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Mitochondrial sirtuins in the heart

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Abstract Sirtuins (SIRTs) are NAD^+-dependent enzymes that catalyze deacylation of protein lysine residues. In mammals, seven sirtuins have been identified, SIRT1–7. SIRT3–5 are mainly or exclusively localized within mitochondria and mainly participate in the regulation of energy metabolic pathways. Since mitochondrial ATP regeneration is inevitably linked to the maintenance of cardiac pump function, it is not surprising that recent studies revealed a role for mitochondrial sirtuins in the regulation of myocardial energetics and function. In addition, mitochondrial sirtuins modulate the extent of myocardial ischemia reperfusion injury and the development of cardiac hypertrophy and failure. Thus, targeting mitochondrial sirtuins has been proposed as a novel approach to improve myocardial mitochondrial energetics, which is frequently impaired in cardiac disease and considered an important underlying cause contributing to several cardiac pathologies, including myocardial ischemia reperfusion injury and heart failure. In the current review, we present and discuss the available literature on mitochondrial sirtuins and their potential roles in cardiac physiology and disease.

Keywords Sirtuin · Mitochondria · Ischemia reperfusion · Heart failure

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Introduction

The heart is highly dependent on continuous delivery of ATP to maintain contractile function. 95 % of cardiomyocyte ATP regeneration stems from oxidative metabolism of fatty acids and carbohydrates within mitochondria, with minor contributions from ketone bodies, amino acids, and other substrates, depending on their availability in the blood serum [1]. Due to the large amount of myocardial ATP turnover (≈ 6 kg per day) and the necessity of metabolic flexibility to adapt to acute and chronic changes in energy demand and serum energy substrate levels, cardiac energetics is the subject of intense regulation by a number of different mechanisms. These mechanisms include hormonal regulation, posttranslational mechanisms, intracellular signaling, transcriptional regulation by transcription factors and transcriptional coactivators, and functional modulation and structural reorganization of chromatin, among others [1, 2].

A recently discovered class of regulators of energy metabolism that receives increasing attention is the sirtuin family of NAD^+-dependent deacylases [3]. The founding member of the sirtuin family, *Saccharomyces cerevisiae* Sir2, is a histone deacetylase that mediates life span extension by calorie restriction in *S. cerevisiae*, *C. elegans*, and *D. melanogaster* [4–7]. Seven mammalian orthologs have been described, SIRT1–7, and SIRT3–5 appear to be mainly or even exclusively located within mitochondria. Protein deacylation by sirtuins requires NAD^+, which is converted to nicotinamide (NAM) and 2′-O-Acyl-ADP-ribose during the enzymatic reaction. Besides the classical deacetylation reaction that is catalyzed by several sirtuins, a number of other posttranslational modifications (or lysine deacylations) have been identified that are catalyzed by sirtuins, including demalonylation, desuccinylation,

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deglutarylation, and delipoylation [8–11]. Since sirtuin function is essentially dependent on the NAD^+/NADH ratio, it is believed that this family of proteins may serve as sensors of the cellular energy status. This may be particularly important within mitochondria, which host a high number of acylated proteins and contain high levels of NAD^+ and NADH [12, 13].

Mitochondrial dysfunction contributes to a number of cardiac pathologies, including pathological hypertrophy, diabetic cardiomyopathy, ischemia reperfusion (IR) injury, and heart failure [14–16]. Despite intense investigation, the underlying mechanisms of mitochondrial dysfunction in cardiac pathologies remain incompletely understood, and the development of therapeutics to improve mitochondrial function turned out to be challenging. In the following article, we will present and discuss the available literature on mitochondrial sirtuins and their role in cardiac physiology and disease.

