Novel Role of Silent Information Regulator 1 in Myocardial Ischemia

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The incidence of cardiovascular diseases is increasing at an alarming rate throughout the world. According to the World Health Report 2010, cardiovascular disease accounts for 17.1 million global deaths per year, or 29% of total deaths worldwide. It is predicted that this number will increase to 23.6 million by 2030. Myocardial ischemia, which is reported to induce irreversible damage to the myocardium, causes a number of cardiovascular diseases, such as myocardial infarction, myocardial hypertrophy, atherosclerosis, and heart failure. Medical treatment that effectively prevents ischemic injury would alleviate the consequent development of cardiac remodeling and failure. Previous studies have indicated that ischemic preconditioning (IPC), caloric restriction, resveratrol preconditioning, and some related factors can prevent ischemic injury to the heart and are cardioprotective. The underlying mechanisms of these interventions appear to be controlled by a nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylase called silent information regulator 1 (SIRT1).

SIRT1 is a member of the class III group of histone deacetylases, collectively called sirtuins. The mammalian sirtuin family consists of 7 members, designated SIRT1 through SIRT7, which are characterized by a conserved 275-amino-acid catalytic core and unique additional N-terminal and C-terminal sequences of variable length. Previous studies have shown that SIRT1 can deacetylate many transcription factors, including forkhead box O (FOXO) transcription factors, p53, nuclear factor-κB (NF-κB), liver X receptor, peroxisome proliferator–activated receptor γ, and brain and muscle Arnt-like protein 1, and nuclear coactivators, as well, including peroxisome proliferator–activated receptor γ coactivator-1α (PGC-1α), cAMP-responsive element-binding protein–regulated transcription coactivator 2, and period homolog 2. SIRT1 also deacetylates serine/threonine kinase 11, endothelial nitric oxide synthase (eNOS), and histones H1, H3, and H4. It has been reported that SIRT1 performs a wide variety of functions in a variety of biological systems, including obesity-associated metabolic diseases, cancer, aging, cellular senescence, cardiac aging and stress, prion-mediated neurodegeneration, inflammation, and placental cell survival. Most importantly, SIRT1 is involved in cardioprotection.

Several studies suggest that SIRT1 plays a role in myocardial ischemia. Pillai and colleagues found that SIRT1 was involved in the effect of fructose feeding on myosin heavy chain (MHC) gene expression and cardiac protection. Additionally, the localization of SIRT1 has been reported to change during cardiac development and under stress conditions. Moreover, Sciarretta and colleagues have reported that SIRT1 plays a role in the cardiac autophagy signaling pathway. In these mechanisms, SIRT1 is controlled by several upstream molecules, such as fructose, Longevinex, nicotinamide phosphoribosyltransferase (Nampt), and microRNA (MIR)-199a. Meanwhile, SIRT1 regulates many downstream factors, including the α-MHC gene promoter, FOXOs, hypoxia-inducible factor (HIF)-1α, PGC-1α, mitogen-activated protein kinase, NF-κB, eNOS, and p53. These results indicate that SIRT1 participates in cardioprotection via a complex signaling network.

The focus of this review is to summarize the latest progress regarding the protective effects of SIRT1 in myocardial ischemia. First, we discuss some important actions of SIRT1 in myocardial ischemia. We then introduce some upstream mediators of SIRT1 and some downstream signaling pathways. Furthermore, we highlight the role of SIRT1 in human heart diseases. Finally, we show several novel potential directions of SIRT1 studies. Collectively, the information compiled here will serve as a comprehensive reference for the actions of SIRT1 in the cardiovascular system identified to date and will hopefully aid in the design of further experimental research and increase the potential of SIRT1 as a therapeutic target in the future.

Role of SIRT1 in Myocardial Actions

Autophagy

Autophagy is a catabolic process whereby long-lived proteins in the cytosol and organelles are sequestered into double-membrane vesicles, termed autophagosomes, and transported to lysosomes for degradation. Autophagy occurs at basal conditions and mediates homeostatic functions in cells. However, autophagy is also induced by stress, such as nutrient
starvation, hypoxia, endoplasmic reticulum stress, and oxidative stress.\textsuperscript{11} Autophagy is progressively activated in mice after 3 days of fasting, which is paralleled by a progressive decline in heart weight.\textsuperscript{12,13} Sciarretta and colleagues have found that fasting activates FOXO through SIRT1-mediated deacetylation, which in turn induces Rab7 expression, thereby stimulating autophagosome-lysosome fusion. The downregulation of FOXO1 or the suppression of FOXO1 deacetylation inhibits starvation-induced autophagy and promotes cardiac dysfunction during fasting.\textsuperscript{14} These results suggest that starvation-induced autophagy activated through the SIRT1-FOXO-dependent mechanism is adaptive for the heart and the cardiomyocytes therein and broadly support the hypothesis that, in response to ischemia and reperfusion, autophagy can maintain the energy status, protein quality control, and organelle function observed under better conditions.

