Sirtuins: to be or not to be in diabetic cardiomyopathy

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Keywords: diabetic cardiomyopathy; inflammation; metabolic dysregulation; myocardial fibrosis; oxidative stress; sirtuin

Abstract

Diabetic cardiomyopathy is the leading cause of death among people with diabetes. Despite its severity and poor prognosis, there are currently no approved specific drugs to prevent or even treat diabetic cardiomyopathy. There is a need to understand the pathogenic mechanisms underlying the development of diabetic cardiomyopathy to design new therapeutic strategies. These mechanisms are complex and intricate, and include metabolic dysregulation, inflammation, oxidative stress, fibrosis and apoptosis. Sirtuins, a group of deacetylase enzymes, play an important role in all these processes and are, therefore, potential molecular targets for treating this disease. In this review, we discuss the role of sirtuins in the heart, focusing on their contribution to the pathogenesis of diabetic cardiomyopathy and how their modulation could be therapeutically useful.

Diabetic cardiomyopathy: the not-so-silent disease

Diabetic cardiomyopathy (DCM) is defined as the occurrence of myocardial dysfunction in people with diabetes that is not directly attributable to coronary artery disease, hypertension or valve disease. It is the leading cause of death among diabetic people, although its prevalence differs among studies, ranging from 20% to 60%, regardless of whether they suffer from type 1 (T1D) or type 2 (T2D) diabetes [1]. DCM is a chronic disease characterized by metabolic dysregulation, in which hyperglycemia and hyperinsulinemia play an indispensable pathogenic role, and that is often accompanied by local inflammation, oxidative stress, mitochondrial dysfunction, **endoplasmic reticulum (ER) stress**, cardiomyocyte apoptosis and **fibrosis** (see Glossary and Box 1) [2]. As a result, the heart develops left ventricular hypertrophy, contractile dysfunction and dilated cardiomyopathy, which impair cardiac output and eventually lead to heart failure. DCM typically manifests initially with **diastolic dysfunction**, although later it may also evolve with systolic dysfunction [2].

Despite the severity and poor prognosis of DCM, there are currently no formal guidelines regarding its management or approved specific pharmacological drugs to treat it. Chronic diseases like diabetes are characterized not only by changes in protein levels, but also by posttranslational modifications, which are of the utmost importance [3]. One such modification is lysine acetylation, which regulates a myriad of cell processes [3]. Sirtuins (SIRT) are a group of enzymes that catalyze the reversible deacetylation of proteins. Accumulating evidence suggests that they play an important role in several of the mechanisms involved in DCM. In this review, we discuss the role of sirtuins in the pathogenesis of DCM to better clarify how their modulation could be therapeutically useful (see Clinician's Corner).

Sirtuins: a tale with seven intricate plots

The main characters: the sirtuin family

The sirtuin family encompasses a group of evolutionarily conserved enzymes that couple the deacetylation of both histone and non-histone lysine residues to nicotinamide adenine dinucleotide $(NAD)^+$ hydrolysis. The resulting dependence on the NAD^+/NADH ratio explains why their activity is closely associated with the energy and redox status of the cell [4]. Seven mammalian sirtuin orthologs have been described (SIRT1-7), with characteristic tissue and subcellular distributions. SIRT1, SIRT6 and SIRT7 are located in the nucleus, SIRT2 is primarily found in the cytoplasm, while SIRT3, SIRT4 and SIRT5 reside mainly in the mitochondrial matrix. However, some sirtuins may shuttle between different subcellular compartments and even display different locations depending on the cell type [5-8].

The plot: what they do

All sirtuins possess conserved NAD^+-binding and catalytic domains, but their different flanking N- and C-terminal regions contribute to specific differences in subcellular localization, enzymatic activity and substrate specificity. SIRT1, SIRT2 and SIRT3 display deacetylase and long-chain deacylase activities, while SIRT4 exhibits ADP-ribosyltransferase, deacylase, substrate-specific deacetylase and lipoamidase activities. SIRT5 shows strong deacylase activity, while SIRT6 presents deacetylase, ADP-ribosyltransferase and long-chain deacylase activities. SIRT7 primarily mediates deacetylation, histone desuccinylation and long-chain deacylation responses. The deacylation catalyzed by sirtuins includes, besides deacetylase activity, desuccinylase, demalonylase, deglutarylase, demyristoylase and depalmitoylase activities [4, 9]. Sirtuins regulate important cell functions associated with physiological as well as pathological conditions. Mitochondrial sirtuins share some functional redundancy and, together, coordinate numerous aspects of mitochondrial function, including the redox balance, metabolism homeostasis and dynamics.