SIRT3 in cardiac physiology and disease

SIRT3 is the most well-characterized mitochondrial sirtuin to date, which catalyzes the classical sirtuin deacetylation reaction. Using quantitative mass spectrometry, Hebert et al. [17] detected a vast number ($\sim$400) of mitochondrial acetyl sites increasing more than twofold in the absence of SIRT3. Bioinformatic analysis of protein networks and biological processes identified an enrichment of acetylated proteins in major mitochondrial processes, including fatty acid metabolism, tricarboxylic acid (TCA) cycle activity, and electron transport. Among these, a number of enzymes have been confirmed to be deacetylated by SIRT3, including isocitrate dehydrogenase 2, glutamate dehydrogenase, complex I (Ndufa9 subunit), and long-chain acyl-CoA dehydrogenase (LCAD) [18–22]. Compatible with predicted and proven metabolic targets of SIRT3, functional studies showed increased mitochondrial respiration rates in HIB1B brown adipocytes overexpressing SIRT3 [23]. Conversely, SIRT3$^{-/-}$ mice exhibit decreased hepatic fatty acid oxidation rates, hepatic ATP production, LCAD activity, OXPHOS complex I–III activity, and ATP levels in kidney and liver [10, 12, 18, 24, 25]. Besides effects on energy metabolism, the mitochondrial antioxidant enzyme manganese superoxide dismutase 2 (MnSOD or SOD2) has also been identified as a SIRT3 target [26, 27]. SIRT3 deacetylates several lysine residues of MnSOD, thereby increasing MnSOD activity and detoxification of superoxide radicals [26, 27]. Thus, it is likely that SIRT3 serves to increase oxidative ATP regeneration by coordinated activation of mitochondrial metabolic pathways, and to increase mitochondrial ROS detoxification.

SIRT3 is highly expressed in the heart [20, 23]. Rates of fatty acid oxidation, glucose oxidation, oxygen consumption, mitochondrial respiration rates, ATP levels, ATP synthesis, and activities of OXPHOS complexes were decreased in hearts of SIRT3$^{-/-}$ mice, accompanied by increased acetylation of numerous proteins of the fatty acid oxidation spiral, TCA cycle, and OXPHOS chain [18, 28]. Based on studies in other tissues and cells and the assumption that deacetylation of SIRT3 generally increases enzymatic activity of its target proteins, these metabolic alterations may suggest that SIRT3 is required to maintain oxidative metabolism in the heart, and that increased acetylation of various metabolic enzymes due to the lack of SIRT3 activity may downregulate oxidative metabolism. This conclusion needs to be drawn carefully though since many proteins are potential SIRT3 targets but the functional effect of SIRT3-mediated deacetylation remains to be tested experimentally for many of them. In addition, although some studies investigated specific lysine residues by site-directed mutagenesis and demonstrated the functional consequence for enzyme activity, most proteins contain several lysine acetylation sites, but not all acetylation sites have been tested for their functional impact on the enzyme activity [18, 19]. In fact, increased acetylation of LCAD in SIRT3$^{-/-}$ tissue has been reported to be increased or decreased [19, 29].

The above-mentioned myocardial metabolic alterations in SIRT3$^{-/-}$ mice are associated with a mild cardiac dysfunction in ex vivo perfused hearts at early ages, which may not yet be readily detectable in vivo by echocardiography due to neurohumoral compensatory mechanisms [28, 30]. When SIRT3$^{-/-}$ mice age, cardiac dysfunction is more pronounced and also detectable by echocardiography [28]. When subjecting SIRT3$^{-/-}$ mice to a chronic increase in energy demand by transverse aortic constriction, the development of cardiac dysfunction is accelerated compared to WT mice, supporting the conclusion that compromised energy substrate utilization may be causally involved in the development of contractile defects in SIRT3$^{-/-}$ mice [28]. SIRT3 may serve as redox-sensitive rheostat that regulates ATP-generating metabolic pathways by coordinated changes in lysine acetylation of various energy metabolic enzymes. Changes in myocardial energy demand would transiently alter the myocardial NAD^+/NADH ratio, and SIRT3 may serve to translate these changes into appropriate changes in protein acetylation to synchronize ATP regeneration with ATP demand. Such changes in energy demand may include physical exercise or physiological changes in blood pressure. NAD^+ levels also oscillate in a circadian fashion in many cell types to synchronize oxidative metabolic pathways with the 24-h fasting and feeding cycle [31]. Thus, it can be speculated that SIRT3 may even be involved in the synchronization of

myocardial energetics with changes in physical activity during day and night times.