**Nucleocytoplasmic Shuttling**

Nucleocytoplasmic shuttling of SIRT1 takes place in cardiomyocytes during stress and has a role in cardioprotection. SIRT1 is expressed in all mammalian cells and was originally identified as a nuclear protein.\textsuperscript{15} However, recent studies have shown that the subcellular localization of SIRT1 differs from cell to cell. Although some cells show nuclear localization of SIRT1, others express it either both in the nucleus and the cytoplasm or in the cytoplasm alone.

In the heart, the nuclear and cytoplasmic localization of SIRT1 was found to be regulated developmentally and under stress conditions.\textsuperscript{16} In the mouse embryonic heart at embryonic day (E) 10.5 and E12.5, when the 4-chambered heart appears, a high level of SIRT1 has been found in the nucleus of myocytes in both the atria and ventricles. SIRT1 expression in the heart further declines with organogenesis. At E16.5, SIRT1 levels in the heart are 21\% of E12.5 levels, and after birth, they remain constant until 27 months of age. In the adult rodent heart, SIRT1 is localized primarily in the cytoplasm and moves to the nucleus during stress conditions. The nuclear localization of SIRT1 in cardiomyocytes was inhibited by the use of the phosphatidylinositol 3-kinase inhibitor, LY294002, which also blocked Akt activation, thus suggesting a possible role for phosphatidylinositol 3-kinase/Akt-mediated phosphorylation in the nuclear translocation of SIRT1.\textsuperscript{17} Likewise, the c-Jun N-terminal kinase 1–mediated phosphorylation of SIRT1 also promotes its translocation into the nucleus.\textsuperscript{18} SIRT1 nuclear translocation was found to be essential for its cytoprotective effects against oxidative stress through the activation of manganese superoxide dismutase (MnSOD),\textsuperscript{19} suggesting that phosphorylation-mediated shuttling in and out of the nucleus could be 1 mechanism by which SIRT1 activity is regulated. These studies also reported the presence of nuclear SIRT1 in different models of heart failure, including the failing hearts of TO-2 hamsters, post–myocardial infarction in rats, and dilated cardiomyopathy in human patients.\textsuperscript{17} Because the nuclear presence of SIRT1 is a feature of the fetal heart and SIRT1 is translocated to the nucleus under pathological conditions, it is intriguing to consider that the nuclear translocation of SIRT1 could have a role in ischemia/reperfusion (IR)–related cardiovascular disease.

**α-MHC Expression**

Pillai and colleagues found that SIRT1 might participate in the expression of α-MHC. Fructose feeding has been shown to induce cardiac α-MHC expression and protect the heart from IR-mediated cell injury. The study was designed to investigate the mechanism involved in the effect of this sugar on MHC gene expression and cardiac protection. Adult mice were fed a 6-propyl-2-thiouracil (PTU) diet or PTU combined with a fructose-rich diet. PTU treatment made animals hypothyroid, which resulted in the total replacement of cardiac α-MHC with the β-MHC isofrom. The addition of fructose to the PTU diet led to a significant level of reexpression of the α-MHC isofrom. A similar induction of α-MHC expression was also observed when the PTU diet was combined with resveratrol, an agonist of SIRT1 deacetylase. An analysis of the heart lysate of these animals indicated that fructose feeding augmented the NAD-to-NADH ratio and the cardiac SIRT1 levels, suggesting a role for SIRT1 in the fructose-mediated activation of the α-MHC isofrom. To determine whether SIRT1 has a direct effect on MHC isoform expression, Pillai and colleagues also generated transgenic mice expressing SIRT1 in the heart. Treatment of these transgenic mice with the PTU diet did not lead to the disappearance of α-MHC, as it did in the nontransgenic animals. SIRT1 overexpression also activated the α-MHC gene promoter in transient transfection assays, thus, confirming a role of SIRT1 in the induction of α-MHC expression. α-MHC has high ATPase activity and accounts for a faster shortening velocity of cardiac myofibers, whereas β-MHC, having low ATPase activity, leads to greater economy of force generation.\textsuperscript{6} Previous studies conducted with transgenic mice and rabbits have indicated that α-MHC hearts have an advantage in stress conditions in comparison with hearts primarily expressing the β-MHC isofrom.\textsuperscript{19,20} These studies have indicated that the induction of α-MHC expression in a failing heart may be beneficial in terms of increasing the myocardial contractility of a hemodynamically challenged heart, thus suggesting that SIRT1 may protect the heart from IR injury via the induction of α-MHC expression.

