Regulation of Akt Signaling by Sirtuins
Its Implication in Cardiac Hypertrophy and Aging

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Abstract: Cardiac hypertrophy is a multifactorial disease characterized by multiple molecular alterations. One of these alterations is change in the activity of Akt, which plays a central role in regulating a variety of cellular processes ranging from cell survival to aging. Akt activation is mainly achieved by its binding to phosphatidylinositol (3,4,5)-triphosphate. This results in a conformational change that exposes the kinase domain of Akt for phosphorylation and activation by its upstream kinase, 3-phosphoinositide–dependent protein kinase 1, in the cell membrane. Recent studies have shown that sirtuin isoforms, silent information regulator (SIRT) 1, SIRT3, and SIRT6, play an essential role in the regulation of Akt activation. Although SIRT1 deacetylates Akt to promote phosphatidylinositol (3,4,5)-triphosphate binding and activation, SIRT3 controls reactive oxygen species–mediated Akt activation, and SIRT6 transcriptionally represses Akt at the level of chromatin. In the first part of this review, we discuss the mechanisms by which sirtuins regulate Akt activation and how they influence other post-translational modifications of Akt. In the latter part of the review, we summarize the implications of sirtuin-dependent regulation of Akt signaling in the control of major cellular processes such as cellular growth, angiogenesis, apoptosis, autophagy, and aging, which are involved in the initiation and progression of several diseases. (Circ Res. 2014;114:368-378.)

Key Words: aging • cardiac hypertrophy • sirtuins

In the quest to live longer and lead a healthy, disease-free life, we have invested enormous resources to understand the mechanisms of the aging process. The only proven approach that is capable of slowing the aging process and aging-associated diseases is calorie restriction.1 One of the most extensively studied signaling pathways associated with nutrition supply is insulin-like growth factor (IGF) signaling.2 The disruption of IGF-1 signaling uniformly extends the lifespan of animal species ranging from yeast to monkeys.3 Accumulating data provide overwhelming evidence that another group of enzymes, called sirtuins, is also sensitive to calorie restriction, and they also regulate IGF signaling.4 One of the key signaling mechanisms that is activated after IGFR receptor (IGF-1R) stimulation is the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, which plays a central role in cellular signaling and regulation of a variety of cellular processes. Recent advancements in cell biology have identified sirtuins as major regulators of Akt activation. In this review, we discuss the mechanisms of activation of Akt signaling by sirtuins and its implications in the development of cardiac diseases and the aging process.

Sirtuin Deacetylases

Lysine acetylation is a reversible post-translational modification process in which histone acetyltransferases transfer the acetyl moiety from acetyl coenzyme A to the ε-amino groups of lysine within a protein, resulting in its charge neutralization. The opposite reaction is performed by another group of enzymes called histone deacetylases, which remove the acetyl moiety from target proteins. Sirtuins belong to class III histone deacetylases, which need nicotinamide adenine dinucleotide for their deacetylation reaction. The name sirtuin originates from the discovery of the yeast gene, Sir2, which was originally described as a regulator of transcriptional silencing of mating-type loci, ribosomal DNA, and lifespan of yeast.3 Subsequently, as more isoforms of this gene were identified, they were named together as sirtuins. Because of dependency of sirtuins on NAD and their ability to deacetylate histones, they are considered sensors of cellular energy status and effectors of gene transcription by controlling acetylation of histones.5 With the identification of more isoforms of sirtuins, it did not take long to realize that sirtuins deacetylate not only histones but also a wide variety of transcription factors, metabolic enzymes, and signaling kinases, thereby controlling their activity.

The mammalian genome encodes 7 sirtuin isoforms (silent information regulator [SIRT] 1 to SIRT7) that vary in their tissue specificity, subcellular localization, enzymatic activity, and targets.6 SIRT1 is the prototype member of this...
SIRT3-transgenic Cardiac-specific overexpression of SIRT3 protects the heart from hypertrophic stimuli by preserving mitochondrial function. Low to moderate overexpression of SIRT1 attenuates age-dependent decline in cardiac functions in mice. However, high levels of SIRT1 expression induce cardiac hypertrophy and heart failure.

SIRT6-deficient mice are more susceptible to ischemic injury, as evidenced by increased myocyte death and exacerbated form of cardiac hypertrophy when subjected to pressure overload. SIRT6-deficient mice also show enhanced contractile function.

SIRT7-deficient mice show no basal cardiac abnormalities at birth but develop hypertrophy, fibrosis, and contractile dysfunction with age. SIRT7-deficient mice are highly susceptible to cardiac hypertrophic stimuli.

Massive concentric cardiac hypertrophy and heart failure were observed in the whole-body and cardiac-specific SIRT6-deficient mice.

SIRT7 deficiency induces spontaneous cardiac hypertrophy and inflammatory cardiomyopathy in mice.

Cardiac-specific overexpression of SIRT3 protects the heart from hypertrophic stimuli by preserving mitochondrial function.

Cardiac-specific overexpression of SIRT6 protects the heart from hypertrophic stimuli by blocking the activation of Akt signaling at the level of chromatin.
this process is equivocal. Comparable to their roles in the aging process, SIRT3 and SIRT6, but not SIRT1, expression blocks the development of cardiac hypertrophy and heart failure. Although SIRT1 activation protects cardiomyocytes from apoptosis and ischemia/reperfusion injury, the overexpression of SIRT1 in mice leads to the development of cardiac hypertrophy and heart failure. Each one of these sirtuin isoforms has been found to target Akt signaling to produce their specific cellular response. Before discussing how sirtuins control Akt activation, a brief description of Akt and its mechanism of activation is provided.