A number of studies suggest that SIRT3 may play a role in the development of cardiac hypertrophy and heart failure. Mice lacking SIRT3 develop spontaneous cardiac hypertrophy [28, 32]. In addition, SIRT3$^{-/-}$ mice show exacerbated cardiac hypertrophy following transverse aortic constriction or treatment with hypertrophy agonists such as phenylephrine, isoproterenol, or angiotensin II, accompanied by increased cardiac fibrosis [28, 30, 32, 33]. Furthermore, agonist-induced cardiac hypertrophy and fibrosis can be blocked by overexpression or activation of SIRT3, suggesting that SIRT3 is needed to prevent stress-induced cardiac hypertrophy [32, 34]. A number of mechanisms may be responsible for exacerbation and inhibition of cardiac hypertrophy by SIRT3 deficiency and overexpression, respectively. One obvious mechanism may be energy depletion due to the impairment of metabolic substrate oxidation, resulting in energy depletion and the inability to adapt to increased energy demands [18, 28]. Another mechanism may include attenuation of the ROS-sensitive mitogen-activated protein kinase (MAPK)/extracellular-signal regulated kinase (ERK) pathway and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt pathways by SIRT3, which are known to play a major role in the development of cardiac hypertrophy [32, 35–38]. Activation and nuclear translocation of forkhead box protein 3a (Foxo3a) into the nucleus and subsequently enhanced expression of MnSOD and catalase may reduce ROS and may thus attenuate signaling of the above-mentioned pathways [32]. Furthermore, SIRT3 may protect from cardiac hypertrophy by activating AMP-activated protein kinase (AMPK). AMPK upregulates catabolic ATP-regenerating pathways in the heart, such as glycolysis and fatty acid oxidation, and simultaneously inhibits anabolic ATP-consuming pathways [39]. AMPK is also capable of inhibiting mTOR, thereby decreasing protein synthesis and inhibiting hypertrophic growth, whereas cardiomyocyte-specific deletion of the AMPK upstream kinase, liver kinase B1 (LKB1), decreases AMPK activity and induces cardiac hypertrophy and dysfunction [40–43]. Pillai et al. [30] demonstrated that SIRT3 can interact with and deacetylate LKB1 in the heart, resulting in the activation of LKB1 and AMPK. Activation of AMPK by exogenous supplementation of NAD$^+$ rescued the heart from ATP depletion and blocked the activation of pro-hypertrophic Akt signaling [30]. Finally, SIRT3$^{-/-}$ but not SIRT3-overexpressing mice develop age-associated cardiac fibrosis, likely due to impaired deacetylation and thus inhibition of glycogen synthase kinase 3β (GSK3β) and subsequent induction of profibrotic transforming growth factor β1 (TGF-β1) signaling [34].

The dependence of SIRT3 on the NAD$^+$/NADH ratio may determine the function of SIRT3 during hypertrophy and heart failure. The NAD$^+$/NADH ratio seems to be increased during compensated cardiac hypertrophy, which should increase SIRT3 activity and may potentially even contribute to induction of SIRT3 in these hearts [28, 44]. This adaptation is likely beneficial and may reflect a feedback mechanism to increase and maintain high rates of ATP synthesis in order to adapt to increased energy demands. In contrast, overactivation of the NAD$^+$-consuming DNA repair enzyme poly(ADP-ribose)-polymerase 1 (PARP-1) may lead to increased consumption and thus depletion of NAD$^+$ during decompensated hypertrophy and heart failure [45]. Indeed, lysine acetylation is increased in human failing hearts, even accompanied by reduced SIRT3 expression [45, 46]. Another attractive hypothesis for decreased NAD$^+$ levels and decreased SIRT3 activity in heart failure may be drawn from the study published by Karamanlidis et al. [47] who generated a mouse model of mitochondrial dysfunction by inactivation of the Ndufs4 gene, resulting in defective complex I activity and heart failure in response to pressure overload stress. Decreased NADH oxidation by complex I resulted in a decreased NAD$^+$/NADH ratio and thus impaired SIRT3 activity, and supplementation with the NAD$^+$ precursor nicotinamide mononucleotide (NMN) normalized the NAD$^+$/NADH ratio, the protein hyperacetylation, and the SIRT3-mediated increase in mPTP sensitivity [47]. Therefore, it may be concluded that a preexisting mitochondrial dysfunction, in this case complex I dysfunction, may lead to a relative decrease in NAD$^+$ depletion, thereby impairing SIRT3 activity and further exacerbating mitochondrial dysfunction. Whatever may be the cause of NAD$^+$ depletion, the resulting impairment in SIRT3 activity and/or reduced SIRT3 expression may exacerbate mitochondrial dysfunction and thus accelerate the development of cardiac failure. Indeed, several studies demonstrated increased hypertrophy, accelerated development of cardiac dysfunction, and decreased survival of SIRT3$^{-/-}$ mice following transverse aortic constriction [28, 32, 33]. Furthermore, exogenous application of NAD$^+$ was capable of maintaining intracellular NAD$^+$ levels and of blocking agonist-induced cardiac hypertrophy, and honokiol treatment, which increases expression and activity of SIRT3, blocked the agonist-induced and pressure overload-induced cardiac hypertrophic response in mice [34]. Thus, prevention of NAD$^+$ depletion during development of heart failure deserves to be explored as a potentially novel and clinically applicable therapeutic strategy [30].

