophagy is activated in response to several kinds of stress and promotes cell survival by removing damaged organelles and preventing activation of the apoptotic machinery. In the heart, autophagy acts as a mechanism for quality control of proteins and is implicated in several important pathophysiologies, including cardiac hypertrophy (21), cardiomyopathies (95), heart failure (90), and ischemic heart disease (33). Autophagy allows for adaptation to nutrient and oxygen deprivation during myocardial ischemia and mediates cell death during reperfusion injury (77). The genes encoding proteins involved in autophagosome formation are regulated by the FoxO family of transcription factors, especially FoxO1 and FoxO3 (73). Sirt1 deacetylates FoxO1, FoxO3, and FoxO4 and regulates FoxO-dependent gene transcription either positively or negatively, leading to stress resistance and organismal longevity. For example, Sirt1 enhances FoxO3’s ability to induce resistance to oxidative stress and cell cycle arrest, whereas it inhibits FoxO3’s ability to induce cell death (14). Sirt1 deacetylates and suppresses the activity of FoxO3a and other mammalian forkhead factors, thereby reducing FoxO-dependent apoptosis (83).

Studies using pharmacological manipulation of Sirt1 support the existence of a link between Sirt1 and autophagy. Resveratrol induces autophagy in cancer cell lines in a Sirt1-dependent manner (82). Resveratrol also exhibits protective effects against apoptosis induced by rotenone and enhances degradation of α-synucleins via autophagy (143). Prolonged administration of Longevinex, a resveratrol derivative, increases autophagy in rat hearts after I/R, accompanied by upregulation of Sirt1 and Sirt3 and nuclear translocation of FoxO1, FoxO3a, and FoxO4. Manipulation of Nampt, a key molecule for the replenishment of the NAD$^+$ pool, modulates autophagy in a rat cerebral artery occlusion model and in the presence of oxygen and glucose deprivation in cultured cortical neurons in a Sirt1-dependent manner (141). The findings that Sirt1 coimmunoprecipitates with Atg proteins provide direct evidence that Sirt1 is involved in regulation of the autophagic machinery. Sirt1 directly regulates autophagy by interacting with and deacetylating autophagy-related proteins such as Atg5, Atg7, and Atg8 in MEFs (56). As described above, Sirt1 also promotes the expression of autophagy-related proteins via deacetylation of transcription factors, such as FoxO family proteins (40).

The role of Sirt1 in autophagy in the heart was recently described. Downregulation of Nampt impairs autophagic flux in cardiomyocytes, raising the possibility that Nampt regulates autophagy via the NAD$^+$-dependent Sirt1 pathway (38). A
more recent study demonstrated that the absence of glucose increased Sirt1-mediated deacetylation, activation, and nuclear translocation of FoxO1 in cardiomyocytes. Sirt1-induced deacetylation of FoxO1 and upregulation of Rab7, a small GTPase mediating late autophagosome-lysosome fusion, are essential for glucose starvation-induced autophagy in cardiomyocytes and play a pivotal role in preserving cardiac function during starvation (Fig. 1) (35). These lines of evidence suggest that Sirt1 is a key regulator of autophagy in the heart and that, in general, enhanced autophagy could be part of a protection mechanism under stress conditions. The Sirt1-FoxO axis modulates autophagy in a cell type-dependent manner in response to multiple stress signals (91, 145). Thus the role of Sirt1 in autophagy in noncardiomyocytes of the heart should be examined in the future. Moreover, other transcription factors that are deacetylated by Sirt1 may also be potential targets in autophagy regulation, as in the recent findings that nuclear p53 induces autophagy whereas cytoplasmic p53 suppresses autophagy (Fig. 1) (134).

