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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***
Cetern of Cardiac Cell biology and Therapeutics, Committee on Molecular Medicine, University of Chicago, Chicago, IL 60637

Abstract

Cardiac hypertrophy is a multifactorial disease characterized by multiple molecular alterations. One of these alterations is change in 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 (PIP3). This results in a conformational change that exposes the kinase domain of Akt for phosphorylation and activation by its upstream kinase PDK1 in the cell membrane. Recent studies have shown that sirtuin isoforms SIRT1, SIRT3 and SIRT6 play an essential role in the regulation of Akt activation. While SIRT1 deacetylates Akt to promote PIP3 binding and activation, SIRT3 controls ROS-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 like cellular growth, angiogenesis, apoptosis, autophagy and aging; which are involved in the initiation and progression of several diseases.

Keywords

Akt; Sirtuins; cardiac hypertrophy; aging; IGF signaling

Introduction

In the quest to live longer and lead a healthy disease free life, we have invested enormous resources to understand the mechanism of aging process. The only proven approach which is capable of retarding the aging process and aging-associated diseases is calorie-restriction[1]. One of the most extensively studied signaling pathways associated with nutrition supply is the insulin like growth factor (IGF) signaling[2]. Disruption of IGF-1 signaling uniformly extends lifespan of animal species ranging from yeast to monkeys[3]. Accumulating data provide overwhelming evidence that another group of enzymes called sirtuins are also sensitive to calorie restriction, and they too regulate IGF signaling[4]. One of the key

Address for Correspondence. Mahesh Gupta, Department of Surgery, University of Chicago, 5841 S. Maryland Avenue, Chicago, IL 60637, mgupta@surgery.bsd.uchicago.edu.

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signaling mechanisms that is activated following insulin like growth factor receptor (IGF-1R) stimulation is the PI3K/AKT pathway, which plays a central role in cellular signaling and regulation of variety of cellular processes. Recent advancements in cell biology have identified sirtuins as major regulators of Akt activation. Here, in this review we will discuss the mechanism 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 where histone acetyltransferases (HATs) transfer the acetyl moiety from acetyl coenzyme A to the -amino groups of lysine (K) within a protein, resulting in its charge neutralization. The opposite reaction is cairred out by another group of enzymes called histyome deacetylases (HDACs), which remove the acetyl moiety from target proteins. Sirtuins belong to class-III HDACs, which need NAD for their deacetylation reaction. Name sirtuin originates from the discovery of the yeast gene, silent information regulator 2 (Sir2), which was originally described as regulators of transcriptional silencing of mating-type loci, ribosomal DNA and lifespan of yeast[5]. Subsequently, as more isoforms of this gene were identified, they were named together as sirtuins. Because of dependency of sirtuins to 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 identification of more isoforms of sirtuins it did not take long to realize that sirtuins not only deacetylate histones, but also a wide variety of transcription factors, metabolic enzymes and signaling kinases, and thereby controlling their activity.

The mammalian genome encodes seven sirtuin isoforms (SIRT1 to SIRT7), which vary in their tissue specificity, subcellular localization, enzymatic activity and targets[6]. SIRT1 is the prototype member of this class which is studied the most. SIRT1 is localized in the nucleus and cytoplasm[7, 8]. Recent studies suggest that, to a lesser extent, SIRT1 is also localized in the plasma membrane, where it up regulates insulin signaling[9]. SIRT1 is implicated in the control of cell survival, apoptosis, autophagy and metabolism[10]. SIRT2 is a cytoplasmic deacetylase which deacetylates tubulin and regulates cytoskeletal reorganization, autophagy and metabolism[11-13]. The sirtuins SIRT3, SIRT4 and SIRT5 are localized in the mitochondria, though a lesser amount of SIRT3 is also present in the nucleus, where it participates in gene regulation[14, 15]. These three isoforms of sirtuins are implicated in regulating several mitochondrial-dependent metabolic pathways, including oxidative phosphorylation, ROS synthesis, urea-cycle, ATP synthesis and apoptosis[14]. SIRT6 is a chromatin associated enzyme involved in deacetylating H3K9 and H3K56, thereby regulating gene expression, cellular metabolism and the inflammatory response[16-19]. SIRT7 is localized in the nucleolus and up-regulates the RNA polymerase I dependent gene transcription[20-23]. Each of these seven sirtuin isoforms has been knocked out in mice. The results indicated that while most inbred SIRT1 knockout mice die postnatally, outbred mice survive to adulthood with retarded body size. SIRT6 knockout mice die nearly one month after birth with characteristics of multi-organ pre-mature aging[19, 24]. Similar to SIRT1, outbred SIRT6 knockout mice survive to adulthood and have retarded body size. Cardiac

phenotypes of all the SIRT knockout and transgenic mice studied so far are summarized in table-1.