SIRT3 may also modulate the extent of myocardial IR injury. In the Langendorff model, seven-month-old mice with heterozygous deletion of SIRT3 (SIRT3$^{+/-}$) showed impaired recovery of cardiac function and larger infarct

size following 25 min of ischemia [48]. Interestingly, recovery from IR injury in seven-month-old SIRT3$^{+/-}$ hearts was similar as in 18-month-old wild-type hearts [48]. Similarly, the extent of increased mitochondrial protein acetylation was similar in seven-month-old SIRT3$^{+/-}$ and 18-month-old wild-type hearts, suggesting that myocardial SIRT3 activity is similar in aged wild-type mice and younger SIRT3$^{+/-}$ mice [48]. In contrast, recovery of contractile function following IR and myocardial infarct size following LAD ligation in vivo were not different between eight-week-old homozygous SIRT3$^{-/-}$ and wild-type mice [49]. These findings may suggest that compensatory mechanisms may attenuate IR injury in homozygous SIRT3$^{-/-}$ mice, or that SIRT3 deficiency may actually not increase IR injury. However, the rather contradictory results of the above-mentioned studies may be compatible when considering the age difference in the animals and the mechanism by which SIRT3 may modulate IR injury, as follows.

SIRT3 has been shown to deacetylate the regulatory component of the mPTP, cyclophilin D (CypD), on lysine 166 [33]. Increased acetylation of cyclophilin D in the absence of SIRT3 is believed to promote interaction of cyclophilin D with the mPTP pore, thereby increasing the sensitivity for mPTP opening [33]. Opening of the mPTP is an essential determinant of myocardial IR injury, and mice lacking SIRT3 exhibit an age-dependent increase in mitochondrial swelling, associated with increased mPTP opening [33]. Bochaton et al. [50] recently showed that SIRT3 overexpression prevented cyclophilin D acetylation, limited mPTP opening, and reduced cell death in H9C2 cells subjected to hypoxia–reoxygenation. They also showed that cardiac ischemic postconditioning could not reduce infarct size and cyclophilin D acetylation in SIRT3$^{-/-}$ mice [50]. These studies support the conclusion that impairment in SIRT3 may increase the sensitivity for mPTP opening and thereby modulate myocardial IR injury. Of interest, a number of studies reported of increased sensitivity of cardiac mitochondria for mPTP opening in aged animals, and in parallel the susceptibility of the heart to damage from IR injury also increases with age [51–58]. In addition, numerous studies demonstrated additional mitochondrial alterations in aged hearts, including structural derangements, increased ROS generation and oxidative damage, and impairment in mitochondrial function [59, 60]. Among others, increased ROS generation is also a powerful trigger for mPTP opening [15]. Thus, it is tempting to speculate that absence of SIRT3 per se may not yet be sufficient to increase the vulnerability to IR injury in young mice. However, in the presence of generally increased susceptibility for mPTP opening and the presence of additional age-related changes in mitochondrial function such as increased mitochondrial oxidative stress, a

decrease in SIRT3 activity may predispose the aged heart for increased opening of the mPTP, in particular following IR.

