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The role of SIRT3 in mitochondrial homeostasis and cardiac adaptation to hypertrophy and aging

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Abstract

Although acetyl-modification of protein lysine residues has been recognized for many decades, the appreciation that this post-translational modification is highly prevalent in mitochondria and plays a pivotal regulatory role in mitochondrial function has only become apparent since 2006. The classical biological stressors that modulate mitochondrial protein acetylation include alterations in caloric levels and redox signaling and the major enzyme orchestrating deacetylation is the mitochondrial enriched sirtuin SIRT3. Overall the action of SIRT3 modulates mitochondrial homeostasis and SIRT3 target proteins include mediators of energy metabolism and mitochondrial redox stress adaptive program proteins. Given these effects, it is not surprising that the role of SIRT3 has begun to be implicated in cardiac biology. This review gives a brief overview of sirtuin biology and then focuses on the role of the SIRT3 regulatory program in the control of cardiac hypertrophy and aging.

Keywords

Mitochondria; SIRT3; Cardiac Hypertrophy; MnSOD

1. Introduction

The high-density of mitochondria in cardiomyocytes reflect the high energy-demand of the heart to maintain contractile function. The functioning of these mitochondria, are in turn tightly aligned to energy transduction and to the control of calcium and redox stress homeostasis to optimize bioenergetic efficiency of the heart [1]. Accordingly, it is not be surprising that cardiac pathology is associated with the disruption of mitochondrial functioning [2, 3] and that innate adaptive programs' to ameliorate cardiac pathology incorporate pathways to resist mitochondrial injury and to restore and rejuvenate mitochondrial functioning [4–6]. Emerging evidence suggests that the post-translational modification (PTM) of mitochondrial proteins themselves may play an important role in mitochondrial homeostasis and in mitochondrial adaptation to biomechanical stressors. The regulatory programs controlling these PTM's are beginning to be explored. In this review, the role of mitochondrial protein deacetylation will be explored as one of these PTM's. Its role in modifying the function of mitochondrial proteins, the regulatory program

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underpinning this modification, the consequences of this deacetylation and the identified cardiovascular sequelae will be reviewed.

2. Mitochondrial modulation by protein deacetylation

Lysine-residue acetylation is a reversible PTM controlled by a diverse family of acetyltransferase enzymes. This modification involves the covalent transfer of an acetyl group from acetyl-coenzyme A to the ϵ -amino group on lysine. The reverse reaction is driven by deacetylase enzymes. Recognition of the dynamic flux and 'coverage' of mitochondrial protein undergoing acetylation/deacetylation was initially identified in 2006 during a proteomic screen of mitochondria extracted from liver tissue from mice comparing the fed to fasted state [7]. The acetylated peptides sequenced in that study aligned to proteins involved in all the major mitochondrial metabolic pathways and to proteins that modulated redox stress control [7]. Subsequent investigations have shown that an even a wider spectrum of mitochondrial proteins are substrates for lysine-residue acetylation and moreover, that the distribution of proteins modified in the mitochondria appear to be tissue/organ specific [8, 9]. To begin to understand the functional consequences of this PTM, the regulatory program orchestrating these events are being explored.

2.1. Regulatory proteins controlling lysine-residue acetylation

The original enzymes identified controlling lysine-residue acetylation and deacetylation were histone acetyltransferases (HATs) and histone deacetylases (HDACs). These reside in the nucleus and cytosol and are instrumental in regulating gene transcription. Numerous acetyltransferase (AT) enzymes that modify histones have recently been shown to function as non-histone AT's. These include the Gcn5-related N-acetyltransferase (GNAT) family such as Gcn5/PCAF and multiple N-acetyltransferase (NAT) proteins, Tat-interactive protein 60 (Tip60) and the p300/CBP (CREB binding protein) family [10–13]. Interestingly, Gcn5 and p300 have both been found to acetylate and inactivate the mitochondrial biogenesis master regulatory coactivator proteins – PGC-1 α and β [14–16].