Recent and increasing lines of evidence have demonstrated an intimate link between Sirt2 and autophagy as well. Overexpression of Sirt2 suppresses lysosome-mediated autophagic turnover by inhibiting aggrecase formation in neuronal cells. Sirt2 knockdown increases basal autophagy and causes mitotic catastrophe malfunction (45). However, the role of Sirt2 in regulating autophagy in the heart remains to be elucidated. Several studies using gain- or loss-of-function of Sirt3 and Sirt6 indicate that Sirt3 and Sirt6 are also involved in autophagy regulation. Sirt3 overexpression enhances autophagy and postpones primary HUVEC apoptosis during long-term oxime LDL treatment (66). Under basal conditions, Sirt3-knockout mouse embryonic fibroblast cells exhibit increased autophagic flux compared with wild-type cells that are not inhibited by JNK inhibitor, indicating that Sirt3-related autophagy is independent of JNK activity (58). Sirt6 overexpression induced autophagy via attenuation of IGF-Akt-mammalian target of rapamycin signaling, and Sirt6 attenuates cigarette smoke extract-induced human bronchial epithelial cell senescence via induction of autophagy (129). However, there is no report of direct evidence of a link between Sirt3 or Sirt6 and autophagy in the heart. As for the other sirtuin family proteins, to date little is known regarding the roles of Sirt4, Sirt5, and Sirt7 in autophagy. Interestingly, however, inhibitors of other types of HDACs, namely, class I and II HDACs, are known to induce autophagy (144).

Sirtuin-Activating Compounds

Sirtuins are considered attractive drug targets because of their obvious role in mediating benefits not only in life span extension related to caloric restriction but also in several diseases, including cancer, inflammation, metabolic disorder, and cardiovascular disease. Some substances, such as flavones, stilbenes, and anthocyanidins, can directly stimulate the enzymatic activity of Sirt1 in vitro through an allosteric mechanism (37). Most of the activators are polyphenolic, with planar multiphenyl rings bearing hydroxyl groups. Resveratrol (3,5,4'-trihydroxystilbene), originally identified in 1940 as a phenolic substance in the white hellebore, is well known as a natural potent activator of Sirt1 (37). It can also be found in red wine and the Japanese knotweed, Polygonum sachalinense (12). To date, more than 3,500 Sirt1-activating compounds (STACs) have been synthesized and analyzed (55). Small molecule Sirt1 activators include first-generation molecules such as resveratrol and polyphenol (37), second-generation STACs such as imidazothiazoles (80), and third-generation STACs such as benzimidazoles and urea-based scaffolds (41). Many studies have shown that STACs mimic the effect of Sirt1 activation upon gene expression in mammals, and these effects were found to be Sirt1 dependent (81). Modulation of sirtuin activity was assessed by the Fluor de Lys fluorescent biochemical assay, the validity of which has been questioned (99). However, several mechanisms of direct regulation of Sirt1 by STACs such as benzimidazoles and urea-based scaffolds (41). One is a model of direct interaction of the compound with enzyme-bound substrates (31). Another is an assisted-allosteric activation in which STACs bind to a substrate-induced exosite on Sirt1 and, in turn, stabilize substrate binding and subsequent deacetylation (41).