During postischemic reperfusion of the heart, mitochondrial NAD$^+$ levels decrease, suggesting that SIRT3 activity may be compromised during reperfusion and may contribute to the extent of IR injury [61, 62]. A number of mechanisms that contribute to IR injury may be contributed to by decreased SIRT3 activity. First, as discussed above, impairment in SIRT3 activity may increase mPTP opening due to decreased deacetylation of cyclophilin D [33]. Second, impairment in SIRT3 activity may result in decreased deacetylation and thus inhibition of MnSOD, thereby impairing detoxification of superoxide. As a consequence, mitochondrial oxidative stress will be increased, which will promote mPTP opening and oxidative damage within mitochondria [26]. Third, decreased SIRT3 activity may lead to a concerted downregulation of mitochondrial oxidative capacity due to hyperacetylation of numerous enzymes participating in the major pathways of mitochondrial metabolic substrate oxidation, resulting in delayed recovery of ATP synthesis during reperfusion and potentially a further increase in ROS generation during reenergization of a defective electron transport chain. Thus, preventing myocardial mitochondrial NAD$^+$ depletion during IR may represent a useful approach to ameliorate detrimental effects of myocardial IR. Indeed, increasing myocardial NAD$^+$ content by overexpression of the rate-limiting enzyme for NAD$^+$ synthesis, nicotinamide phosphoribosyltransferase (Nampt), or by administration of NMN, resulted in reduced myocardial infarct size in response to ischemia reperfusion [63, 64].

Taken together, the available studies on SIRT3 in the heart suggest potentially protective effects of endogenous SIRT3 or SIRT3 activation in the heart. SIRT3 may protect against the development of pathological hypertrophy and may prevent decompensation to overt heart failure. SIRT3 may also protect against IR injury in the heart. These potentially beneficial effects of SIRT3 and SIRT3 activation as well as proposed underlying mechanisms of these beneficial effects are summarized in Fig. 1.

SIRT4 in cardiac physiology and disease

Much fewer studies have been published on SIRT4. In contrast to SIRT3, the in vitro deacetylation activity seems to be much lower; however, other posttranslational modifications have been reported for SIRT4, such as delipoylation and ADP-ribosylation, although the latter one is thought to be a side reaction of the main deacylation activity without significant physiological relevance [8, 65–68]. Similar to SIRT3, SIRT4 exerts a number of
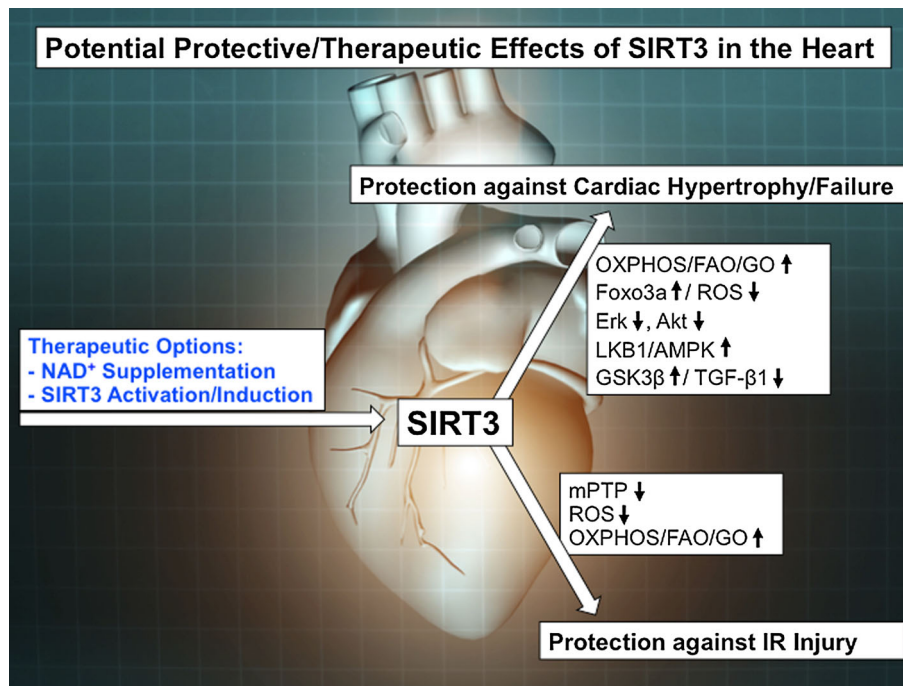
Fig. 1 Potential protective/therapeutic effects of SIRT3 in the heart. NAD^+ supplementation and/or activation of SIRT3 and/or induction of SIRT3 expression may protect against the development of cardiac hypertrophy and failure by increasing mitochondrial oxidative capacity, increasing nuclear translocation of Foxo3a and detoxification of ROS, inhibition of prohypertrophic Erk and Akt signaling, activation of AMPK, and inhibition of profibrotic TGF-β1 signaling. Protection against IR injury may be mediated by desensitization of mPTP opening, increased detoxification of ROS, and increased mitochondrial oxidative capacity. *OXPHOS* oxidative phosphorylation, *FAO* fatty acid oxidation, *GO* glucose oxidation