The mammalian deacetylases are grouped according to phylogenic and sequence homology. The mammalian class I and II and IV enzymes predominantly function as HDACs and employ zinc as a cofactor [17]. The sirtuins are designated as class III deacetylases and are NAD⁺-dependent enzymes. The founding member of the sirtuin enzymes is yeast Sir2, which silences chromatin via deacetylation of histones [18]. Sir2 enzymes have been shown to mediate lifespan extension in yeast, worms and flies and are postulated to function, in part, via the modulation of mitochondrial function [19]. Mammals have 7 sirtuin enzymes designated as SIRT1 through SIRT7 and functionally predominantly as non-histone deacetylases. They have distinct tissue distributions and subcellular localizations which together contribute to their diverse biological and non-histone functions [20]. The mammalian sirtuins are further phylogenetically divided by homology of their amino acid core domain [21]. The mitochondrial enriched SIRT3 clusters with SIRT1 and SIRT2 in subclass I. These three enzymes show closest homology to yeast Sir2 and exhibit the most robust deacetylase activity. The additional mitochondrial enriched sirtuins SIRT4 and SIRT5 are assigned to subclasses II and III, and exhibit weaker ADP-ribosyltransferase and deacetylase activities respectively (Reviewed [22]). Additionally, emerging data shows that SIRT5 may function in lysine residue desuccinylation and demalonylation [23]. The functional consequence of these alternate modifications of mitochondrial protein lysine residues has not yet been determined and will not be discussed further in this review. Also, as mitochondrial enriched acetyltransferase enzymes have also not been elucidated, the predominant function of the acetyl-lysine PTM in the mitochondrial has been explored in the context of deacetylation. Of the two active mitochondrial deacetylases SIRT3 has been established as the major enzyme [24, 25] and its targets and the relevant biological

consequences of the activity of SIRT3 will be discussed in this review. The only target of SIRT5 functioning as a deacetylase identified to date, is a component of the urea cycle [26], and whether this isoform is relevant to cardiac biology as a deacetylase enzyme, or via its alternate activities has not been established.

2.2. Mediators of sirtuin activation

As sirtuin activity is dependent on NAD^+ , it is not surprising that sirtuin activation is directly linked to the energetic and redox status of the cell as measured by the ratio of $\text{NAD}^+:\text{NADH}$, by the absolute levels of NAD^+ , NADH , and by the NAD^+ catabolite nicotinamide [27, 28]. Interestingly, nicotinamide itself inhibits sirtuin activity and nicotinamide-depletion during NAD biosynthesis inversely activates sirtuins [29].

The NAD biochemistry in the context of sirtuin control has recently been reviewed [30]. However, in brief, the NAD salvage pathway using nicotinamide as the precursor is the dominant pathway for NAD synthesis in mammalia. Here two intermediary steps in NAD generation are initiated by the conversion of nicotinamide to nicotinamide mononucleotide (NMN) via the nicotinamide phosphoribosyltransferase (NAMPT) enzyme. Nicotinamide/nicotinic acid mononucleotide adenylyltransferase (NMNAT) then converts NMN to NAD . These biochemical pathways are most well characterized in the nucleus, and are pivotal for the activity of SIRT1 [31]. NAMPT has been identified as the rate-controlling step in NAD biosynthesis in that overexpression of *Nampt* but not *Nmnat* increased cellular NAD levels [32]. The investigation into the biology of NAD in the mitochondria has begun to be explored, and the identification of a mitochondrial-enriched NMNAT isoform, i.e. NMNAT3 supports the concept of subcellular compartment specific functioning of NAD biosynthesis [33, 34]. Moreover, mitochondrial NAD^+ levels can now be measured by mass spectroscopy and have been used to show that the metabolic stress of fasting increases mitochondrial NAMPT with a concomitant rise in mitochondrial NAD^+ levels [35].

2.3. Identified targets of SIRT3

The advancing technology of immuno-affinity peptide capture coupled to mass spectroscopy [7] has enabled the identification of growing numbers of SIRT3 substrates [36]. The functional characterization of these substrates has also been advanced by coupling proteomics to metabolomics to identify 'roadblocks' in metabolic pathways which uncover enzymes modified by SIRT3 [37]. The major pathways modulated by SIRT3 activity include the mitochondrial catabolism of substrates for the generation of reducing equivalents and ammonia, the control of the electron transfer chain and multiple pathways in the control of redox stress and the oxidation of reactive aldehydes. An overview of the characterized SIRT3 targets is reviewed here.