Denouement: sirtuins are multi-talented proteins

SIRT1: cardioprotection beyond metabolism regulation

SIRT1 is highly expressed in the heart. Protein substrates of SIRT1 include histones, transcription factors, DNA repair proteins and factors associated with **autophagy** [10], through which it modulates cardiac metabolism, stress responses, apoptosis, DNA repair, inflammation and mitochondrial function [11]. Several types of pathophysiological stress modulate SIRT1 expression and activity in the heart, either through the regulation the transcription factors controlling its mRNA expression (E2F transcription factor 1 [E2F1], Forkhead box class O [FOXO]1 and FOXO3) [12] or by the direct control of its enzymatic activity via post-translational modifications (methylation, nitrosylation, phosphorylation and sumoylation) [13].

By regulating the activity of many cytoplasmic proteins (phosphoinositide 3-kinase [PI3K]/AKT, AMP-activated protein kinase [AMPK], peroxisome proliferator-activated receptor [PPAR]α, PPARγ coactivator-1α [PGC-1α] and protein tyrosine phosphatase [PTP]1B), SIRT1 ameliorates metabolism in diabetes, thereby contributing to improve DCM (Figure 1) [2, 13-15]. SIRT1 also exerts a strong protective effect against oxidative stress by attenuating the production of reactive oxygen species (ROS) and, consequently, reducing cardiomyocyte apoptosis. Mitochondrial manganese-dependent superoxide dismutase (SOD2), the major ROS detoxifying enzyme, is transcriptionally upregulated by

SIRT1 through deacetylation and the subsequent nuclear translocation of FOXO1, as well as through the activities of hypoxia-inducible factor (HIF)-2α and FOXO4 [16]. SIRT1 also attenuates oxidative stress by upregulating thioredoxin 1 and catalase [12]. SIRT1 prevents cardiomyocyte apoptosis by reducing caspase-3 activity and the expression of the pro-apoptotic protein BCL2-associated X protein (BAX) through FOXO activation [17] and through the deacetylation and inhibition of poly (ADP-ribose) polymerase (PARP) activity, which preserves NAD^+ levels [18]. Furthermore, SIRT1 protects cardiomyocytes via the expression of autophagy-related genes in a process that depends on the activation of FOXO1 and FOXO3 [12].

SIRT1 also displays anti-inflammatory activity by promoting the binding of PPARα to the pro-inflammatory transcription factor nuclear factor-κB (NF-κB) [19], as well as through the deacetylation of the K310 residue of the NF-κB p65 subunit, which results in the inhibition of its transcriptional activity [20].

SIRT2: friend or foe in cardiac pathophysiology?

SIRT2 is abundantly expressed in metabolically active tissues [21], but its role in the heart is yet to be elucidated. Genetic or pharmacological inhibition of SIRT2 in insulin-resistant myotubes has been shown to activate AKT and increase insulin-stimulated glucose uptake [21, 22], suggesting that its downregulation might improve insulin sensitivity (Figure 2). In addition, several studies have indicated that SIRT2 attenuates apoptosis, oxidative stress damage and inflammation, the latter probably due to K310 deacetylation and the subsequent deactivation of p65/NF-κB [23-25]. SIRT2 overexpression renders cardiomyocytes more susceptible to cell death during ischemia/reperfusion (I/R) injury [26]. However, SIRT2 also protects mice from angiotensin II-induced **cardiac hypertrophy** and fibrosis through the deacetylation of liver kinase B1 (LKB1), which promotes AMPK activity [27], and nuclear factor in activated T-cells (NFAT) [28]. In any case, the effects of SIRT2 appear to be specific for each cell type or stimulus, and might even play a double-faced role in the same cell type.