effects on energy metabolism. SIRT4 has been shown to inhibit glutamate dehydrogenase in pancreatic beta-cells by ADP-ribosylation, resulting in decreased insulin secretion in response to amino acids [67]. SIRT4 also coimmuno-precipitates with insulin-degrading enzyme and may thereby suppress insulin secretion in response to glucose, suggesting that SIRT4 regulates systemic glucose disposal by modulating insulin secretion [66]. Furthermore, SIRT4 enzymatically hydrolyzes the lipoamide cofactors from the E2 component dihydrolipoyllysine acetyltransferase (DLAT) of the pyruvate dehydrogenase (PDH) complex, thereby diminishing PDH activity and compromising intracellular glucose oxidation [8]. Interestingly, the authors also showed that SIRT4 catalytic efficiency for lipoyl–lysine and biotinyl–lysine modifications may be superior to its deacetylation activity. Besides effects on glucose disposal and utilization, SIRT4 also regulates lipid metabolism due to the ability to deacetylate malonyl-CoA decarboxylase (MCD) at K471, thereby inhibiting its activity and increasing malonyl-CoA levels [69]. Conversely, mice lacking SIRT4 display elevated MCD activity, decreased malonyl-CoA levels, resulting in increased fatty acid oxidation and decreased lipogenesis in skeletal muscle and white adipose tissue [69]. These mice show increased exercise tolerance and are protected against diet-induced obesity [69]. Similarly, knockdown of SIRT4 also resulted in increased fatty acid oxidation and cellular respiration in liver and muscle cells, maybe driven by increased expression of genes encoding for enzymes of fatty acid oxidation [70]. Interestingly, $SIRT4^{-/-}$ mice display increased rates of fatty acid oxidation and increased expression of PPARα and fatty acid oxidation genes in the liver, and increased oxidation rates seem to require functional SIRT1, suggesting a cross talk between mitochondrial and nuclear sirtuins [71]. Finally, it has been shown that SIRT4 expression is induced by numerous genotoxic agents and represses the metabolism of glutamine into the TCA cycle, thereby impairing TCA cycle anaplerosis [72]. Thus, the metabolic role of SIRT4 may be to inhibit oxidation of glucose and fatty acids, and may impair TCA cycle anaplerosis and function, which should result in impaired ATP synthesis and energy depletion.

Although SIRT4 is highly expressed in the heart, studies investigating the metabolic function of SIRT4 in the heart are lacking. Nevertheless, some studies suggest that SIRT4 may modulate the outcome following myocardial ischemia reperfusion. Liu and colleagues demonstrated in H9C2 rat heart myoblasts that hypoxia downregulates the expression of SIRT4 [73]. In this study, knockdown of SIRT4 decreased cell viability, increased caspase activation, and