Table 1. Diverse roles of sirtuins in the heart

<table>
<thead>
<tr>
<th>Roles of Sirtuins in the Heart</th>
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<tbody>
<tr>
<td>Energy metabolism</td>
</tr>
<tr>
<td>Sirt2, Sirt4, Sirt5, Sirt6, and Sirt7: unknown</td>
</tr>
<tr>
<td>Cardiac hypertrophy</td>
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<tr>
<td>Sirt6: antihypertrophic ● Sirt2, Sirt4, Sirt5, and Sirt7: unknown</td>
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<tr>
<td>Heart failure</td>
</tr>
<tr>
<td>Sirt2, Sirt4, Sirt5, Sirt6, and Sirt7: unknown</td>
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<tr>
<td>Ischemia-reperfusion injury</td>
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<tr>
<td>Sirt5: protective (hypoxia) ● Sirt5 and Sirt7: unknown</td>
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<tr>
<td>Oxidative stress</td>
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<tr>
<td>Sirt2: oxidative stress ↓ or ↑ ● Sirt3: oxidative stress ↓</td>
</tr>
<tr>
<td>Sirt5, Sirt6, and Sirt7: protective against oxidative stress ● Sirt4: unknown</td>
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<tr>
<td>Cell death</td>
</tr>
<tr>
<td>Sirt2: positive regulator</td>
</tr>
<tr>
<td>Autophagy</td>
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<tr>
<td>Sirt4, Sirt5, and Sirt7: unknown</td>
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Sirtuins are closely involved in energy metabolism, cardiac hypertrophy, heart failure, ischemia-reperfusion injury, oxidative stress, cell death, and autophagy. Silent mating-type information regulation (Sirt) 1 and Sirt3 have been well investigated and documented. Overall, Sirt1 and Sirt3-7 are thought to have beneficial effects in cardiovascular disease, whereas Sirt2 appears to be detrimental. Upregulation of Sirt1 may have detrimental effects in the heart in response to pressure overload. FFA, free fatty acid.
Of the numerous STACs analyzed, resveratrol is still one of the most potent Sirt1 activators. However, resveratrol has many off-target effects unrelated to sirtuins, such as antioxidant effects, and negatively regulates proinflammatory cytokines and growth factors (99, 100). Resveratrol also activates AMPK through competitive inhibition of cAMP-degrading phosphodiesterases (100). Although it remains unclear whether the beneficial effects of resveratrol in the heart are solely mediated by Sirt1, based on numerous reports, resveratrol does appear to be beneficial to the treatment of cardiovascular disease. Resveratrol scavenges free radicals and chelates copper, thereby reducing oxidation of LDL particles, a contributing factor to the development of coronary heart disease (12). Resveratrol exerts diverse effects on the vasculature by increasing expression of endothelial and inducible NO synthase and inhibiting vascular smooth muscle cell proliferation and expression of vascular cell adhesion molecules (VCAMs) (19).

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SRT1720, which is structurally unrelated to resveratrol, stimulates 750% Sirt1 activity at a concentration of 10 μM and binds Sirt1 at the same site as resveratrol (amino acids 183–225 in the NH2-terminus) (80). SRT1720 reduces myocardial infarction by 75% (75). Sirt1 activators have the most potent Sirt1 activators. However, resveratrol has many off-target effects unrelated to sirtuins, such as antioxidant effects, and negatively regulates proinflammatory cytokines and growth factors (99, 100). Resveratrol also activates AMPK through competitive inhibition of cAMP-degrading phosphodiesterases (100). Although it remains unclear whether the beneficial effects of resveratrol in the heart are solely mediated by Sirt1, based on numerous reports, resveratrol does appear to be beneficial to the treatment of cardiovascular disease. Resveratrol scavenges free radicals and chelates copper, thereby reducing oxidation of LDL particles, a contributing factor to the development of coronary heart disease (12).

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Sirt1 regulates aging and resistance to oxidative stress in the heart. Sirt3 also appears to be a potential target for cardiovascular disease treatment, mainly because of its protective effects upon mitochondrial function. Although Sirt4-7 also have beneficial effects in cardiovascular disease, Sirt2 appears to have the opposite effect in the heart (Table). So far, Sirt1 and Sirt3 have been well investigated and documented, and activating compounds for these proteins have therapeutic potential for cardiovascular diseases such as metabolic disease, ischemic heart disease, and heart failure. However, the balance between the dosage of the longevity mechanism and the pathogenesis of the cardiovascular disease must be carefully evaluated to obtain the beneficial effects but eliminate the undesirable side effects of sirtuins.

An important unresolved question in the field of sirtuin research in the heart is whether sirtuins act through direct modification of histones and, if so, at what regions of the genome. Most of the observations relating acetyl-transferring enzymes to cardiac phenotype have been made without an understanding of the totality of molecular targets. Further investigations are needed to address the molecular mechanisms mediating the actions of sirtuins in the heart and elucidate their functional significance.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

S.M. and J.S. conception and design of research; S.M. prepared figures; S.M. and J.S. drafted manuscript; S.M. and J.S. edited and revised manuscript; S.M. and J.S. approved final version of manuscript.

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