**Akt Isoforms and Their Functions**

Akt, also called protein kinase B because of its similarity with protein kinase A and C, is a serine/threonine kinase involved in the regulation of a variety of cellular functions, including metabolism, glucose uptake, proliferation, and protein synthesis, all assigned toward the single goal of cell survival. Mammals have 3 isoforms of Akt, designated Akt1, Akt2, and Akt3, all having >80% homology at the amino acid level. In vivo function of these isoforms is deduced by generating mouse mutants that lack each one of these isoforms or a combination of these. The Akt1-null mouse is growth-restricted, with proportional decrease in organ size, and shows shorter lifespan because of exacerbated apoptosis when subjected to oxidative stress. Akt2-deficient mice show reduced insulin sensitivity, whereas Akt3-null mice exhibit a 20% to 25% reduction in brain size and weight, partly because of a significant reduction in cell size and number. Combined deficiency of Akt1 and Akt2 in mice results in neonatal lethality, severe growth deficiency, muscle atrophy, and defects in adipogenesis as well as in skin and bone development. Mice deficient in both Akt1 and Akt3 are embryonically lethal and show defects in the development of the nervous system, cardiovascular system, and vasculature. Akt2-null and Akt3-null mice have normal embryonic development but are growth-restricted, with smaller brain and testis size. They also have impaired glucose metabolism. These observations underscore the unique function as well as functional redundancy among the 3 Akt isoforms. For additional information, the cardiac phenotypes of Akt knockout and transgenic mice are summarized in the Table.

**Mechanisms of Akt Activation**

Akt activation is a multistep process. It involves binding of Akt to membrane lipids, recruitment of Akt to the plasma membrane, and phosphorylation of Akt by the upstream kinase, 3-phosphoinositide-dependent protein kinase 1 (PDK1), which is also localized in the plasma membrane. Structurally, Akt consists of 3 domains, an N-terminal pleckstrin homology (PH) domain, a kinase domain, and a hydrophobic C-terminal regulatory domain. For its basal activation, Akt needs to be phosphorylated at T308 by PDK1. When Akt is inactive, intramolecular interaction between PH and kinase domains prevents the accessibility of PDK1 to T308. For PDK1 to access the kinase domain of Akt, the latter needs to undergo a significant conformational change. This happens only when the PH domain of Akt binds to phosphatidylinositol (3,4,5)-triphosphate (PIP3) molecule, which is generated from PIP2 by the activation of PI3K. PIP3 generation takes place in the inner leaflet of the plasma membrane. Once the PH domain of Akt binds to PIP3, it undergoes a conformational change, exposing its kinase domain to its upstream kinase PDK1, resulting in T308 phosphorylation and a 100-fold increase in Akt activity. For maximal activation, Akt needs to be phosphorylated at yet another site, S473, by mTORC2. mTORC2 is a multiprotein complex that consists of mTOR, Rictor, mSin1, and Protor-1. Phosphorylation of Akt at S473 further increases its activity by 10-fold. Thus, cumulatively, T308 and S473 phosphorylation augments Akt activity by 1000-fold from the basal level in response to growth factor stimulation and, customarily, these phosphorylation sites are regarded as surrogate markers of Akt activity.

**Factors That Prime Akt Phosphorylation**

Two critical steps occur before PDK1-dependent phosphorylation and activation of Akt. These are transportations of Akt to the plasma membrane and binding of Akt to PIP3. The PH domain of Akt regulates both these steps. One of the seminal studies that linked defects in Akt PH domain to disease conditions is the finding that a mutation (E17K) in PH domain increases the affinity of Akt for PIP3 and enhances Akt membrane localization. These changes render Akt hyperactive even in unstimulated NIH3T3 cells, hence, promoting their proliferation and survival. The E17K mutation of Akt induces leukemia in mice; in humans, this is associated with breast, colon, and ovarian cancers. A recent study elaborated these observations using the role of lysine ubiquitination in the activation of Akt.

**Ubiquitination Recruits Akt to the Plasma Membrane**

Ubiquitination is a reversible post-translational modification that covalently attaches ubiquitin protein to lysine residues of the target protein. This reaction was originally associated with protein recycling because ubiquitin-labeled proteins are directed to proteasome-mediated degradation. Recent studies impart degradation-independent functions to ubiquitination, including kinase activation. Tumor necrosis factor receptor–associated factor 6, an E3 ubiquitin ligase, was shown to monoubiquitinate Akt in the PH domain in response to IGF stimulation of cells. This modification helps to recruit Akt to the plasma membrane. However, the activation of Akt by tumor necrosis factor receptor–associated factor 6–mediated ubiquitination was independent of its ability to bind to PIP3, suggesting that ubiquitination does not regulate Akt binding to PIP3. In this report, it was also shown that the enhanced membrane trafficking of E17K mutants of Akt is due to the tumor necrosis factor receptor–associated factor 6–mediated ubiquitination of this additional lysine residue, leading to overall hyperubiquitination of Akt, thus promoting its tumorigenic activity. Recently, another E3 ligase, the S-phase kinase–associated protein 2, was found to be critical for ErbB receptor–mediated Akt ubiquitination and membrane recruitment in response to EGF stimulation of cells, suggesting that different growth factors target distinct E3 ligases for ubiquitination and activation of Akt.
SIRT1-Mediated Deacetylation Regulates Akt Binding to PIP3 and Activation