increased the amount of apoptotic cells, whereas SIRT4 overexpression increased cell viability, inhibited caspase activation, and decreased the amount of apoptotic cells, suggesting that downregulation of SIRT4 during hypoxia may contribute to increased apoptotic cell death in response to hypoxia and ischemia reperfusion [73, 74]. In contrast, suppression of SIRT4 by siRNA protects against induction of mPTP opening and mPTP-dependent ROS production in HeLa cells, suggesting that suppression of SIRT4 may exert beneficial effects during hypoxia and ischemia reperfusion injury [75]. The impact of SIRT4 suppression during hypoxia and ischemia reperfusion on mitochondrial oxidative capacity and cardiac recovery remains speculative. Inhibition of SIRT4 activity could be adaptive since recovery of myocardial energetics may be improved following hypoxia and ischemia due to disinhibition of glucose and fatty acid oxidation. In contrast, increased oxidative capacity may increase mitochondrial ROS generation during reenergization of the electron transport chain shortly after the onset of reperfusion and may thereby exaggerate IR injury. Studies investigating myocardial IR injury in mice with genetic manipulation of SIRT4 expression are much needed to understand the role of SIRT4 in the context of IR in the heart.

SIRT5 in cardiac physiology and disease

Similar to SIRT4, SIRT5 posseses only weak deacetylase activity. SIRT5 has been found to deacetylate carbamoyl phosphate synthetase 1 (CPS1), thus participating in the detoxification of ammonia [76]. Cytochrome c and peroxiredoxin 1 were identified as further deacetylation targets of SIRT5, potentially suggesting a role for SIRT5 in apoptosis, mitochondrial respiration, and redox regulation [22, 77, 78]. More importantly though, SIRT5 has been shown to function as an efficient protein lysine desuccinylase and demalonylase [79–81]. Park and colleagues identified 2565 succinylation sites on 779 proteins using systematic profiling of the mammalian succinylome, and Rardin et al. identified 1190 succinyllysine sites on 252 proteins, 56 % of which seem to be SIRT5 target proteins [13, 81]. Pathway analyses revealed that many proteins identified to be SIRT5 targets are involved in mitochondrial metabolic pathways, including amino acid degradation, the TCA cycle, fatty acid oxidation, pyruvate decarboxylation, and ATP synthesis [13, 81]. Regarding malonylation, Nishida et al. identified 1137 malonyllysine sites across 430 proteins, with 120 proteins that also showed increased malonylation in SIRT5$^{-/-}$ animals. A pathway analysis identified glycolysis as the top SIRT5-regulated pathway [82]. While numerous potential SIRT5 targets have been identified, elucidation of the functional

consequences and physiological relevance appears challenging, and controversial results have been reported. Lack of SIRT5 was shown to decrease palmitate oxidation in mouse embryonic fibroblasts and primary hepatocytes of SIRT5$^{-/-}$ mice, and to impair the activity of enoyl-CoA hydratase α (ECHA) in the heart, suggesting that SIRT5 may serve to increase fatty acid oxidation [83]. Less conclusive are data on mitochondrial respiration and glucose utilization. Overexpression of SIRT5 increased ATP production and oxygen consumption in HepG2 cells, but a higher mitochondrial respiration rate was also observed in hepatocytes of SIRT5$^{-/-}$ mice and in 293T cells with SIRT5 knockdown [13, 84]. Regarding glucose utilization, decreased glycolytic flux was reported in primary hepatocytes from SIRT5$^{-/-}$ mice, maybe due to increased malonylation of glyceraldehyde-3-phosphate dehydrogenase, whereas knockdown of SIRT5 increased PDH activity due to increased succinylation of multiple PDH subunits [13, 82]. Besides metabolism, SIRT5 desuccinylated and activated superoxide dismutase 1 (SOD1) and may thus boost cellular antioxidant activity [85]. Finally, SIRT5 has been shown to catalyze protein lysine glutarylation. Tan et al. identified 229 proteins with hyperglutarylation in liver tissue of SIRT5$^{-/-}$ mice, and a biological pathway analysis of the lysine glutarylome revealed again fatty acid metabolism, TCA cycle, and aerobic respiration as major pathways, although the functional consequences for each pathway remain to be elucidated [9].