**Myocardial Hypertrophy**

SIRT1 has been confirmed as a key factor in myocardial hypertrophy. An initial study conducted with SIRT1 overexpression in cardiomyocytes showed that it protected cells from death in response to serum starvation, but, at the same time, it caused an overall increase in cardiomyocyte size.\textsuperscript{21} This study also showed that blocking SIRT1 activity with inhibitors increased the propensity of cardiomyocyte to die but prevented myocyte hypertrophy in response to stress stimuli, thus implying that SIRT1 promotes cardiomyocyte growth under stress conditions through cytoprotective effects. These observations are supported by other reports demonstrating increased SIRT1 levels in hypertrophied and failing hearts.\textsuperscript{22,23} Studies performed with a cardiac-specific SIRT1 transgenic mouse model showed that SIRT1 exhibits hormesis; depending on the magnitude of SIRT1 expression, it can be either beneficial or harmful. Low to moderate SIRT1 expression (2.5- to 7.5-fold over endogenous levels) was found to be protective against the age-dependent increase in cardiac hypertrophy, apoptosis, and cardiac dysfunction, whereas 12.5-fold
SIRT1 overexpression induced dilatation, hypertrophy, and cardiac failure.22 A low level of SIRT1 overexpression was also shown to reduce infarct size and improve cardiac function in a mouse model of myocardial infarction.23 These observations strengthen the perception that SIRT1 is a progrowth and prosurvival molecule for cardiomyocytes, and, hence, its expression needs to be tightly controlled to obtain desirable effects. The study also showed that the SIRT1 homozygous knockout mice were more susceptible to cell death induced by IR injury.24 Sundaresan and colleagues7 demonstrated that whole-body SIRT1-knockout mice have smaller hearts than their wild-type littermates and are resistant to the development of cardiac hypertrophy induced by hypertrophic agonists. These mice also show reduced activation of the fetal gene program, a lack of cardiomyocyte hypertrophy, and impaired Akt-signaling following the infusion of hypertrophic agonists, thus suggesting that SIRT1 is needed for the induction of the cardiac hypertrophic program and implying that SIRT1 mediates compensated myocardial hypertrophy during IR.7

Upstream Mediators

Nicotinamide Phosphoribosyltransferase

Nampt is a rate-limiting enzyme in the mammalian NAD+ salvage pathway and has been proposed to be a functional equivalent of pyrazinamidase/nicotinamidase 1 in mammals.26 Nampt upregulation increases the cellular NAD+ level and enhances the transcriptional regulatory activity of the catalytic domain of SIRT1 in mouse fibroblasts.27 Because the histone deacetylase activity of SIRT1 is NAD+ dependent, Nampt downregulation causes SIRT1 suppression.

It has been reported that the action of SIRT1 in autophagy is mediated by Nampt. Recent evidence suggests that SIRT1 stimulates autophagy through the deacetylation of antithymocyte globulins in cancer cells.28 SIRT1 downregulation increased p62 accumulation, suggesting that it suppresses autophagic flux, thereby mimicking the effect of Nampt downregulation in cardiomyocytes. Furthermore, downregulation of both Nampt and SIRT1 did not show additive effects on the inhibition of autophagic flux, consistent with the notion that Nampt may inhibit autophagic flux through SIRT1 suppression. Further investigation is required to elucidate the role of SIRT1 in mediating the effect of Nampt downregulation on autophagy in cardiomyocytes. An electron transport chain present in lysosomes maintains a proton gradient at the expense of NADH.29 Decreases in NAD+ may directly inhibit the function of lysosomes, which may also contribute to the suppression of autophagy induced by Nampt downregulation.

One study reported that Nampt expression in the heart significantly reduced the size of a myocardial infarction after ischemia and IR, thus suggesting that Nampt has a protective function in the heart via SIRT1. Because the downregulation of endogenous Nampt after ischemia and IR is normalized in Tg-Nampt, the results suggest that Nampt downregulation has a causative role in mediating myocardial injury during ischemia or IR. Because Nampt downregulation decreases cellular ATP content, increases apoptosis, and inhibits autophagy, researchers speculate that all of these contribute to myocardial injury after IR. Reperfusion after prolonged ischemia causes a release of NAD+ from mitochondria through the mitochondrial permeability transition pore opening and the subsequent downregulation of NAD+,30 which would exacerbate myocardial damage by IR. Because the detrimental effects of Nampt downregulation in cardiomyocites appear to be rescued by exogenously applied NAD+, increasing the cellular level of NAD+ by targeting Nampt may be a promising strategy to reduce IR injury. Increased Nampt provides protection against cell death and requires an intact mitochondrial NAD+ salvage pathway. These results demonstrate that Nampt is an important upstream factor of SIRT1 and accelerates cardioprotection during ischemic stress.