As SIRT3 is activated by caloric restriction or fasting, it is not surprising that SIRT3 deacetylates and activates numerous mitochondrial enzymes that facilitate the conversion of acetate to acetyl-CoA for energy production in extra-hepatic tissues and for the generation of ketones in the liver. The respective enzymes activation by SIRT3 deacetylation in modulating these pathways include acetyl-CoA synthetase 2 [38, 39] and 3-hydroxy-3-methylglutaryl CoA synthase 2 [40]. Additional metabolic pathway targets in the mitochondria include glutamate dehydrogenase which facilitates the oxidative deamination of glutamate to α -ketoglutarate, and the citric acid cycle enzyme isocitrate dehydrogenase 2 [24, 41]. SIRT3 has also been linked to the activation of fatty-acid β -oxidation with the most robust functional characterization showing SIRT3-dependent activation of long-chain acyl-CoA dehydrogenase [37, 42]. As the final common denominator in energy production from the mitochondrial derived reducing equivalents many investigators have explored whether SIRT3 activates components of the electron

transfer chain. SIRT3 activation does increase oxidative phosphorylation [25, 43, 44] and has been shown to deacetylate and activate enzymes in complex I and II of the ETC [43–45] with additional deacetylation of proteins in complex V [41, 46]. An additional metabolic pathway induced by SIRT3 under caloric restricted conditions is the urea cycle to detoxify ammonia during amino acid metabolism. The enzyme identified here is ornithine transcarbamoylase [37], although this pathway is probably not operational in the heart.

Additional programs in the mitochondria have also been shown to be modulated by SIRT3 activation under caloric-restricted conditions. Here, SIRT3 activation results in the deacetylation and inactivation of the mitochondrial ribosomal protein L10 (MRPL10) [47]. This results in an NAD-dependent inhibition of mitochondrial protein synthesis, which could be considered an energy-sparing response under nutrient restricted conditions. A pivotal mitochondrial reactive oxygen species scavenging enzyme MnSOD has also been shown to be activated by SIRT3 mediated deacetylation to reduce levels of superoxide [48–50]. The functional consequences of this ameliorative effects in the heart [51–53], as described in greater detail below, and has been described for other degenerative conditions [54]. Interestingly, the activation of citric acid cycle intermediate isocitrate dehydrogenase 2 by SIRT3 also ameliorates age-associated degeneration but here the beneficial effect was attributed to the increase in reduced glutathione levels [55]. The mitochondrial matrix peptidyl-prolyl isomerase cyclophilin D (Ppif) has also been identified as a substrate for SIRT3 mediated deacetylation [53, 56, 57]. Similarly to MRPL10, the deacetylation of cyclophilin D inhibits its activity. Our understanding of the function of cyclophilin D has expanded in recent years to not only include a role in increasing susceptibility to mitochondrial permeability transition (MPT) [58, 59], but also to the regulation of mitochondrial calcium efflux with the concordant regulation of Ca^{2+} -dependent mitochondrial enzyme activities [60]. An additional, albeit indirect, effect of SIRT3-dependent inactivation of cyclophilin D, is the dissociation of hexokinase II from the mitochondria, which plays a role in SIRT3-induced oxidative phosphorylation [56]. The role of SIRT3 mediated inactivation of cyclophilin D in the heart is described in the cardiac specific section below.

In light of the stress-ameliorative effects assigned to SIRT3 function as discussed, we would expect that SIRT3 displays adaptive effects. However, its role in tolerance against cellular stressors is not completely defined. Under certain conditions, SIRT3 has been shown to be necessary for cell survival under genotoxic stress condition in a NAD^{+} -dependent manner [35] and its overexpression in cardiomyocytes support that it may have anti-apoptotic effects [51]. In contrast, SIRT3 has been found to participate in Bcl-2 and JNK2-mediated apoptosis in several human cancer cell line [61], and Kaempferol, a natural flavonoid, induces apoptosis in K562 and U937 cell lines via activation of SIRT3 [62].