Similar to the other mitochondrial sirtuins, SIRT5 is highly expressed in cardiac tissue. A very recent study by Sadhukhan et al. [83] revealed that succinyl-CoA is the most abundant acyl-CoA molecule in the heart, suggesting that lysine succinylation may be relevant for cardiac physiology. Indeed, mice with deletion of SIRT5 showed cardiac contractile dysfunction, pathologic hypertrophy, and increased fibrosis [83]. Analysis of the cardiac succinylome in SIRT5$^{-/-}$ mice also suggested mitochondrial energy metabolic pathways to be regulated by SIRT5, and that myocardial energy depletion may contribute to cardiac dysfunction in SIRT5$^{-/-}$ mice, potentially contributed to by hypersuccinylation and inhibition of ECHA [83]. Thus, similar to SIRT3, SIRT5 may be required to maintain cardiac function and energetics. The study also implies that SIRT5 may protect from the development of cardiac hypertrophy, although gain-of-function studies need to be conducted for further conclusions.

Again similar to SIRT3, lack of SIRT5 exacerbates IR injury. Myocardial infarct size was increased, and recovery of contractile function was impaired in hearts of SIRT5$^{-/-}$ mice following IR [86]. IR injury was restored to WT levels by inhibition of succinate dehydrogenase, which was hypersuccinylated and probably more active in SIRT5$^{-/-}$

hearts [13, 86]. Since succinate accumulates during ischemia and drives mitochondrial ROS generation at the onset of reperfusion by increasing reverse electron transport in the electron transport chain, the authors proposed that inhibition of SDH may restore IR injury to WT levels by reducing succinate-driven ROS production [86, 87]. Interestingly, SIRT5 is downregulated in cardiomyocytes upon oxidative stress, and SIRT5 knockdown results in a marked reduction in cell viability, a significant increase in the number of apoptotic cells, and increased activity of caspase 3 activity [73]. Oxidative stress and apoptosis are frequently observed in cardiac pathologies, such as IR injury and heart failure. Thus, although only few studies are available on SIRT5 in the heart, the currently available data strongly suggest a significant role for SIRT5 in cardiac physiology and pathology that needs to be investigated.

Conclusions

Mitochondrial function and energetics are essential for cardiac function and integrity, and impairment in mitochondrial biology contributes to cardiac pathologies such as IR injury and heart failure. Mitochondrial sirtuins are major regulators of mitochondrial energetics and modulate ROS detoxification and permeability transition, and several studies suggest that mitochondrial sirtuins modulate the cardiac response to IR and various stressors. SIRT3 may exert protective effects during IR and the development of cardiac hypertrophy and failure. Few data are available on SIRT4 and SIRT5 in the heart. Nevertheless, these studies suggest that SIRT5 may also exert beneficial effects during IR, whereas the role of SIRT4 during IR remains to be elucidated. While pharmacological modulation of mitochondrial sirtuins appears like a promising therapeutic approach, a number of unresolved issues remain to be addressed. Many potential targets that have been identified in loss-of-function models and using screening assays for posttranslational modifications need to be confirmed as definite targets of mitochondrial sirtuins. Although quite laborious, it would be desirable to elucidate the functional consequence of each lysine acylation (e.g., aceytlation, succinylation, or malonylation) on a single and promising target protein to elucidate the functional modulation due to altered lysine acylation. Some studies reported opposite functional effects by posttranslational modifications of the same protein (e.g., LCAD), which may potentially be related to distinct acylation patterns of the same protein, resulting in different functional outcomes [19, 29]. Furthermore, we do not understand why the mitochondrion hosts three different sirtuins which, at least in part, seem to have targets in similar mitochondrial pathways. The NAD^+ dependence of all mitochondrial sirtuins may suggest that

SIRT3–5 may comprise an enzymatic system which may serve as a redox-sensitive rheostat that adapts cellular energy production to energy demand, among other functions. The relative and functional contribution of each sirtuin remains however unresolved to date. Understanding of their interplay is important though if modulation of sirtuins should be considered as a therapeutic option. Nonetheless, modulation of mitochondrial sirtuin activity may represent a promising approach to alter mitochondrial biology in cardiac disease and may promote further research in the field of mitochondrial medicine.

Compliance with ethical standards

Conflict of interest The authors declare that they do not have any conflict of interest related to this manuscript.

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