In addition, Nampt may exert cardioprotection via another signaling pathway. It has been demonstrated that the NADH/NAD+ ratio can regulate the expression of sultonylurea receptor 2A, a KATP channel regulatory subunit.31 Researchers have found that the sole overexpression of the sultonylurea receptor 2A protein generates a cardiac phenotype with more sarcolemmal KATP channels, which is essential for preconditioning-induced cardioprotection, thus, increasing resistance to hypoxia and ischemia.32–36 Therefore, it is possible that the sultonylurea receptor 2A-mediated pathway that is regulated by Nampt acts assists in SIRT1-related cardioprotection.

Resveratrol

The discovery of resveratrol stems from an interesting phenomenon. The population of France consumes foods high in saturated fats, yet experiences fewer cardiovascular pathologies. This is known as the French paradox and may be explained in part by their daily consumption of red wine.37 Resveratrol (3,5,4-trihydroxystilbene) is prominent in red wine, and it is an antioxidant that has been shown to have many beneficial effects and to function as a caloric restriction mimetic. Similar to caloric restriction, resveratrol can maintain mitochondrial integrity, reduce insulin-like growth factor-1, activate SIRT1, and increase the lifespan of organisms from yeast to mammals.38,39 Resveratrol also improves the outcome after ischemic episodes in the heart and brain40,41 and requires SIRT1 to mediate ischemic protection.42 Resveratrol-activated pathways have been shown to protect against ischemia by modulating excitotoxicity, mitochondrial functioning, blood vessel integrity, and nitric oxide signaling.43–46

The effect of the endogenous SIRT1 activation achieved by resveratrol treatment has been studied in different models of cardiac hypertrophy. Resveratrol treatment limits the phenylephrine-induced hypertrophic response of isolated cardiomyocyte cultures.47 Resveratrol also reduces fatty lesions, myocardium vacuolization, degeneration, and inflammation in mice on a high calorie diet.38 The prefeeding of rats for 14 days with resveratrol resulted in the protection of hearts from the deleterious effects of pressure overload–mediated cardiac hypertrophy.48 Likewise, resveratrol feeding improves cardiac function in diabetic mice.49 Further, the oral administration of resveratrol to TO-2 hamsters has been shown to suppress fibrosis, preserve cardiac function, and significantly improve survival.17 All these studies correlated the cardioprotective effects of resveratrol with its ability to activate SIRT1. Based on the data available to date, it appears that resveratrol
treatment may be beneficial in the management of cardiac hypertrophy and cell death.

One significant action of resveratrol treatment under ischemic conditions is the mediation of inflammatory signaling pathways. Inflammation during IR causes the breakdown of the vascular endothelium, leading to increased membrane permeability and hemorrhage. Resveratrol has been reported to maintain blood vessel integrity after ischemic injury by modulating leukocyte activity and reducing the expression of inflammatory enzymes. For example, matrix metalloproteinase-9 is an endopeptidase expressed during ischemia that induces inflammatory signaling and leads to blood vessel degradation. A major source of matrix metalloproteinase-9 during ischemia is leukocytes, which bind to the endothelium, infiltrate the ischemic tissue, and initiate the inflammatory response. After myocardial ischemia, resveratrol has been shown to suppress molecules that mediate leukocyte adhesion, such as NF-κB, a major mediator of inflammatory signaling. It has been reported that NF-κB can be inhibited by SIRT1 deacetylation, which suggests that SIRT1 may have anti-inflammatory effects. Resveratrol has been further shown to protect coronary arterial endothelial cells against oxidative stress by activating nuclear factor-E2-related factor-2, a transcription factor that regulates the genes involved in antioxidant defense.