In the last several years, sirtuins received significant attention because of their roles in regulating aging process, and their responsiveness to calorie restriction[1]. Calorie restriction and physical exercise robustly increase expression levels of SIRT1, SIRT3 and SIRT6[25-28]. Among them, the expression levels of SIRT3 and SIRT6 have been linked with longevity of mammals, whereas the role of SIRT1 in this process is equivocal[29-32]. Same as for their roles in the aging process, SIRT3 and SIRT6 expression blocks the development of cardiac hypertrophy and heart failure, but not SIRT1[9, 33, 34]. Although SIRT1 activation protects cardiomyocytes from apoptosis and ischemia-reperfusion injury, overexpression of SIRT1 in mice leads to development of cardiac hypertrophy and heart failure[35, 36]. Each one of these sirtuin isoform has been found to target Akt signaling to produce their specific cellular response[9, 33, 34]. Before we discuss how sirtuins control Akt activation, a brief description of Akt and its mechanism of activation is discussed below.

Akt isoforms and their functions

Akt, also called protein kinase B due to 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 towards a single goal of cell survival[37, 38]. Mammals have three isoforms of Akt, designated as Akt1, Akt2 and Akt3, all having greater than 80% homology at the amino acid level[39]. *In vivo* function of these isoforms is deduced by generating mouse mutants that lack each one of these isoforms or in combination. Akt1 null mouse is growth retarded with proportional decrease in organ size and shows shorter lifespan due to exacerbated apoptosis when subjected to oxidative stress[40, 41]. Akt2 deficient mice show reduced insulin sensitivity, whereas Akt3 null mice exhibit a 20-25% reduction in brain size and weight, partly due to a significant reduction in cell size and number[42, 43]. Combined deficiency of Akt1 and 2 in mice results in neonatal lethality, severe growth deficiency, muscle atrophy and defects in adipogenesis as well as in skin and bone development[44]. Mice deficient in both Akt1 and Akt3 are embryonically lethal, and show defects in the development of nervous system, cardiovascular system and vasculature[45]. Akt2 and Akt3 null mice have normal embryonic development but are growth retarded with a smaller brain and testis size. They also have impaired glucose metabolism[46, 47]. These observations underscore the unique function as well as functional redundancy among the three Akt isoforms. For additional information we have summarized the cardiac phenotype of Akt knockout and transgenic mice in table-1.

Mechanism 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 PDK1 which is also localized in the plasma membrane. Structurally, Akt consists of three domains, an N-terminal PH domain followed by a kinase domain and a hydrophobic C-terminal regulatory domain. For its basal activation, Akt needs to be phosphorylated at T308 by PDK1[48]. When Akt is inactive, intra molecular interaction between the PH and

kinase domains prevents accessibility of PDK1 to T308[49]. In order 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 PIP3 molecule, which is generated from PIP2 by activation of PI3-kinase (PI3K)[50]. 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[51, 52]. For maximal activation, Akt needs to be phosphorylated at yet another site S473 by mTORC-2[53]. mTORC2 is a multi-protein complex that consists of mTOR, Rictor, mSIN1 and Protor-1[54]. 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 the surrogate markers of Akt activity[55, 56].

Factors that prime Akt phosphorylation

Two critical steps occur prior to PDK1-dependent phosphorylation and activation of Akt. These are (1) Transportation of Akt to the plasma membrane and (2) 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 the PH domain increases the affinity of Akt for PIP3 and enhances Akt membrane localization. These changes render Akt hyperactive even in un-stimulated NIH3T3 cells, and hence promoting their proliferation and survival[57]. The E17K mutation of Akt induces leukemia in mice, and in humans this is associated with breast, colon and ovarian cancers[57]. A recent study elaborated these observations to the role of lysine ubiquitination in the activation of Akt[58].

Ubiquitination recruits Akt to the plasma membrane

Ubiquitination is a reversible posttranslational modification that covalently attaches ubiquitin protein to lysine residues of the target protein. This reaction was originally associated with protein recycling as ubiquitin labeled proteins are directed to proteasome-mediated degradation. Recent studies impart degradation independent functions to ubiquitination including kinase activation[57]. TRAF6 an E3 ubiquitin ligases 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[58]. Activation of Akt by TRAF6-mediated ubiquitination was however 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 TRAF6-mediated ubiquitination of this additional lysine residue, leading to overall hyper-ubiquitination of Akt, thus promoting its tumorigenic activity[58]. More recently another E3 ligase, the Skp2 was found to be critical for ErbB-receptor-mediated Akt ubiquitination and membrane recruitment in response to EGF stimulation of cells, thus suggesting that different growth factors target distinct E3 ligases for ubiquitination and activation of Akt[59].

SIRT1-mediated deacetylation regulates Akt-binding to PIP3 and hence activation

Another post translational modification that regulate 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 deacetylated form of PDK1 displayed opposite results, thus suggesting that acetylation-dependent regulation could be a common mechanism controlling activity of the 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, and thereby increasing the activity of Akt. SIRT1-mediated deacetylation is also necessary for the phosphorylation of IRS2 by the IR kinase in hepatocytes[60]. These findings suggest that SIRT1 upregulates insulin signaling and Akt activation at multiple levels. A model describing roles of the PH domain acetylation and ubiquitination for regulating Akt activation is presented in Figure 2.