SIRT3: a multi-talented enzyme at the crossroads of mitochondrial function and cardiac metabolism

SIRT3 exhibits robust deacetylase activity on proteins associated with the oxidative balance, fatty acid (FA) oxidation, glycolysis, amino acid metabolism, the tricarboxylic acid (TCA) cycle, the electron transport chain (ETC) and mitochondrial turnover and biogenesis [13, 29]. Recent studies indicate that,

at least in the heart, SIRT3 localizes in the mitochondria, the cytoplasm and the nucleus, where it also displays enzymatic activity [30].

Homozygous SIRT3-knockout mice have been reported to exhibit a marked hyperacetylation of mitochondrial proteins, which is not observed in SIRT4- or SIRT5-knockout mice [31]. These mice display reduced rates of FA oxidation, glucose oxidation, oxygen consumption, the mitochondrial respiration rate, ATP synthesis and the activity of oxidative phosphorylation complexes in the heart [32, 33]. Unexpectedly, SIRT3-knockout mice show normal cardiac function, although this worsens after the induction of cardiac hypertrophy. The hearts of these mice display increased glycolysis, abnormal lipid accumulation, energy depletion, impaired contractile function and fibrosis upon stress [32, 34]. SIRT3 promotes the TCA cycle and the generation of ATP [35]. SIRT3 activates the first enzyme of the pyruvate dehydrogenase complex (PDC), pyruvate dehydrogenase E1α (PDHA1) [29], which catalyzes the oxidation of pyruvate into acetyl-CoA that subsequently enters the TCA cycle and regulates anaerobic glycolysis by deacetylating and activating lactate dehydrogenase A (LDHA), a key enzyme in determining the metabolic fate of pyruvate, the end-product of glycolysis (Figure 3) [36]. In cardiomyocytes, SIRT3 promotes AMPK activity by LKB1 deacetylation, thus inhibiting the phosphorylation of glycogen synthase kinase (GSK)3β and upregulating glucose transporter 4 (GLUT4) [37].

SIRT3 deacetylates FOXO3, subsequently increasing the expression of the antioxidant enzymes SOD2 and catalase in the heart [33]. Indeed, it is capable of directly deacetylating several lysine residues of SOD2, thereby increasing its activity [38]. SIRT3 also indirectly reduces ROS production by increasing the efficiency of the ETC and promoting the TCA cycle, which increases NADPH production in the mitochondria [29]. NADPH is necessary to form reduced glutathione, an essential cofactor for mitochondrial glutathione peroxidase in the scavenging of ROS [16]. In the heart, SIRT3 deacetylates cyclophilin D [12], which inhibits the opening of the mitochondrial permeability transition pore (mPTP), thereby reducing ATP depletion and the release of pro-apoptotic factors from the mitochondria, subsequently preventing cardiomyocyte cell death [13, 16].

Overall, the data suggest that SIRT3 acts as a redox-sensitive rheostat that is required for preserving oxidative metabolism and increasing energy production (ATP synthesis) in the mitochondria for the maintenance of proper function in the heart. SIRT3 can also have important cardioprotective effects through mitochondrial ROS detoxification and carrying out anti-inflammatory, anti-fibrotic and anti-

apoptotic actions [39-41]. These actions may result in the inhibition of pro-hypertrophic transcription factors (GATA-binding protein 4 [GATA4] and NFAT and translation factors [29], which might explain why SIRT3 overexpression prevents cardiac hypertrophy whilst SIRT3 knockout causes interstitial fibrosis and cardiac hypertrophy in mice [33].

SIRT4: the last puzzle of mitochondrial sirtuins

SIRT4 is known to interact with fewer proteins than the other sirtuins, even though it has been associated with several pathways controlling oxidative balance, FA metabolism, glycolysis and amino acid catabolism. It shows very low NAD^+-dependent deacetylase activity, instead displaying lipoamidase activity and strong ADP-ribosyltransferase activity [29, 42]. The role of SIRT4 in the heart has been poorly investigated, but studies performed with knockout mice have demonstrated that it is tightly associated with energy metabolism in other tissues, where it inhibits glucose and FA oxidation, thus resulting in impaired ATP synthesis and energy depletion [43, 44]. SIRT4 also suppresses the inflammatory and oxidative stress responses in human chondrocytes [45], and the progression of high-fat diet (HFD)-induced hepatic steatosis and fibrosis in the liver [46].