Another post-translational modification that regulates Akt activity is reversible acetylation. Under basal conditions, Akt is acetylated in various tissues, including heart, liver, brain, and skeletal muscle, and this modification suppresses Akt activity. The amino acids that underwent acetylation were identified as K14 and K20, both located in the PH domain of Akt (Figure 1). Deacetylation of these lysines by SIRT1 is necessary for the binding of Akt to PIP3 and for its membrane localization and activation.9 In this study, it was also shown that the PH domain of another kinase, PDK1, is acetylated. This modification hampered the binding of PDK1 to PIP3, whereas the deacetylated form of PDK1 displayed opposite results, suggesting that acetylation-dependent regulation could be a common mechanism controlling the activity of membrane lipid-binding proteins. On a similar note, another study found insulin receptor substrate 2 (IRS2) as a constitutively acetylated protein.60 IRS2 is a receptor-associated downstream effector of IGF-1R signaling. Lysine acetylation inhibits IRS2 activity, and SIRT1-dependent deacetylation increases its activity, thereby increasing the activity of Akt. SIRT1-mediated deacetylation is also necessary for phosphorylation of IRS2 by the insulin receptor (IR) kinase in hepatocytes.46 These findings suggest that SIRT1 upregulates insulin signaling and Akt activation at multiple levels. A model describing the roles of PH domain acetylation and ubiquitination for regulating Akt activation is presented in Figure 2.

Figure 1. Reversible lysine acetylation in the pleckstrin homology (PH) domain regulates Akt activation. Crystal structure of the PH domain of Akt (top). Acetylated lysine residues (Lys14 and Lys20) are shown in purple and phosphatidylinositol 3,4,5 triphosphate (P IPs3) in green. Both lysines are in close proximity to the binding pocket for PIP3. Schematic activation of Akt by silent information regulator (SIRT) 1 (bottom). Lysine acetylation of the PH domain makes Akt incapable to bind to PIP3, leading to inactivation of the kinase. SIRT1-dependent deacetylation promotes Akt–PIP3 binding and hence phosphorylation and activation of Akt by the upstream kinases (for details, see Sundaresan et al9).

SIRT3 Blocks ROS-Mediated Hyperactivation of Akt Signaling

Another sirtuin analogue implicated in regulating Akt activity and the aging process is SIRT3. SIRT3 is a mitochondrial deacetylase regulating a variety of mitochondrial functions and, therefore, is considered to be a mitochondrial fidelity protein.61 SIRT3-knockout mice do not show any noticeable phenotype at birth, but they are sensitive to stress stimuli. Because of this, it is thought that SIRT3 does not play a role in the development but rather it fine tunes the activity of its mitochondrial substrates by lysine deacetylation to protect cells from stress. SIRT3 regulates the activity of several mitochondrial enzymes, including antioxidant manganese superoxide dismutase 2 and enzymes of the electron transport chain, NADH dehydrogenase (ubiquinone) 1α subcomplex 9 in complex I, and succinate dehydrogenase complex subunit A in complex II.62–65 SIRT3-knockout mice manifest ≈50% reduced cellular ATP and increased ROS levels in many tissues, including liver and heart.63 SIRT3 knockout hearts also exhibited robust cardiac hypertrophic response after infusion of hypertrophy agonist. However, SIRT3-overexpressing transgenic hearts were resistant to hypertrophic stimuli and showed no signs of Ras–Akt activation.33 Thus, SIRT3 indirectly controls hyperactivation of Akt by regulating mitochondrial ROS production and ROS-mediated Ras–PI3K–Akt activation (Figure 2).

SIRT6 Negatively Regulates Akt Signaling at the Level of Chromatin

Recently, yet another sirtuin analogue, SIRT6, received considerable importance for its role in maintaining cellular homeostasis and regulating aging and associated diseases. SIRT6-knockout mice have shortened lifespans with metabolic defects.66–68 H3K9 and H3K56 are the 2 histone substrates of SIRT6.66–68 By deacetylating H3K9, SIRT6 controls the expression of genes, including telomere maintenance, DNA repair, inflammation, and metabolism.66,69–71 SIRT6 binds to nuclear factor-κ light chain enhancer of activated B cells and hypoxia-inducible factor 1α transcription factors to negatively regulate their target gene transcription.69,71 Recently, it was shown that SIRT6 directly controls IGF/Akt signaling at the level of chromatin through deacetylation of H3K9.34 Whole body SIRT6-knockout mice spontaneously developed cardiac hypertrophy by 2 to 3 months of age. Consistent with this observation, SIRT6 levels were reduced in different mouse models of cardiac failure as well as in human failing hearts. All these hearts showed robust activation of many transcription/translational factors and growth factors and their receptors related to IGF/Akt signaling, including IGF-1R, IR, IGF-2R, IGF-2, IRS1/2, Akt, Foxo1, mTOR, GSK3, myc, β-catenin, Elf4E, p70S6P, and S6P (Figure 3). IGF-1 levels were, however, downregulated in SIRT6-deficient hypertrophied hearts. Increased activation of IGF/Akt signaling in these hearts was due to increased expression of IGF-2, which can bind to IGF-1R, IGF-2R, and IR. In SIRT6-deficient hearts, SIRT1 was also elevated, which is necessary for deacetylation and activation of Akt. Further studies provided evidence that SIRT6 physically interacts with c-Jun and suppresses its transcriptional activity. Under stress and pathological conditions, cellular SIRT6 levels are reduced,
leading to derepression of c-Jun activity and thereby increasing expression of IGF-Akt signaling–related genes harboring c-Jun binding sites in their promoters (Figure 3). In accordance with this finding, another study reported the incidence of chronic inflammation in SIRT6-knockout mice by 7 to 8 months of age as a result of increased activity of c-Jun. Another report by Kanfi et al observed a 15% increase in median lifespan in male transgenic mice overexpressing SIRT6. This enhanced longevity of male mice was again linked to alterations in IGF/Akt signaling–related genes. All these studies provided strong evidence that SIRT6 is an endogenous negative regulator of IGF/Akt signaling at the level of chromatin. These studies together demonstrated that sirtuins act as master regulators of IGF/Akt signaling by establishing their control both at transcriptional and post-translational levels. Other factors that activate or terminate Akt signaling are summarized in Online Table I.