SIRT3 blocks ROS-mediated hyper activation of Akt signaling

Another sirtuin analogue implicated in regulating Akt activity and the aging process is SIRT3. SIRT3 is a mitochondrial deacetylase regulating variety of mitochondrial functions and therefore 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 reason it is believed that SIRT3 does not play a role in the development, but rather it fine tunes the activity of mitochondrial substrates by lysine deacetylation to protect cells from stress. SIRT3 regulates activity of several mitochondrial enzymes including antioxidant MnSOD and enzymes of the electron transport chain, NDUFA9 in complex I and SDHA in complex II[62-65]. SIRT3KO mice manifests nearly 50% reduced cellular ATP and increased ROS levels in many tissues including liver and heart[63]. Since increased ROS levels are known to activate Ras oncogene, which indirectly activates Akt via activation of PI3K and increased synthesis of PIP3, in SIRT3KO hearts robust activation of Ras and Akt was found[33]. These hearts also exhibited robust cardiac hypertrophic response following infusion of hypertrophy agonist. On the other hand SIRT3 over expressing 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. SIRT6KO

mice have shortened lifespan with metabolic defects[19]. H3K9 and H3K56 are the two histone substrates of SIRT6[6667, 68]. By deacetylating H3K9, SIRT6 controls the expression of genes including telomere maintenance, DNA repair, inflammation and metabolism[66, 69-71]. SIRT6 binds to NF-kB and HIF1α transcription factors to negatively regulate their target gene transcription[70, 71]. Most recently, it was shown that SIRT6 directly controls IGF/Akt signaling at the level of chromatin through deacetylation of H3K9[34]. SIRT6 knockout mice spontaneously developed cardiac hypertrophy by 2-3 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 (R), 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). The IGF-1 levels were, however, downregulated in SIRT6 deficient hypertrophied hearts. Increased activation of IGF/Akt signaling in these hearts was due to increased binding of IGF-2, which can bind to IGF-1R, IGF-2R and insulin receptor (IR). In SIRT6-deficient hearts, SIRT1 was also elevated, which is needed for deacetylation and activation of Akt. Further studies provided evidence that SIRT6 physically interacts with c-Jun, recruiting it to the chromatin and suppressing transcriptional activity of c-Jun. Under stress and pathological conditions, cellular SIRT6 levels are reduced, leading to de-repression 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 accordant with this finding, another study reported the incidence of chronic inflammation in SIRT6 knockout mice by 7-8 months of age as a result of increased activity of c-Jun[72]. Another recent report by Kanfi et al observed a 15% increase in median lifespan in male transgenic mice over expressing SIRT6[30]. 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 the transcriptional and posttranslational levels. Other factors which activate or terminate Akt signaling are summarized in a supplement table (see supplement table).

Implications of Akt/SIRT interaction in cardiac hypertrophy

Akt represents one of the most potential therapeutic targets to meet clinical needs of medicine today. We have discussed how sirtuins act as master regulators of IGF/Akt signaling by regulating its activity at the 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, four conditions which influence the 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 level of SIRT1 (7.5 fold) attenuated age-dependent increase in cardiac hypertrophy[73]. In the pressure overload model of cardiac hypertrophy, haploinsufficiency of SIRT1 was found to be protective and

over expression of SIRT1 exacerbated the cardiac dysfunction[74]. We also observed increased cardiac protection in SIRT1 knockout mice in response to agonist induced cardiac hypertrophy[75]. This effect is associated with reduced Akt signaling in the heart.

SIRT3 and SIRT6 are two other sirtuins, whose role in cardiac hypertrophy is elucidated. SIRT3 knockout mice spontaneously developed cardiac hypertrophic phenotype at adult hood[33, 76]. Over expression of SIRT3 or maintenance of endogenous SIRT3 levels by treating mice with NAD blocked the agonist induced cardiac hypertrophic response in mice[33, 77]. As mentioned above lack of SIRT3 or its reduced activation was associated with increased ROS levels and activation of Akt signaling[33, 77]. Similar to SIRT3, SIRT6 also acts as an antihypertrophic molecule. Cardiac specific over expression of SIRT6 protected mice from pressure overload and agonist-induced hypertrophy. This was achieved by down regulation the IGF/Akt signaling by the interaction of SIRT6 with c-Jun, resulting in deacetylation of histone 3 at Lys9 (H3K9)[34]. These findings reinforce the possible interplay between sirtuins and Akt in modulating cardiac hypertrophic response.

Role of SIRT/Akt in angiogenesis

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[78]. When cells proliferate or grow, oxygen demand also increases[79]. If the supply of oxygen is less, hypoxic tissues secrete growth factors and chemokines that stimulate endothelial cells to proliferate, differentiate and migrate, a process termed as sprouting and branching[80, 81]. The SIRT1 and Akt pathways play a cardinal role in this process[82]. In the heart, during development of physiologic hypertrophy even though cardiomyocytes grow in size, they are adequately nourished by the development of new capillaries. Contrary to this, during pathologic cardiac hypertrophy, cardiomyocyte growth outweighs capillary density, resulting in the supply of less nutrients and oxygen to the growing cardiomyocyte[83]. 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[84]. Similarly, SIRT1 deficient zebra fish also showed dys-regulated endothelial sprouting, vessel navigation and vascular patterning[84]. Although the role of SIRT1 in cardiac angiogenesis has not been studied, acute activation Akt in the heart induces angiogenesis whereas chronic activation inhibits the same[83].