Resveratrol has been shown to strongly stimulate SIRT1 deacetylase activity in a dose-dependent manner by increasing its binding affinity to both the acetylated substrate and NAD+. Biochemical and structural modeling studies have shown that resveratrol binds directly to SIRT1, thereby inducing a conformational change in the SIRT1 protein configuration. However, recent research has suggested that resveratrol is not a direct activator of SIRT1. In vitro studies demonstrated that SIRT1 was activated by resveratrol when an artificial, fluorescent acetyl-peptide was used as a substrate for deacetylation. Otherwise, resveratrol was unable to activate SIRT1 when the same peptide substrate lacked the covalently linked fluorophore. These recent studies are highly controversial and do not exclude the possibility that SIRT1 is activated indirectly by resveratrol. For example, SIRT1 was shown to be phosphorylated on its carboxy-terminal end at serine 659 and serine 661 by the protein kinase creatine kinase 2, an enzyme that can be regulated by resveratrol. Similarly, other studies showed that sumoylation at lysine 734 or JNK2 phosphorylation at serine 27 increased SIRT1 protein deacetylase activity. No studies have yet observed resveratrol-mediated posttranslational modifications of SIRT1. However, resveratrol activates numerous pathways and could therefore modulate SIRT1 activity by regulating these signaling cascades.

Longevinex

Although resveratrol has been shown to possess diverse health benefits, several recent reports have demonstrated conflicting results on some aspects of its effects, including its antiaging properties. Considerable debate appears to exist on the dose and bioavailability of resveratrol, leading to the controversies on its effectiveness. To solve this problem, Mukherjee and colleagues designed a study with a resveratrol formulation that contained resveratrol supplemented with 5% quercetin and 5% rice bran phytate (commercially known as Longevinex). In their study, Longevinex-treated hearts, irrespective of the treatment duration, revealed superior cardiac performance, reduced infarct size, and the induction of survival signals, as evidenced by an increased B-cell lymphoma gene 2 (Bcl2)/Bax ratio and enhanced Akt phosphorylation. However, LC3-II and Beclin were significantly enhanced after 3 months of Longevinex treatment, suggesting that autophagy occurred only after feeding Longevinex to rats for a prolonged period of time. Corroborating the autophagy results, SIRT1 was significantly increased only after 3 months of Longevinex treatment, suggesting that enhanced SIRT1 expression correlated with the induction of autophagy. Additionally, Longevinex caused the phosphorylation and nuclear translocation of FOXO1, FOXO3a, and FOXO4, indicating the involvement of FOXOs in autophagy. Because SIRT1 and FOXOs are reliable markers of longevity, the results appear to suggest that Longevinex induces longevity after prolonged feeding via the induction of autophagy, whereas it converts death signals into survival signals and provides cardioprotection within a relatively shorter period. This research provides a novel target for decreasing IR injury by the use of Longevinex treatment.

Downstream Pathways

Forkhead Box O

One of the well-documented targets of SIRT1 in cardiomyocytes is FOXO. FOXO is the mammalian homolog of Daf16 and has been implicated in the SIRT1-mediated longevity of Caenorhabditis elegans. In mammals, there are 4 evolutionarily conserved FOXO family members, FOXO1, FOXO3, FOXO4, and FOXO6. They control various cellular processes such as cell cycle arrest, reactive oxygen species production, DNA repair, and apoptosis. SIRT1 regulates FOXO activity either positively or negatively depending on the target gene or cell type. SIRT1 deacetylates and activates FOXOs, which synthesize antioxidants, such as MnSOD and catalase, thereby promoting cellular resistance against oxidative stress. SIRT1-transgenic mice display a retarded aging phenotype in the heart owing to its ability to induce MnSOD and catalase expression through the activation of FOXOs. In a mouse myocardial infarction model, the SIRT1-mediated upregulation of FOXO prevented cellular injury by activating prosurvival factors, such as thioredoxin-1 and Bcl-xL, and suppressing the activity of proapoptotic molecules such as Bax and cleaved caspase3. Similarly, the resveratrol-mediated activation of SIRT1 increased MnSOD levels in cardiomyocytes and suppressed fibrosis, preserved cardiac function, and significantly improved the survival of TO-2 hamsters. Chen and colleagues researched the role of the resveratrol-SIRT1 signaling pathway in cardioprotection. They found that SIRT1 has a dual effect on FOXO1 function: SIRT1 increases FOXO1’s ability to induce cell cycle arrest but inhibits FOXO1’s ability to induce cell death. This effect could be reversed by SIRT1 inhibition. The results of their study indicate that resveratrol inhibits apoptosis via the SIRT1-FOXO1 pathway in H9c2 cells, thus suggesting a potential target for preventing ischemic cardiovascular disease, particularly in patients with coronary artery disease.
Hypoxia-Inducible Factors

HIFs are a group of transcription factors involved in mediating protective adaptations. SIRT1 activation of HIF-2α increases the expression of erythropoietin, a regulator of red blood cell production and angiogenesis. The IPC elevation of erythropoietin protein expression has been shown to play a key role in IPC-mediated protection. SIRT1 activation of HIF-2α has also been shown to enhance the expression of the mitochondrial reactive oxygen species scavenger MnSOD, which further shows that SIRT1 can regulate multiple mechanisms to mediate protection against oxidative stress. HIF-1α is regulated by MIR-199a, and SIRT1 is an additional target of MIR-199a, suggesting a conceivable relationship between HIF-1α and SIRT1.