Implications of Akt/SIRT Interaction in Cardiac Hypertrophy

Akt represents one of the most potential therapeutic targets to meet the clinical needs of medicine today. We discussed how sirtuins act as master regulators of IGF/Akt signaling at transcriptional and post-translational levels. Here, we discuss more about how sirtuin/Akt interaction influences cardiac hypertrophic phenotype. Additionally, we discuss how sirtuin–Akt interplay modulates angiogenesis, apoptosis, autophagy, and aging, 4 conditions that influence disease aggressiveness in cardiac hypertrophy.
The role of SIRT1 in cardiac hypertrophy is complex. SIRT1 levels are upregulated in response to pressure overload and oxidative stress. High levels (12.5-fold) of SIRT1 expression induced cardiac hypertrophy and heart failure, whereas low levels of SIRT1 (7.5-fold) attenuated age-dependent increase in cardiac hypertrophy. In the pressure overload model of cardiac hypertrophy, haploinsufficiency of SIRT1 was found to be protective and overexpression of SIRT1 exacerbated cardiac dysfunction. We also observed increased cardiac protection in SIRT1-knockout mice in response to agonist-induced cardiac hypertrophy. This effect is associated with reduced Akt signaling in the heart.

SIRT3 and SIRT6 are 2 other sirtuins whose roles in cardiac hypertrophy have been elucidated. SIRT3-knockout mice spontaneously developed cardiac hypertrophic phenotype during adulthood. The overexpression of SIRT3 or maintenance of endogenous SIRT3 levels by treating mice with NAD blocked agonist-induced cardiac hypertrophic response in mice. As mentioned, the lack of SIRT3 or its reduced activation was associated with increased ROS levels and activation of Akt signaling. Similar to SIRT3, SIRT6 also acts as an antihypertrophic molecule. Cardiac-specific overexpression of SIRT6 protected mice from pressure overload and agonist-induced hypertrophy. This was achieved by downregulating the IGF/Akt signaling by the interaction of SIRT6 with c-Jun, resulting in deacetylation of H3K9. These findings reinforce the possible interplay between sirtuins and Akt in modulating cardiac hypertrophic response.

Role of SIRT/Akt in Angiogenesis

The growth and development of an organ is dependent on the coordinated reinforcement of new vasculature to the newly formed cells necessary for providing essential nutrients, macromolecules, and oxygen. When cells proliferate or grow, oxygen demand also increases. If the supply of oxygen is less, then hypoxic tissues secrete growth factors and chemokines, stimulating endothelial cells to proliferate, differentiate, and migrate, a process termed sprouting and branching. SIRT1 and Akt pathways play a cardinal role in this process. In the heart, during the development of physiological hypertrophy, although cardiomyocytes grow in size, they are adequately nourished by the development of new capillaries. Contrary to this, during pathological cardiac hypertrophy, cardiomyocyte growth outgrows capillary density, resulting in the supply of less nutrients and oxygen to the growing cardiomyocyte. SIRT1 plays a critical role in regulating sprouting angiogenesis and vascular growth. SIRT1-deficient mice displayed impaired ability to develop new blood vessels in response to angiogenic signals. Similarly, SIRT1-deficient zebra fish also showed dysregulated endothelial sprouting, vessel navigation, and vascular patterning. Although the role of SIRT1 in cardiac angiogenesis has not been studied, acute activation of Akt in the heart induces angiogenesis, whereas chronic activation inhibits the same.

One of the key factors participating in vasculature development and growth is nitric oxide (NO). NO synthesized from endothelial cells by endothelial NO synthase (eNOS) promotes vasodilation and protects vessels from atherosclerotic stimuli. eNOS is a target of both Akt and SIRT1. Akt activates eNOS by phosphorylation, and SIRT1 does the same by deacetylation, thereby functionally linking SIRT1 with Akt for maintaining endothelial cellular function and angiogenesis.

Although the role of other sirtuins in angiogenesis has not yet been explored, studies using mouse embryonic fibroblast and cancer cell lines demonstrate that SIRT3 destabilizes hypoxia-inducible factor 1α during hypoxia to reduce transcription of its proangiogenic gene vascular endothelial growth factor A. Also, a recent study implicated the role of SIRT6 in the regulation of endothelial cell function. Depletion of SIRT6 reduced the proliferation and increased the senescence of endothelial cells. This effect of SIRT6 is again associated with lower levels of eNOS mRNA and protein, thus suggesting that, similar to IGF/Akt-related genes, SIRT6 may also regulate the expression of eNOS at the level of chromatin.