One of the key factors participating in vasculature development and growth is nitric oxide. Nitric oxide synthesized from endothelial cells by endothelial nitric oxide synthase (eNOS), promotes vasodilatation 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[84, 85], thereby functionally linking SIRT1 with Akt for maintaining the endothelial cellular function and agiogenesis[86].

Although the role of other sirtuins in angiogenesis is not yet explored, studies utilizing MEFs and cancer cell lines demonstrate that SIRT3 destabilizes HIF1α during hypoxia to reduce transcription of its pro-angiogenic gene VEGF-A[87]. 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 same as for IGF/AKT related genes, SIRT6 may also regulate the expression of eNOS at the level of chromatin[88].

Role of SIRT/Akt in apoptosis

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[89]. Corollary, defects in apoptotic pathways are associated with a variety of human diseases like cancer, neurodegeneration and cardiac hypertrophy[89-91]. Apoptosis plays an imperative role in the development of heart failure. Studies carried out using rabbit as a model system has demonstrated that ischemia reperfusion injury is associated with extensive apoptosis (14%) of cardiomyocytes[92]. In human failing hearts, apoptosis rate ranging from 0.12 to 0.70% is reported[93]. 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 level of apoptosis (23 myocytes/10^5) was sufficient to induce dilated cardiomyopathy and heart failure[94].

About the role of sirtuins in cardiomyocyte apoptosis, SIRT1 plays an anti-apoptotic role and contributes to hearts tolerance to 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 SIRT1, rather than the cytoplasmic, that has the antiapoptotic activity[8]. Increased nuclear SIRT1 levels were observed in the cardiomyocytes of TO-2 hamster failing hearts, 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 I/R injury[95]. 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. Besides, mice over expressing nuclear Akt were also protected against ischemia-reperfusion injury[96].

Studies conducted to explore the mechanism behind cytoprotective effects of nuclear SIRT1 have shown that it upregulates activity of antioxidants and downregulates proapoptotic molecules[35]. SIRT1 upregulates the expression of cardioprotective molecules including MnSOD, TrX1 and Bcl-xL[35]. In addition, SIRT1-mediated deacetylation can negatively regulate the activity of proapoptotic molecules including Bax and p53[35, 97]. Both SIRT1 and SIRT3 can deacetylate Ku70 to sequester Bax away from mitochondria thus inhibiting apoptosis[98, 99]. In this process, Akt may help to maintain cellular Ku70 levels by preventing its Hdm2-mediated degradation[100].

Another step where SIRT1 and Akt can cooperate to regulate cellular survival is modification of the activity p53. P53 is an acetylated protein and this post-translational modification is indispensable for its function[101]. Deacetylation of p53 by SIRT1 renders it

inactive[101]. Deacetylated p53 binds to Mdm2, an E3 ubiquitin ligase which promotes the proteasome-mediated degradation of p53. Akt acts synergistically in this process by phosphorylating Mdm2 at S166 and S186 and promoting its association with p53[102]. Another sirtuin which has been studied for its in 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 SIRT2KO mice or wild-type mice treated with AKG2, a specific pharmacologic inhibitor of SIRT2, were protected from ischemic injury[103]. These studies suggest the contrasting roles of sirtuins in the regulation of cardiomyocyte apoptosis.

Role of SIRT/Akt in Autophagy

Autophagy is a catabolic response, where cells degrade their own components through lysosomes. This process removes dysfunctional proteins and organelles[104]. 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 under a given circumstance 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)[104]. SIRT1 can directly interact with and deacetylate several Atg proteins, including Atg5, Atg7 and Atg8, leading to activation of these proteins[105]. In cardiomyocytes, glucose deprivation upregulates the activity of SIRT1 and its downstream target FOXO1, and both these factors are needed for increased autophagic flux[106]. 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[106].

The role of autophagy in heart is complex; however, evidence suggests that autophagy may be an adaptive mechanism under most conditions[107]. Autophagy is found to be up-regulated in human failing hearts caused by dilated cardiomyopathy resulting from valvular diseases or ischemic heart disease[108]. The results obtained from use of animal models of cardiac diseases have shown contrasting results in terms of the role of autophagy in cardiac protection. Autophagosome nucleation requires beclin1 (Atg6)[109]. In the heart, beclin1 heterozygous knockout mice showed reduced autophagy and displayed blunted pathologic cardiac remodeling in response to aortic banding as well as to ischemia reperfusion injury[110, 111]. Beclin1 is shown to be down regulated in the SIRT1 knockout mice, thus again indicating the possible role of SIRT1 in regulating the autophagy process[112]. Contrary to this, cardiac-specific deletion of ATG5, another target of SIRT1, lead to development of cardiac hypertrophy and failure, and dominant negative ATG5 mutant abolished the cardioprotective effects of autophagy inducing drug, chloramphenicol[113, 114]. In the rat myocardial infarction model, blocking autophagy by use of bafilomycin led to exacerbated cardiac dysfunction[115]. In another study, glucose deprivation or ischemia induced autophagy helped to promote cell survival[110]. Also intermittent fasting, an intervation known to induce SIRT1, helped to reduce infarct size by 2 fold in the rat myocardial infraction model[116]. Based on these reports it appears that increased autophagy is a physiological or pathological