Peroxisome Proliferator–Activated Receptor γ Coactivator-1α

PGC-1α is a member of a family of transcription coactivators that have activities in mitochondrial biogenesis, antioxidation, growth factor signaling regulation, and angiogenesis. Tan and colleagues found that almost all the signaling pathways activated by resveratrol involve PGC-1α activity. Moreover, it has been demonstrated that resveratrol can mediate an increase in PGC-1α activity. These significant conditions support the hypothesis that resveratrol exerts a pharmacological preconditioning effect by activating PGC-1α. Resveratrol can strongly stimulate SIRT1 activity, thus, showing that SIRT1 prevents IR injury via a PGC-1α–related pathway. Other studies have indicated that SIRT1 directly activates PGC-1α, the transcriptional coactivator associated with protection against oxidative stress. SIRT1-dependent PGC-1α expression was shown to reduce infarct size and improve neurologic scores after middle cerebral artery occlusion. Rodents lacking PGC-1α displayed enhanced hippocampal neurodegeneration in response to oxidative stress and exhibited decreased reactive oxygen species scavenger expression after transient global ischemia. This suggests that SIRT1 may protect against ischemia-induced oxidative damage by activating PGC-1α.

Other Downstream Targets of SIRT1

In addition to the previously mentioned downstream pathways, there are other downstream targets of SIRT1 in the myocardial ischemia procedure. In neonatal rat ventricular cardiomyocytes subjected to a simulated IR model, Becatti and colleagues demonstrated that SIRT1 overexpression positively affects the mitogen-activated protein kinase pathway. Vahtola and colleagues reported an increase in the number of SIRT1-positive nuclei in the infarct area of Goto-Kakizaki rats, and the acetylation of p53 at the SIRT1-preferred site was decreased. In an isolated myocardial IR model of SIRT1-overexpressing mice, Nadtochiy and colleagues found that the phosphorylation of eNOS was significantly higher in SIRT1+++ hearts than in SIRT1+/− hearts. Alternatively, SIRT1 is known to deacetylate and inhibit NF-κB and p65; however, the results of Nadtochiy and colleagues also showed a significant increase in the level of p65 acetylation in SIRT1+/− hearts relative to SIRT1+++ hearts. We have provided a table to summarize these target genes (and the downstream biological effects) that are potentially regulated by SIRT1 in myocardial ischemia, along with an experimental model and bibliographic references (Table).

Table. The Target Genes and Downstream Biological Effects That Are Potentially Regulated by SIRT1 in Myocardial Ischemia

<table>
<thead>
<tr>
<th>Target Genes</th>
<th>Experimental Models</th>
<th>Downstream Biological Effects</th>
<th>First Author, Year, and Reference No.</th>
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<tbody>
<tr>
<td>FOXOs</td>
<td>Mouse myocardial infarction model</td>
<td>The SIRT1-mediated upregulation of FOXO prevented cellular injury by activating prosurvival factors and suppressing the proapoptotic molecules</td>
<td>Hsu et al, 2010</td>
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<tr>
<td></td>
<td>Hypoxia model of H9c2 cells</td>
<td>SIRT1 increased FoxO1’s ability to induce cell cycle arrest but inhibited FoxO1’s ability to induce cell death</td>
<td>Chen et al, 2009</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>Hypoxia model of neonatal rat ventricular cardiomyocytes</td>
<td>SIRT1 is a direct target of miR-199a and is responsible for downregulating prolyl hydroxylase 2, which is required for stabilization of HIF-1α.</td>
<td>Rane et al, 2009</td>
</tr>
<tr>
<td>MAPK</td>
<td>Simulated IR model of neonatal rat ventricular cardiomyocytes</td>
<td>SIRT1 overexpression positively affects the MAPK pathway–via Akt/ASK1 signaling–by reducing p38 and JNK phosphorylation and increasing ERK phosphorylation.</td>
<td>Becatti et al, 2012</td>
</tr>
<tr>
<td>p53</td>
<td>Myocardial infarction model of GK rats</td>
<td>The acetylation of p53 at the SIRT1-preferred site was decreased.</td>
<td>Vahtola et al, 2010</td>
</tr>
<tr>
<td>NF-κB and eNOS</td>
<td>Isolated myocardial IR model of SIRT1-overexpressing mice</td>
<td>The phosphorylation of eNOS was significantly higher and the level of p65 acetylation was significantly lower in SIRT1+++ hearts compared with SIRT1+/− hearts</td>
<td>Nadtochiy et al, 2011</td>
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ASK1 indicates apoptosis signal-regulating kinase 1; eNOS, endothelial nitric oxide synthase; ERK, extracellular regulated protein kinase; FOXO, forkhead box O; GK, Goto-Kakizaki; HIF-1α, hypoxia-inducible factor-1α; IR, ischemia/reperfusion; MAPK, mitogen-activated protein kinase; MHC, myosin heavy chain; NF-κB, nuclear factor-κB; and SIRT1, silent information regulator 1.