Role of SIRT/Akt in Apoptosis

The proper development of an organism is dependent on the balance between cell death and cell growth. Apoptosis or programmed cell death is a well-orchestrated, gene-regulated suicide program by which unwanted or harmful cells are removed from the system. Corollary defects in apoptotic pathways are associated with a variety of human diseases such as cancer, neurodegeneration, and cardiac hypertrophy. Apoptosis plays an imperative role in the development of heart failure. Studies performed using rabbits as a model system have demonstrated that ischemia/reperfusion injury is associated with extensive apoptosis (14%) of cardiomyocytes. In human failing hearts, an apoptosis rate ranging from 0.12% to 0.70% has been reported. This small level of apoptosis is considered sufficient to cause heart failure based on the observation that in the hearts with conditionally active caspase 3, even very low levels of apoptosis (23 myocytes/105 nuclei) were sufficient to induce dilated cardiomyopathy and heart failure.

With regard to the role of sirtuins in cardiomyocyte apoptosis, SIRT1 plays an antiapoptotic role and contributes to the heart’s tolerance of oxidative stress. This effect of SIRT1 seems to be governed by its ability to shuttle between nucleus and cytoplasm under stress conditions. It is the nuclear, rather than the cytoplasmic, SIRT1 that has the antiapoptotic activity. Increased nuclear SIRT1 levels were observed in the cardiomyocytes of TO-2 hamster failing hearts, in a rat model of myocardial infarction, and in dilated cardiomyopathy patients as a compensatory mechanism to protect cells from death stimuli. In another study, reduced levels of nuclear SIRT1 were reported in aging hearts, and this was associated with impaired SIRT1 activation and reduced protection of the heart from ischemia/reperfusion injury. In agreement with this, nuclear Akt also appeared to be antiapoptotic. In cardiomyocytes, nuclear expression of Akt blocked apoptosis induced by staurosporine, deoxyglucose, and hypoxia. In addition, mice overexpressing nuclear Akt were also protected against ischemia/reperfusion injury.

Studies conducted to explore the mechanisms behind the cytoprotective effects of nuclear SIRT1 have shown that it
upregulates the activity of antioxidants and downregulates proapoptotic molecules. SIRT1 upregulates the expression of cardioprotective molecules, including manganese superoxide dismutase 2, redox status of thioredoxin-1, and B-cell lymphoma extra large. In addition, SIRT1-mediated deacetylation can negatively regulate the activity of proapoptotic molecules, including Bcl2-associated X protein and p53. Both SIRT1 and SIRT3 can deacetylate Ku70 to sequester Bcl2-associated X protein away from mitochondria, thus inhibiting apoptosis. In this process, Akt may help to maintain cellular Ku70 levels by preventing its Hdmi2-mediated degradation.

Another step in which SIRT1 and Akt can cooperate to regulate cellular survival is modification of p53 activity. p53 is an acetylated protein, and this post-translational modification is indispensable for its function. Deacetylation of p53 by SIRT1 renders it inactive. Deacetylated p53 binds to Mdm2, an E3 ubiquitin ligase, which promotes proteasome-mediated degradation of p53. Akt acts synergistically in this process by phosphorylating Mdm2 at S166 and S186 and promoting its association with p53. Another sirtuin that has been studied for its role in regulating cardiac myocyte survival is SIRT2. In contrast to the antiapoptotic role of SIRT1, ablation of SIRT2 was found to be beneficial in ischemia/reperfusion models. The hearts of SIRT2 knockout mice or wild-type mice treated with AKG2, a specific pharmacological inhibitor of SIRT2, were protected from ischemic injury. These studies suggest contrasting roles of sirtuins in the regulation of cardiomyocyte apoptosis.

Role of SIRT1/Akt in Autophagy
Autophagy is a catabolic response in which cells degrade their own components through lysosomes. This process removes dysfunctional proteins and organelles. Under stress situation, autophagy serves as a mechanism to maintain cellular metabolism by degrading damaged proteins, organelles, as well as undamaged components that are not essential for cell survival to generate amino acids and fatty acids for ATP production. Autophagy involves several sequential steps, including autophagosome nucleation, elongation, lipidation, and degradation, which are controlled by autophagy-related genes (Atgs). SIRT1 can directly interact with and deacetylate several Atg proteins, including Atg5, Atg7, and Atg8, leading to the activation of these proteins. In cardiomyocytes, glucose deprivation upregulates the activity of SIRT1 and its downstream target FOXO1, and both these factors are needed for increased autophagic flux. Cardiac-specific overexpression of a FOXO mutant, which cannot interact with SIRT1, or cardiac-specific deletion of FOXO1 significantly reduced autophagic flux, thus suggesting a role of SIRT1 in regulating autophagy in the heart.

The role of autophagy in the heart is complex; however, evidence suggests that autophagy may be an adaptive mechanism under most conditions. Autophagy is found to be upregulated in human failing hearts caused by dilated cardiomyopathy, resulting from valvular diseases or ischemic heart disease. The results obtained from the use of animal models of cardiovascular diseases contrast in terms of the role of autophagy in cardiac protection. Autophagosome nucleation requires beclin1 (Atg6). In the heart, beclin1 heterozygous knockout mice showed reduced autophagy and displayed blunted pathological cardiac remodeling in response to aortic banding as well as to ischemia/reperfusion injury. Beclin1 was shown to be downregulated in SIRT1-knockout mice, thus indicating the possible role of SIRT1 in regulating the autophagy process. Contrary to this, cardiac-specific deletion of ATG5, another target of SIRT1, led to the development of cardiac hypertrophy and failure, and dominant-negative ATG5 mutant abolished the cardioprotective effects of autophagy-inducing drug chloramphenicol. In the rat myocardial infarction model, blocking autophagy by use of bafilomycin led to exacerbated cardiac dysfunction. In another study, glucose deprivation or ischemia-induced autophagy helped to promote cell survival. Also, intermittent fasting, an intervention known to induce SIRT1, helped to reduce infarct size by 2-fold in the rat myocardial infarction model. Based on these reports, it seems that increased autophagy is a physiological or pathological response to promote myocardial cell survival and largely depends on the nature and extent of cellular stress.