response to promote myocardial cell survival largely depends on the nature and extend of the cellular stress.

A direct role of sirtuins other than SIRT1 in the regulation of autophagy is not studied so far. But evidence suggests that autophagy may be associated with increased activation of SIRT6, because the transcriptional factors, NFkB and AP1, whose activity is negatively regulated by SIRT6, are shown to be positive regulators of autophagy[117, 118]. 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[119]. Further studies using cardiomyocytes are needed to elucidate the conditions where sirtuins and Akt crossover to regulate autophagy.

Sirtuins, Akt and Aging

Calorie restriction is the only proven approach to lessen the aging process[1]. Both, SIRT1 and IGF/Akt signaling pathways are regulated by nutrition supply and both pathways are suggested to be involved in regulation of lifespan in many organisms. Many reports suggest that the health benefits of calorie restriction are mediated through activation of sirtuins; however a role of SIRT1 in this process is disputed. SIRT1 knockout mice failed to increase physical activity during calorie restriction[120]. Also, calorie restriction exacerbated the decreased survivability of SIRT1 null mice, suggesting a positive role of SIRT1 in mediating effects of calorie restriction[121]. In contrast, over expression of SIRT1 did not extend replicative lifespan of human fibroblasts or prostrate epithelial cells, rather caused replicative senescence in response to cellular stress[7, 122]. Also calorie restriction and/or mutations in the yeast Akt homologue; Sch9 causes dramatic chronological lifespan extension in yeast lacking Sir2[123].

One of the family of transcription factors whose activity is regulated by SIRT1 and which play a role in the aging process is Foxo[124, 125]. Consistent with the ambiguous role of SIRT1 in lifespan extension, SIRT1 can positively and negatively regulate activity of the Foxo family of factors. SIRT1 activates Foxo1 and Foxo3 by deacetylation, which promotes nuclear localization of these factors[126, 127]. Contrary to this, SIRT1 can also hamper Foxo3a activity by making it a target for skp2-mediated ubiquitination and degradation[128]. In this process Akt can synergies with SIRT1 by phosphorylating Foxo isoforms which prevents their translocation to the nucleus, thereby abolishing their transcriptional function[129].

In our studies we found that SIRT1-mediated deacetylation positively regulates the activity of Akt upon growth factor stimulation of cells[9]. We therefore propose that in the presence of growth (insulin) signaling, SIRT1 activates Akt, resulting in the phosphorylation of Foxo. This event will expel Foxo from the nucleus thereby inhibiting its activity. In the absence of insulin signaling lack of Akt-mediated phosphorylation and SIRT1-mediated deacetylation will facilitate localization of Foxo into the nucleus, where it promotes 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 where nutrients are ample, SIRT1 promotes Akt signaling and cellular senescence. It should be also noted that apart from direct activation of Akt, SIRT1 can

activate IGF signaling by release of insulin from pancreas or by decreasing the expression of IGFBP, an inhibitory modulator of IGF signaling[130, 131].

As for the role of other sirtuins is concerned, health benefits of calorie restriction were also found to be mediated through activation of SIRT3 and SIRT6. Mice lacking SIRT3 failed to show benefits of calorie restriction with regard to aging associated hearing loss[132]. Similarly, protective effects of calorie restriction on oxidative stress were diminished in SIRT3 knockout mice due to reduced activity of MnSOD[26]. SIRT3 activation has been also linked with life-span extension in humans as polymorphism in the SIRT3 gene prompter which causes gene activation was found associated with longevity of the man[29, 133].

So far, SIRT6 is the only sirtuin whose increased expression conclusively extends the lifespan of mammals. Whole body SIRT6 knockout mice develop aging phenotype, and SIRT6 over expressing mice have an extended lifespan, compared to their wild-type littermates[30, 70]. Interestingly, SIRT6 increases longevity by inhibiting IGF signaling. Transgenic mice over expressing SIRT6 shows 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 Foxo3[30]. In the heart SIRT6 can suppress the expression of IGF/Akt signaling-related genes by interacting with c-Jun and deacetylating histone H3K9[34]. 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 expression of pro-inflammatory genes[72]. For the reasons that cardiac hypertrophy and inflammation are associated with aging, it is conceivable to believe that SIRT6 is an anti-aging sirtuin whose up-regulation 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 potentially lethal effects on the organism. Although both Akt and SIRT1 complement each other in function, the consequence of their interaction and its implications is not yet fully understood. Other than T308, components of mTORC complex could also be regulated by SIRT1 at the posttranslational 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, SIRT1 may have yet another regulatory function at the level of phosphatases which need to be studied. More than 250 mammalian proteins possess the PH domain, and the findings showing that acetylation regulates the activity of two 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 counter balance each other as 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. Activation of SIRT1 also seems to be a complex process since we found that SIRT1 activates Akt only in the presence of growth factors. It is well established that SIRT1 gets activated in conditions