A new function of SIRT3 that extends our understanding of its context specific effects has also recently been uncovered following the identification of mitochondrial aldehyde dehydrogenase 2 (ALDH2) as a deacetylase substrate [36]. Here, the modification by SIRT3 did not alter ALDH2 activity, but rather enabled an allosteric interaction [63] whereby the deacetylated lysine residue facilitated the binding of the reactive metabolite of the xenobiotic acetaminophen to ALDH2. In this context, instead of the absence of SIRT3 giving rise in increased redox stress as described previously [46, 49, 55], the maintenance of ALDH2 acetylation enhanced resistance to acetaminophen induced hepatotoxicity [35]. This mechanism is proposed to function in the enhanced susceptibility to acetaminophen-induced injury under fasting and caloric restricted conditions [64, 65], nutrient states known to promote the activation of sirtuin enzymes.

Taken together these finding shows that SIRT3-dependent deacetylation of mitochondrial proteins function in a target substrate specific manner to: increase protein activity; to inhibit protein function and to modulate allosteric binding of reactive metabolites to SIRT3-substrates. These diverse effects are consistent with the concept that SIRT3 functions to fine-tune multiple metabolic programs in response to caloric and redox stressors [22, 66]. In extra-mitochondrial compartments, deacetylation has been shown to enable ubiquitination of the same lysine residue to modulate protein stability [67] and acetylation has been shown to facilitate protein-protein interactions [68]. Taken together this myriad of effects of the modulation of lysine-residue acetylation is exposing very intriguing biological effects of this PTM. Figure 1 shows a schematic outline the emerging role of acetylation/deacetylation on protein functioning.

3. Cardiac mitochondrial protein deacetylation

Despite the role of SIRT3 in modulating mitochondrial function, no cardiac phenotype is discernable in young SIRT3 knockout mice [24, 42]. Nevertheless, numerous biomechanical stressors and aging do uncover the homeostatic role of SIRT3 in cardiac biology. A role of SIRT3 in controlling cardiac function in aging is quite intriguing due to the predominant degenerative contribution to cardiac dysfunction and as polymorphisms in SIRT3 gene are linked to survival in the elderly [69, 70]. In this regard, the Ang II type 1 receptor (AT1A) knockout mouse which display increased life span is also associated with upregulation of SIRT3 expression in the kidney [71]. Biomechanical stressor and age-associated effects of SIRT3 are discussed below, following a brief review of how the regulatory control of SIRT3 is modified in the heart in response to biomechanical and redox stressors.

3.1. NAD biology in the heart

Firstly, levels of Nampt, the rate limiting enzyme in NAD salvage pathway is downregulated in numerous cardiac pathologies including cardiac ischemia and pressure overload [72]. In parallel generation of a cardiac specific transgene driving Nampt expression is protective against ischemia-reperfusion injury, has ameliorative effects on mitochondrial energetics and blunts the extent of apoptosis in the heart [72]. Whether these effects are exclusively due to activation of SIRT1 with indirect mitochondrial effects or whether the induction of Nampt modulates SIRT3 activity was not directly ascertained in that study. Nevertheless, in a separate study where NAD was administered to SIRT1 and SIRT3 deficient mice, anti-hypertrophic effect of NAD was found to be dependent on SIRT3 and not on SIRT1 [73]. The mechanism identified in this latter study shows that the AMPK signaling pathway is activated by NAD-dependent SIRT3 activation.

3.2. Cardiac SIRT3 regulation

The recognition that SIRT3 is modulated during cardiac stressors was initially noted in primary cardiomyocytes exposed to hypertrophic agonists [51]. The induction of SIRT3 levels by these agonists and in response to pressure overload and exercise was confirmed in the murine heart [52, 74]. Although the effects of aging on cardiac SIRT3 levels have not been determined, it has been shown that SIRT3 levels are reduced in the vastus lateralis muscle bed in elderly sedentary individuals compared to young sedentary controls [75]. Additionally, endurance exercise results in the induction of SIRT3 levels [74, 75] and this regulation can overcome the age-associated decline in skeletal muscle SIRT3 levels [75].

3.3. Direct cardiac consequences of SIRT3 manipulation

The SIRT3 targets characterized to date would support that the disruption of SIRT3 may have consequences regarding cardiac functioning. As alluded to earlier, young SIRT3 knockout mice do not have any obvious phenotype and Tong and colleagues suggest that

SIRT3 knockout mice have normal treadmill performance [74]. However, in-line with the role of SIRT3 in fine tuning mitochondrial homeostasis, it is beginning to emerge that bioenergetic stressors such as prolonged starvation may uncover the role of SIRT3 in biology [42].