The Role of SIRT1 in Human Heart Diseases

Data also suggest that SIRT1 has a role in many human heart diseases. Tanno and colleagues found that normal human cardiomyocytes predominantly expressed SIRT1 in their cytoplasm, and chronic heart failure induced a nuclear translocation of SIRT1 in cardiomyocytes. The nuclear accumulation of SIRT1 is likely to be an adaptive mechanism of cardiomyocytes against heart failure, as indicated by the potent cell protective effect of nuclear SIRT1 against reactive oxygen species. Shan and colleagues reported that SIRT1 participates in the development of congenital heart disease. Carter and colleagues demonstrated that SIRT1 has an effect on aortic stenosis by inhibiting resistin expression. It has also been demonstrated that SIRT1 interacting with Notch signaling can participate in bicuspid aortic valve disease.
Most importantly, SIRT1 has a significant connection with ischemia-related myocardial diseases. Research data indicated that monocytic SIRT1 expression is reduced in patients with stable coronary artery disease and acute coronary syndromes. Moreover, SIRT1 plays an important role in the prevention of atherosclerosis. Zeng and colleagues reported that SIRT1 may prevent the formation and progression of atherosclerosis by enhancing the liver X receptor-ATP–binding cassette, A1/ABCG1/C-C chemokine receptor type 7, and inhibiting the NF-κB pathways.

Above all, both animal experiments and human studies demonstrated that SIRT1 has a credible protective function on myocardial ischemia injury, manifesting the significance of the investigation of SIRT1.

Potential Directions

Among many recent studies on SIRT1, some recent findings indicate potential directions of future studies. They may be of underlying value in the treatment of IR injury.

Recently, MIRs have been shown to play a role in SIRT1-related signaling pathways. Rane and colleagues reported that MIR-199a is acutely downregulated in cardiac myocytes following a decline in oxygen tension. The reduction is required for the rapid upregulation of its target, HIF-1a. Replenishing MIR-199a during hypoxia inhibits HIF-1a expression and its stabilization of p53 and thus reduces apoptosis. However, MIR-199a knockdown during normoxia results in the upregulation of HIF-1a and SIRT1 and reproduces hypoxia preconditioning. SIRT1 is also a direct target of MIR-199a and is responsible for downregulating prolylhydroxylase 2, which is required for the stabilization of HIF-1a. Thus, they concluded that MIR-199a is a master regulator of a hypoxia-triggered pathway and can be exploited for preconditioning cells against hypoxic damage. These data demonstrated a functional link between the 2 key molecules that regulate hypoxia preconditioning and longevity. Additionally, Salloum and colleagues reported that MIRs do affect cardioprotection, and they noted that MIRs can drive SIRT1 expression to some extent. We believe that MIRs have immense potential in the treatment of cardiovascular diseases via SIRT1-related pathways.

Caloric restriction is also a research direction. Prolonged caloric restriction improves the recovery of left ventricle function and limits infarct size after IR. Additionally, it restores the protective effects of IPC. The cardioprotection afforded by prolonged caloric restriction is associated with changes in the subcellular localization of SIRT1, and nitric oxide synthase activity is necessary for the increase in nuclear SIRT1 content during prolonged caloric restriction. These results suggest a possible pathway through which caloric restriction regulates the activity of eNOS, thus mediating SIRT1 expression, leading to cardioprotection.

In 2010, Nadtochiy and colleagues discussed lysine deacetylation in IPC and the upstream regulation of SIRT1. They observed more lysine deacetylation in IPC and an elevation in SIRT1 activity. The investigation demonstrated that SIRT1 protein levels do not change in IPC, although SIRT1 sumoylation does occur. SIRT1 inhibitors can restrain IPC-mediated lysine deacetylation and IPC-induced cardioprotection. The research suggests a potential relationship between SIRT1 and lysine deacetylation.