where growth factors are depleted. How the same deacetylase performs contradictory functions under different cellular conditions, is intriguing and worth further studying? 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 like calorie restriction and diseases associated with abnormal cellular growth. Implications of a direct role of SIRT6 in cellular processes like apoptosis, angiogenesis and autophagy needs 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 in the management of malignant diseases. From a cardiac hypertrophy perspective, where cell growth needs to be regulated without cell death, it seems that magnitude and cellular distribution of SIRT1 could influence cardiac phenotype. We believe that activation of SIRT1 in the presence of growth factor signaling can exacerbate hypertrophic response, hence warrants caution while taking food supplements to enhance its activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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the sirt3 gene, human silent information regulator sir2 homologue, and survivorship in the elderly. Experimental gerontology. 2003; 38:1065–1070. [PubMed: 14580859]

Non-Standard abbreviations and Acronyms

IGF	insulin like growth factor
IGF-1R	insulin like growth factor receptor
PI3K	Phosphatidylinositide 3-kinase
HAT	histone acetyltransferases
SIRT	silent information regulator
ROS	Reactive oxygen species
PDK1	3-phosphoinositide dependent protein kinase-1
PIP3	Phosphatidylinositol (3,4,5)-triphosphate
mTOR	mammalian target of rapamycin
TRAF6	TNF receptor associated factor
Skp2	S-phase kinase-associated protein 2
PH domain	Pleckstrin homology domain
IRS2	Insulin receptor substrate 2
MnSOD	Manganese Superoxide dismutase 2
NDUFA9	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 9
SDHA	Succinate dehydrogenase complex, subunit A
Ras	Rat sarcoma
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B cells
HIF1α	Hypoxia-inducible factor 1-alpha
H3K9	histone 3 at Lys9
eNOS	endothelial nitric oxide synthase
VEGF-A	Vascular endothelial growth factor A
TrX1	Redox status of thioredoxin-1
Bcl-xL	B-cell lymphoma-extra large
Bax	Bcl2 associated X protein
KO	Knock out
Atgs	autophagy related genes
FOXO1	Forkhead box protein O1
AP1	activator protein 1
IGFBP	Insulin-like growth factor-binding protein

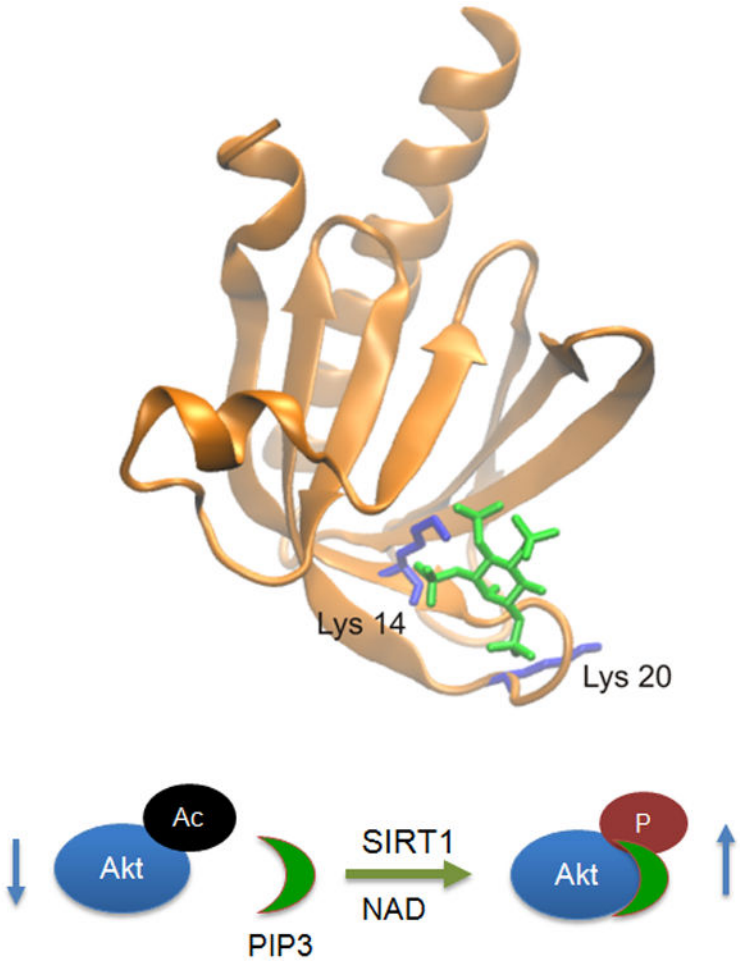

Figure 1. Reversible lysine acetylation in the PH domain regulates Akt activation

Upper panel: crystal structure of the PH domain of Akt. Acetylated lysine residues (Lys14 and Lys20) are shown in purple and PIP3 in green. Both lysines are in close proximity to the binding pocket for PIP3. Lower panel shows schematic activation of Akt by SIRT1. 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 ref 9).