A direct role of sirtuins other than SIRT1 in the regulation of autophagy has not been studied to date. But evidence suggests that autophagy may be associated with increased activation of SIRT6, because transcriptional factors, nuclear factor κ light chain enhancer of activated B cells, and activator protein 1, whose activity is negatively regulated by SIRT6, are shown to be positive regulators of autophagy. Regarding the possible connection of sirtuins with Akt, recent reports show that chronic Akt activation worsens aging-induced cardiac hypertrophy and myocardial contractile function through loss of autophagic regulation. Further studies using cardiomyocytes are needed to elucidate the conditions in which sirtuins and Akt cross-over to regulate autophagy.

Sirtuins, Akt, and Aging
Calorie restriction is the only proven approach to delay the aging process. Both SIRT1 and IGF/Akt signaling pathways are regulated by nutrition supply, and both pathways are suggested to be involved in the regulation of lifespan in many organisms. Many reports suggest that the health benefits of calorie restriction are mediated through the activation of sirtuins; however, a role of SIRT1 in this process is disputed. SIRT1-knockout mice failed to increase physical activity during calorie restriction. Also, calorie restriction exacerbated the decreased survivability of SIRT1-null mice, suggesting a positive role of SIRT1 in mediating the effects of calorie restriction. In contrast, the overexpression of SIRT1 did not extend replicative lifespan of human fibroblasts or prostrate epithelial cells, but rather caused replicative senescence in response to cellular stress. Also, calorie restriction or mutations in yeast Akt homologue, Sch9, caused dramatic chronological lifespan extension in yeast lacking Sir2.

One of the families of transcription factors whose activity is regulated by SIRT1 and that plays a role in the aging process is Foxo. Consistent with the ambiguous role of SIRT1 in lifespan extension, SIRT1 can positively and negatively regulate the activity of the Foxo family. SIRT1 activates Foxo1 and Foxo3 by deacetylation, which promotes the nuclear localization of these factors. Contrary to this, SIRT1 can also hamper
Foxo3a activity by making it a target for S-phase kinase–associated protein 2–mediated ubiquitination and degradation. In this process, Akt can synergise with SIRT1 by phosphorylating Foxo isoforms, which prevents their translocation to the nucleus, thereby abolishing their transcriptional function. In our studies, we found that SIRT1-mediated deacetylation positively regulates the activity of Akt on growth factor stimulation of cells. Therefore, we propose that in the presence of growth (insulin) signaling, SIRT1 activates Akt, resulting in phosphorylation of Foxo. This event will expel Foxo from the nucleus, thereby inhibiting its activity. In the absence of insulin signaling, the lack of Akt-mediated phosphorylation and SIRT1-mediated deacetylation will facilitate localization of Foxo into the nucleus, where it promotes the transcription of genes involved in promoting endurance, stress resistance, and longevity, thus suggesting that SIRT1 may promote longevity under calorie-restricted or growth factor–depleted conditions. But, in conditions in which nutrients are ample, SIRT1 promotes Akt signaling and cellular senescence. It should be noted that, apart from the direct activation of Akt, SIRT1 can activate IGF signaling by release of insulin from pancreas or by decreasing the expression of IGF-binding protein, an inhibitory modulator of IGF signaling.

Regarding the role of other sirtuins, health benefits of calorie restriction were also found to be mediated through the activation of SIRT3 and SIRT6. Mice lacking SIRT3 failed to show benefits of calorie restriction with regard to aging-associated hearing loss. Similarly, the protective effects of calorie restriction on oxidative stress were diminished in SIRT3-knockout mice because of reduced activity of manganese superoxide dismutase. SIRT3 activation has been linked with lifespan extension in humans because polymorphism in the SIRT3 gene promoter, which causes gene activation, was found to be associated with longevity of humans.

To date, SIRT6 is the only sirtuin whose increased expression conclusively extends the lifespan of mammals. Whole-body SIRT6-knockout mice developed aging phenotype, and SIRT6-overexpressing male mice had extended lifespan compared with their wild-type littermates. Interestingly, SIRT6 increases longevity by inhibiting IGF signaling. Transgenic mice overexpressing SIRT6 showed lower serum levels of IGF-1, which caused reduced activation of the IGF-1 signaling pathway, including reduced activity of Akt and reduced phosphorylation of Foxo1 and 3. In the heart, SIRT6 can suppress the expression of IGF/Akt signaling–related genes by interacting with c-Jun and deacetylating histone H3K9. Through this mechanism, SIRT6 blocked cardiac hypertrophic response in various mouse models of cardiac hypertrophy (Figure 3). Similarly, by inhibiting c-Jun, SIRT6 was reported to block the expression of proinflammatory genes. For the reason that cardiac hypertrophy and inflammation are associated with aging, it is conceivable to think that SIRT6 is an antiaging sirtuin whose upregulation may help to impede the development of many diseases.