Figure. The potential upstream regulators of SIRT1, and a variety of downstream target genes, as well, and their related effects implicated in the myocardial protection. The regulation of gene expression by SIRT1 deacetyltransferase activity allows for the activation and inhibition of signaling pathways involved in myocardial protection. CR indicates caloric restriction; eNOS, endothelial nitric oxide synthase; FOXO, forkhead box O; HIF-2a, hypoxia-inducible factors-2a; MAPK, mitogen-activated protein kinase; MH, myocardial hypertrophy; MHC, myosin heavy chain; MIR, microRNA; MnSOD, manganese superoxide dismutase; NF-κB, nuclear transcription factor κB; NAD+, nicotinamide adenine dinucleotide; Nmp1, nicotinamide phosphoribosyltransferase; PGC-1α, peroxisome proliferator–activated receptor γ coactivator 1α; and SIRT1, sirtuin 1.
It is encouraging to know that in addition to resveratrol, another component of white wine, n-tirosol [2-(4-hydroxyphenyl) ethanol], also exhibits cardioprotective effects. Samuel and colleagues evaluated the effect of tyrosol treatment on myocardial ischemic stress. Tyrosol-treated rats demonstrated reduced infarct size and improved myocardial function. They also observed a significant increase in the phosphorylation of Akt, eNOS, and FOXO3a. In addition, tyrosol induced the expression of SIRT1. These findings suggest that tyrosol induces myocardial protection against ischemia-related stress by inducing survival and longevity proteins, particularly SIRT1, and that it may be considered as a new target for anti-aging therapy for the heart.

**Conclusions**

Above all, we proposed a hypothesis to connect all of the pathways and to simulate the probable process of SIRT1-related cardioprotection in myocardial ischemia. When the heart is under ischemic stress, the absence of oxygen and calories promotes the expression of SIRT1. In addition, the activation of the phosphatidyl inositol 3-kinase/Akt and e-Jun N-terminal kinase 1 pathways facilitates SIRT1 translocation to the nucleus. After nucleocytoplasmic shuttling, SIRT1 begins to regulate several target genes: (1) SIRT1 activates the α-MHC gene promoter, thus enhancing α-MHC expression, providing higher ATPase activity and a faster shortening velocity of cardiac myofibers; (2) SIRT1 activates FOXOs, which in turn induce Rab7 expression, thereby stimulating autophagosome-lysosome fusion to maintain homeostatic functions in cells; (3) activation of FOXO1 alters the cell cycle to prevent apoptosis, as SIRT1 increases FOXO1’s ability to induce cell cycle arrest but inhibits FOXO1’s ability to induce cell death; (4) SIRT1 promotes myocardial hypertrophy and reduces infarct size via a FOXO3a-related pathway; (5) SIRT1 increases the expression of erythropoietin via a HIF-2a-mediated pathway, leading to red blood cell production and angiogenesis, thus improving blood supply; and (6) through several pathways, such as FOXOs, HIFs, PGC-1α, mitogen-activated protein kinase, NF-κB, eNOS, and p53, SIRT1 increases the expression of various antioxidants to resist oxidative stress. In studies of obesity-associated metabolic diseases, cancer, prion-mediated neurodegeneration, inflammation, and placental cell survival, other mediators have been reported to participate in SIRT1-dependent actions, including liver X receptor, peroxisome proliferator–activated receptor γ, cAMP-responsive element–binding protein–regulated transcription coactivator 2, period homolog 2, serine/threonine kinase 11, etc. However, all of these mediators have not been investigated in myocardial ischemia; therefore, further investigation is necessary. Meanwhile, these activities constitute an interactive network that protects the heart against ischemia. In this network, we can find that different pathways have intersections, and these intersections may suggest new targets of myocardial ischemia.

In summary, increasing lines of evidence suggest that SIRT1 protects the heart from ischemia-related injury. Stimulating SIRT1 appears to be a promising modality for reducing the level of ischemia-related injury. At present, the data indicate that a complex network of signaling mechanisms is involved in SIRT1 mediation (Figure). Its numerous regulators and signaling pathways provide researchers with many chances to explore its mechanism. However, there are many unsolved issues regarding the function of SIRT1 in the heart. Undoubtedly, more work is needed to understand the role of SIRT1 in cardiac cell biology before it can be considered as a valuable therapeutic target for translational studies of myocardial ischemia.

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**Disclosures**

None.

**References**


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