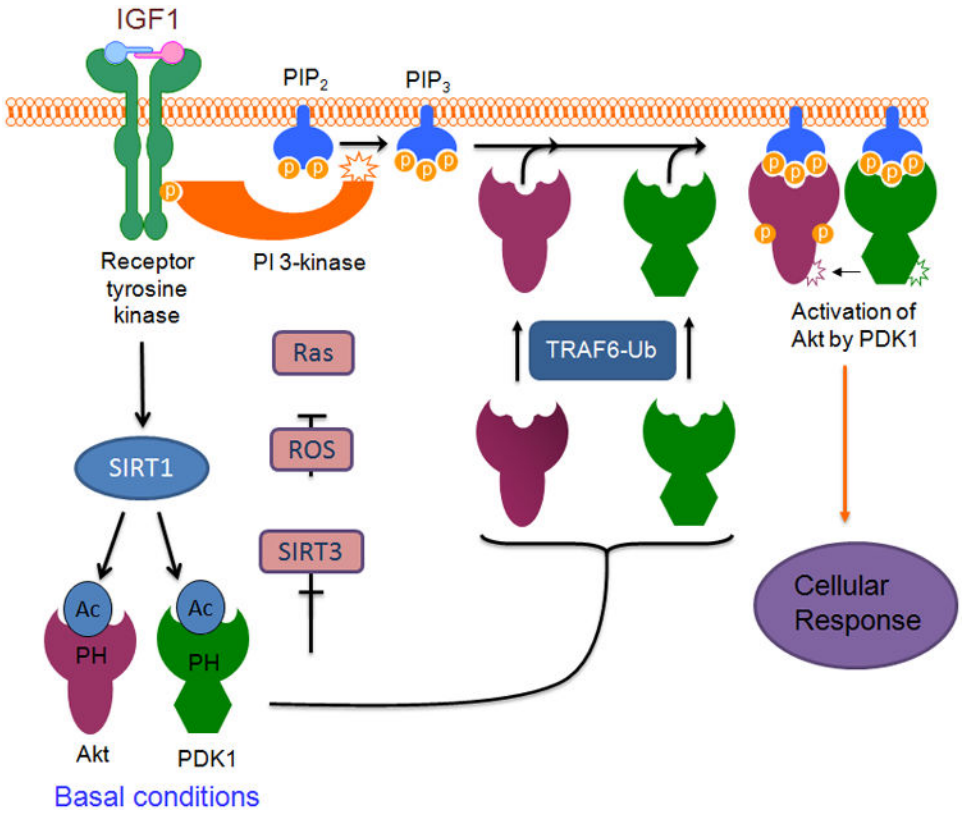

Figure 2. A model illustrating the role of SIRT1 and SIRT3 in regulating Akt activation

Under basal conditions the PH domains of Akt and PDK1 are acetylated, leading to inhibition of their binding to PIP3 and hence inactivation. During growth factor stimulation of cells, SIRT1 binds to and deacetylates Akt and PDK1 PH domians. This change enables them to bind to PIP3 which is generated from PIP2 by activation of PI3K during growth factor stimulation of cells. The PH domain deacetylation is likely to make lysine residues available for TRAF6-mediated ubiquitination, thereby facilitating ubiquitination-mediated transport of the protein to the plasma membrane (ubiquitination of PDK1 is not shown yet, but based on the PH domain acetylation studies, it seems also subject to ubiquitination, same as Akt). At the level of plasma membrane PDK1 phosphorylates and activates Akt leading to cellular response (for details see ref 9). Under oxidative stress Akt can be hyper-activated by ROS-mediated Ras activation, which activates PI3K. In this condition SIRT3 blocks Akt activation by suppressing ROS production from mitochondria (for details see ref 9 and 33).