**Future Perspective**

Akt and sirtuins regulate the very basis of cellular functioning, and alterations in their functions have potential lethal effects on the organism. Although both Akt and SIRT1 complement each other in function, the consequence of their interaction and its implications are not yet fully understood. Other than Akt and PDK1, the components of mTORC complex could also be regulated by SIRT1 at the post-translational level. How acetylation modulates these phosphorylation events will provide a deeper insight into the acetylation-mediated regulation of Akt activity. Furthermore, acetylation is known to regulate the activity of phosphatases, suggesting that SIRT1 may have yet another regulatory function at the level of phosphatases. More than 250 mammalian proteins possess the PH domain, and the finding that acetylation regulates the activity of 2 PH domain proteins, Akt and PDK1, could be a prelude to the existence of a similar mechanism in other PH domain proteins. The presence of SIRT1 in the plasma membrane also suggests that many other molecules in the membrane could be a target of SIRT1. Acetylation and ubiquitination counterbalance each other because both modifications occur in lysine residues, and acetylated lysine residues are immune to ubiquitination. This suggests the existence of an intricate interplay between acetylation and ubiquitination in the activation of Akt. The activation of SIRT1 also seems to be a complex process because we found that SIRT1 activates Akt only in the presence of growth factors. It is well-established that SIRT1 gets activated in conditions in which growth factors are depleted. How the same deacetylase performs contradictory functions under different cellular conditions is intriguing and worth studying further. SIRT1 and SIRT6 seem to contradict each other in cell signaling pathways associated with cellular growth. It will be fascinating to study their relationship in conditions such as calorie restriction and diseases associated with abnormal cellular growth. Implications of a direct role of SIRT6 in cellular processes, such as apoptosis, angiogenesis, and autophagy, need to be studied with the aim that inhibitors or activators of these molecules will emerge as promising drugs for the treatment of cancer and cardiac hypertrophy. The synergistic effects of SIRT1 and Akt inhibitors and SIRT6 activator could have a profound effect on the management of malignant diseases. From a cardiac hypertrophy perspective, in which cell growth needs to be regulated without cell death, it seems that the magnitude and cellular distribution of SIRT1 could influence cardiac phenotype. We think that the activation of SIRT1 in the presence of growth factor signaling may exacerbate hypertrophic response and may warrant caution while using food supplements to enhance its activity.

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**Disclosures**

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Regulation of Akt Signaling by Sirtuins: Its Implication in Cardiac Hypertrophy and Aging
Vinodkumar B. Pillai, Nagalingam R. Sundaresan and Mahesh P. Gupta

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## Supp. Table I: Molecules regulating Akt activity and cardiac functions.