This same concept is becoming evident in the heart, where the overall aging process elucidates that SIRT3 deficiency gives rise to increased cardiac dilatation [53], and where the introduction of pressure overload results in maladaptive cardiac hypertrophy in SIRT3 knockout mice [52, 53]. The mechanisms underpinning these pathologies align with the prior functions attributable to SIRT3 that have been described above, and include the increased propensity to calcium-induced mitochondrial permeability and to the increased generation of reactive oxygen species [52, 53]. The ameliorative role of SIRT3 in modulating these stressors is shown in cardiomyocytes where the overexpression of SIRT3 promotes anti-apoptotic programs [51] and in cardiac-restricted SIRT3 transgenic mice, where excess SIRT3 blunts reactive oxygen species levels and enhances the activity of ROS scavengers including SOD2 and catalase [52].

An interesting additional mechanism whereby SIRT3 deficiency could potentially contribute to the pathophysiology of cardiac hypertrophy is the role of this sirtuin in fat metabolism [42]. As the loss of metabolic plasticity with the downregulation of fatty acid oxidation (FAO) is synonymous with cardiac pressure-overload mediated decompensation [76, 77], it is feasible that the downregulation of FAO in SIRT3 knockout mice may play a role in the pressure-overload and aging maladaptive phenotype in the heart. This concept has not been explored and warrants direct investigation. Figure 2 shows the identified and potential roles whereby SIRT3 functions to modulate cardiac adaptation to pressure overload.

4. Conclusions

Our understanding of the biology of SIRT3 mediated modulation of mitochondrial homeostasis has blossomed over the last 5 years. During this time numerous targets of SIRT3 deacetylation have been identified and uncover the wide array of mitochondrial pathways regulated by the activity of this sirtuin. The bulk of emerging evidence shows that SIRT3 plays an adaptive role in mitochondrial and cellular homeostasis including in the heart. The pursuit of sirtuin agonists continues [78] and their role in cardiac pathology may prove to be a fruitful avenue for future cardiac therapeutics.

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Abbreviations

ALDH2	aldehyde dehydrogenase 2
AMPK	adenosine monophosphate kinase
CREB	cyclic AMP response element binding protein
GNAT	Gcn5-related N-acetyltransferase
HAT	histone acetyltransferase
HDAC	histone deacetylase
LCAD	long chain acetyl-CoA dehydrogenase
MnSOD	manganese superoxide dismutase

MPT	mitochondrial permeability transition
NAD	nicotinamide adenine dinucleotide
NAMPT	nicotinamide phosphoribosyltransferase
NMN	nicotinamide mononucleotide
NMNAT	NMN adenylyltransferase
PTM	post translational modification
PGC-1	peroxisome proliferator activated receptor gamma coactivator 1
ROS	reactive oxygen species
TCA	tricarboxylic acid
Tip60	Tat-interactive protein 60

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Highlights

- SIRT3 is a mitochondrial enriched protein lysine residue deacetylase
- Deacetylation of mitochondrial proteins modifies target protein function
- SIRT3 target proteins control mitochondrial energetics and ROS levels
- SIRT3 ameliorates pressure-overload and aging associated cardiac dysfunction

Figure 1.

Schematic of consequences of SIRT3 mediated lysine-residues deacetylation of mitochondrial proteins. The classic physiologic activators of SIRT3 include caloric restriction and redox signaling. In the cardiovascular system, pressure overload and hypertrophic agonists similarly upregulate and activate SIRT3. The consequences of target protein deacetylation are pleiotropic including: activation or inactivation of distinct enzymes; exposure of the lysine residue for interaction with other PTM's including for e.g. ubiquitination, or methylation; the acetylation of lysine residue facilitates binding to bromodomains of other proteins and deacetylation has recently been shown to enable reactive metabolites to bind to lysine-residues.

Figure 2.

The established role of SIRT3 in the heart. SIRT3 activation in response to hypertrophic signaling increases the activity of MnSOD and inhibits cyclophilin D. SIRT3 has also been shown to increase fatty acid oxidation in the heart with increased LCAD activity. Whether this substrate metabolism control alters cardiac pathology has not been ascertained. The cellular consequences of SIRT3 activation and the known cardiac effects of SIRT3 activation prevent adverse remodeling in response to thoracic aortic banding and prevent age-associated decline in left ventricular function are shown.