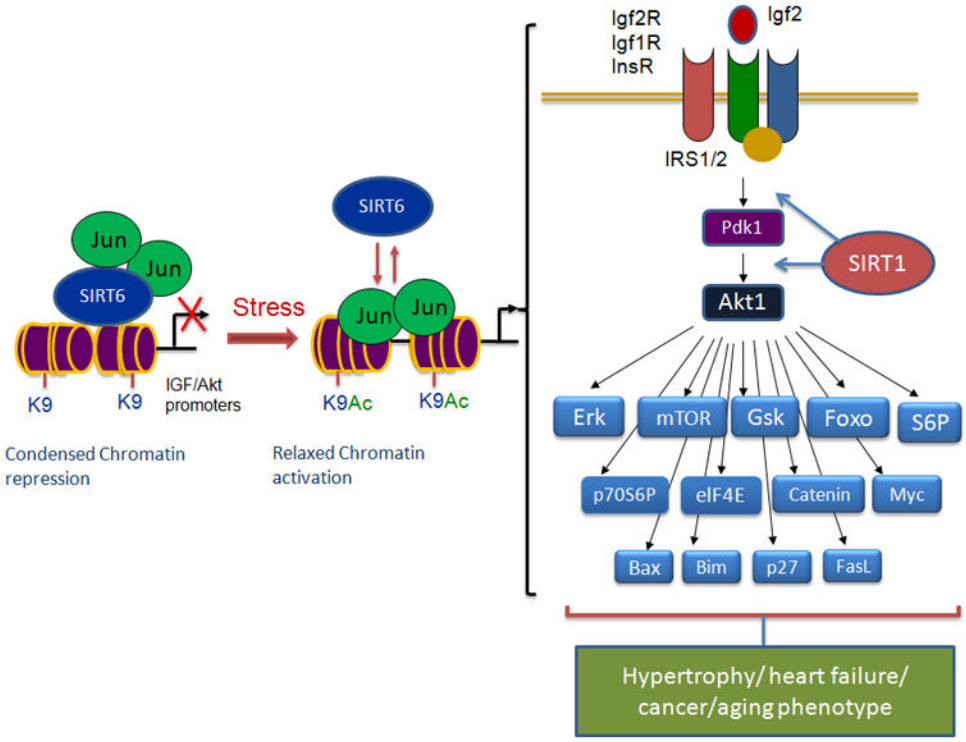

Figure 3. Schematic model showing SIRT6-depdendent transcriptional regulation of IGF/Akt signaling-related genes

Under basal conditions SIRT6 binds to chromatin as a complex with c-jun and deacetylates histones (H3K9), leading to chromatin condensation and repression of the IGF/Akt signaling-related genes. Under pathological stress, SIRT6 levels are reduced leading to increased acetylation of H3K9 at the promoters of IGF/Akt signaling related genes and c-jun mediated transcriptional activation. IGF/Akt signaling related genes which are activated in SIRT6KO hearts are shown on the right side of the figure (For details see ref 34).

Table 1
Cardiac phenotype of Akt and Sirtuins knockout and transgenic mice

Mice genotype	Cardiac Phenotype	Refs
Akt1-KO	Akt1-KO mice show reduced cardiac muscle mass and display defects in physiological growth. Mice develop an exacerbated form of cardiac hypertrophy when subjected to pressure overload.	25-27
Akt2-KO	Akt2-deficiency does not affect cardiac mass, but induces insulin resistance, mitochondrial and cardiac contractile dysfunction. Akt2-KO mice are more sensitive to myocardial injury as evidenced by increased myocyte death and inflammatory cardiomyopathy.	25, 28-32
Akt3-KO	No obvious cardiac phenotype noted.	33
Akt1-transgenic	Cardiomyocyte specific Akt expression increases myocyte size and induces cardiac hypertrophy. But Akt1 transgenic mice are protected from injury caused by transient cardiac ischemia.	34-39
Akt3-transgenic	Cardiac-specific over expression of Akt3 induces cardiac hypertrophy, and contractile dysfunction together with increasing susceptibility of the heart to cardiac injury.	40
Nuclear-Akt1 transgenic	Transgenic hearts exhibit increased number of smaller cardiomyocytes that relates with cardiomyocyte survival and enhanced contractile function.	41, 42
SIRT1-KO	Whole-body SIRT1-deficient mice show reduced heart size and are resistant to develop cardiac hypertrophy. Contrary, cardiomyocyte specific SIRT1-KO mice do not show any cardiac abnormalities at basal conditions; however, they are more susceptible to ischemic injury. Partial deficiency of SIRT1 attenuated pressure overload induced cardiac hypertrophy and failure.	43-45
SIRT2-KO	No basal cardiac abnormalities are noted, but SIRT2-KO mice are protected from ischemic injury due to attenuated programmed apoptosis in the heart.	46
SIRT3-KO	SIRT3-KO mice show no basal cardiac abnormalities at birth, but develop hypertrophy, fibrosis and contractile dysfunction with age. SIRT3 deficient mice are highly susceptible to cardiac hypertrophic stimuli.	47-49
SIRT6-KO	Massive concentric cardiac hypertrophy and heart failure were observed in the whole-body and cardiac-specific SIRT6 deficient mice.	50
SIRT7-KO	SIRT7-deficiency induces spontaneous cardiac hypertrophy and inflammatory cardiomyopathy in mice.	51
SIRT1-transgenic	Low to moderate over expression of SIRT1 attenuates age-dependent decline in cardiac functions in mice. However, high level of SIRT1 expression induces cardiac hypertrophy and heart failure.	43, 44, 52
SIRT3-transgenic	Cardiac specific over expression of SIRT3 protects the heart from hypertrophic stimuli by preserving mitochondrial function.	48
SIRT6-transgenic	Cardiac specific over expression of SIRT6 protects the heart from hypertrophic stimuli by blocking activation of Akt signaling at the level of chromatin.	50