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Action</th>
<th>Cardiovascular functions</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI (3,4,5) P3-dependent protein kinase-1 (PDK1)</td>
<td>PDK1 phosphorylates Akt at T308 residue and increases its activity.</td>
<td>PDK1 deficiency induces heart failure in mice due to reduced cardiomyocyte size, cardiac muscle mass and increased sensitivity of cardiomyocytes to hypoxia.</td>
<td>1-3</td>
</tr>
<tr>
<td>Mammalian target of rapamycin Complex 2 (mTORC2)</td>
<td>mTORC2 phosphorylates Akt at S473 residue and enhances its catalytic activity.</td>
<td>mTORC2 mediates pro-survival signaling in adult cardiomyocytes.</td>
<td>4, 5</td>
</tr>
<tr>
<td>Inositol hexakisphosphate kinase 1 (IP6K1)</td>
<td>IP6K1 produces diphasphoinositol pentakiphosphate which competes with Akt PH domain for binding to PIP3.</td>
<td>Inhibition of IP6Ks enhances Akt activity in mesenchymal stem cells to improve their therapeutic efficacy for treating myocardial infarction.</td>
<td>6, 7</td>
</tr>
<tr>
<td>Inositol polyphosphate multikinase (IPKs)</td>
<td>IPK physiologically generates PIP3 to activate Akt.</td>
<td>Expresses mostly in the developing heart. Homozygous IPK2-null mice are smaller than normal controls, and they die embryonically during E9.5–E10.</td>
<td>8, 9</td>
</tr>
<tr>
<td>PTEN</td>
<td>PTEN negatively regulates intracellular levels of PIP3 and thereby inhibits Akt activity.</td>
<td>Muscle-specific deletion of PTEN induces basal cardiac hypertrophy, accompanied with mild reduction in LV systolic function. However, cardiac specific deletion of PTEN protects mice from post MI cardiac remodeling.</td>
<td>10-14</td>
</tr>
<tr>
<td>SH2 domain-containing inositol 5’-phosphatases (SHIP); Inositol polyphosphate-5-phosphatase (INPP5)</td>
<td>Inositol 5- phosphatases hydrolyze PI (3,4,5) P3 and thereby negatively regulates the growth factor-mediated activation of Akt.</td>
<td>INPP5f knockout mice exhibit exaggerated hypertrophy with reactivation of the fetal genes during cardiac stress.</td>
<td>15-18</td>
</tr>
<tr>
<td>PH domain leucine-rich repeat protein phosphatases (PHLPP-1 and -2)</td>
<td>PHLPP protein phosphatases inhibit Akt by dephosphorylating it at S473 residue.</td>
<td>PHLPP-1 knockout cardiomyocytes show increased survival during ischemia/ reperfusion injury due to increased activity of Akt.</td>
<td>19-23</td>
</tr>
<tr>
<td>Protein kinase C-related kinase 2 (PRK2)</td>
<td>PRK2 directly binds and inhibits Akt by preventing phosphorylation at T308 and S473 residues.</td>
<td>The functional role of PRK2 in the heart is not known.</td>
<td>24</td>
</tr>
<tr>
<td>Protein phosphatase 2A (PP2A)</td>
<td>PP2A dephosphorylates Akt at T308 residue.</td>
<td>PP2A hyper activation leads to contractile dysfunction in the heart</td>
<td>25-28</td>
</tr>
<tr>
<td>PHLDA3</td>
<td>PHLDA3, the PH domain-only protein, directly interferes with binding of membrane lipids to Akt, thereby inhibiting Akt activity.</td>
<td>Not studied in cardiomyocytes.</td>
<td>29</td>
</tr>
<tr>
<td>Protein</td>
<td>Description</td>
<td>Cardiomyocyte Study</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
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<tr>
<td>TCL1</td>
<td>TCL1 oncogene binds to the PH domain of Akt, promotes nuclear transport, and enhances its kinase activity.</td>
<td>Not studied</td>
<td></td>
</tr>
<tr>
<td>Zyxin</td>
<td>Zyxin, a cytoskeletal LIM-domain protein targets Akt into the nucleus and promotes Akt activity.</td>
<td>Zyxin promotes cardiomyocyte survival.</td>
<td></td>
</tr>
<tr>
<td>Carboxyl-terminal modulator protein (CTMP)</td>
<td>CTMP binds to the carboxyl-terminal of Akt at the plasma membrane and inhibits phosphorylation of Akt at T308 and S473.</td>
<td>Not studied</td>
<td></td>
</tr>
<tr>
<td>TRAF6 E3 Ligase</td>
<td>TRAF6-mediated lysine-63 ubiquitination of the PH domain promotes Akt membrane recruitment, and phosphorylation upon growth-factor stimulation of cells.</td>
<td>Muscle specific deletion of TRAF6 inhibits skeletal muscle wasting in mice. Endothelial deficiency of TRAF6 attenuated the development of atherosclerosis in a mouse model.</td>
<td></td>
</tr>
<tr>
<td>Skp2-SCF E3 ligase</td>
<td>Skp2-SCF E3 ligase poly-ubiquitinates Akt to promote membrane recruitment in response to EGF stimulation of cells.</td>
<td>Not studied</td>
<td></td>
</tr>
<tr>
<td>NEDD4-1 E3 ligase</td>
<td>Controls lysine-63 ubiquitin-dependent trafficking of phosphorylated AKT to perinuclear region, where it is released into cytoplasm or imported into the nucleus.</td>
<td>Not studied</td>
<td></td>
</tr>
<tr>
<td>Tetratricopeptide repeat domain 3 (TTC3) E3 ligase</td>
<td>TTC3 binds to phosphorylated Akt, facilitates its ubiquitination and degradation within the nucleus.</td>
<td>Not studied</td>
<td></td>
</tr>
<tr>
<td>Poly(ADP-ribose) polymerase-1 (PARP1)</td>
<td>Inhibition of PARP-1 increases Akt phosphorylation.</td>
<td>PARP-1 deficiency protects mice from angiotensin II-induced cardiac hypertrophy, ischemia reperfusion injury and diabetic cardiomyopathy.</td>
<td></td>
</tr>
<tr>
<td>BSD domain–containing signal transducer and Akt interactor (BSTA)</td>
<td>BSTA-Akt1 interaction promotes the mTORC2 - Akt1 association and phosphorylation of Akt1 at S473 during growth factor stimulation.</td>
<td>Not studied</td>
<td></td>
</tr>
<tr>
<td>Cylindromatosis factor (CYLD)</td>
<td>Deubiquitination of Akt by CYLD suppresses growth factor–mediated ubiquitination, membrane recruitment and activation.</td>
<td>CYLD activation inhibits inflammation and proliferation of vascular cells.</td>
<td></td>
</tr>
<tr>
<td>SIRT1</td>
<td>SIRT1 mediated deacetylation promotes Akt-PIP3 binding, membrane recruitment, and phosphorylation upon growth-factor stimulation.</td>
<td>Chronic SIRT1 activation induces cardiac hypertrophy and heart failure in mouse models. However, short term activation protects the heart from ischemia reperfusion injury.</td>
<td></td>
</tr>
<tr>
<td>SIRT2</td>
<td>SIRT2-mediated deacetylation of Akt</td>
<td>SIRT2 depletion reduces TNFα</td>
<td></td>
</tr>
<tr>
<td><strong>Promotes its phosphorylation.</strong></td>
<td><strong>Stimulated necrosis, thus reduces ischemia reperfusion injury in the mouse heart.</strong></td>
<td><strong>References:</strong></td>
<td></td>
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</tr>
<tr>
<td><strong>O-GlcNAcase</strong></td>
<td>O-GlcNAcylations of Akt disrupts its interaction with PDK1, thereby inhibiting Akt phosphorylation at T308 residue.</td>
<td>55, 56</td>
<td></td>
</tr>
<tr>
<td><strong>Glutaredoxin</strong></td>
<td>Glutaredoxin, a protein disulfide oxidoreductase reduces oxidative modification of Akt, thereby maintaining Akt phosphorylation status.</td>
<td>57-61</td>
<td></td>
</tr>
</tbody>
</table>

**References:**