SIRT5: the missing link in metabolic dysregulation in diabetic cardiomyopathy?

SIRT5 is ubiquitously expressed, although its expression in the heart is comparatively high relative to other tissues [47]. It is regarded as a mitochondrial matrix protein, but it may also localize to the cytosol and nucleus [47-49]. This sirtuin shows weak deacetylase activity and is primarily known for carrying out NAD^+-dependent deglutarylation, demalonylation and desuccinylation [29].

SIRT5 represses the activity of PDC directly by desuccinylating several of its subunits (Figure 2) [48], as well as indirectly by deacetylating signal transducer and activator of transcription 3 (STAT3) [50]. In the liver, SIRT5 regulates the activities of diverse enzymes to increase ketone body synthesis [51]. This is important, since ketone bodies are an important source of energy for the heart under fasting conditions. SIRT5-mediated desuccinylation also inhibits the activity of cardiac succinate dehydrogenase (SDH) within the TCA cycle, which contributes to the protection of the heart from I/R injury due to reduced superoxide production [52]. It is noteworthy that SIRT3 and SIRT5 cooperate in deacylating very long-chain acyl-CoA dehydrogenase (ACADVL) to promote FA oxidation [53]. SIRT5 demalonylates glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and other glycolytic enzymes to promote the glycolytic flux [48]. A recent study investigating with left ventricular hypertrophy models has also linked SIRT5 deficiency with a decrease in ATP production and subsequent AMPK activation, a fact which contributes to cardiac protection under stress [54].

Given that succinyl-CoA is the most abundant acyl-CoA molecule in the heart [49], it is not surprising that SIRT5 plays an important role in cardiac function. Studies on SIRT5-knockout mice have shown significant protein hyperacylation and hypersuccinylation in the heart, although only mild cardiac dysfunction is observed in the absence of any stress [49, 52]. However, these mice display severe cardiac hypertrophy with aging or in response to chronic pressure overload [47, 49]. This is probably due to a reduction in both FA and glucose oxidation and a decrease in the mitochondrial NAD^+/NADH ratio and ATP production, which result in a greater impairment of systolic function and favor the development of pathological cardiac hypertrophy. In cardiomyocytes, SIRT5 also boosts the cell antioxidant capacity and prevents apoptosis by desuccinylating and activating copper- and zinc-dependent superoxide dismutase (SOD1), increasing NADPH generation through the activation of isocitrate dehydrogenase 2 (IDH2) and decreasing the activity of SDH by desuccinylation, and increasing glucose-6-phosphate dehydrogenase (G6PD) activity via deglutarylation [55, 56]. Another study suggested that SIRT5 could deacetylate FOXO3 in lung epithelial cells and, thus, promote the expression of additional antioxidant genes [57]. The deacetylation of cytochrome c and peroxiredoxin by SIRT5 further reinforces its anti-apoptotic role in the heart [12].

SIRT6: the great unknown in the heart

SIRT6 regulates chromatin remodeling, genome stability and gene transcription through its mono-ADP-ribosyltransferase and histone deacetylase activities [13]. It is highly expressed in the myocardial tissue, where it regulates glucose and lipid homeostasis and plays a protective role [4]. It acts as a negative endogenous regulator of cardiac hypertrophy and heart failure by suppressing JUN transcriptional activity, which dampens the pro-hypertrophic insulin-like growth factor (IGF)-AKT signaling pathway (Figure 4) [58], and by suppressing the expression and activity of NFATc4 [59]. SIRT6 improves cardiomyocyte stress resistance through several mechanisms: AMPKα activation, B-cell lymphoma 2 (BCL2) upregulation, as well as reductions in AKT activity, cell oxidative stress and inflammation [60]. This anti-inflammatory effect depends on the histone H3 deacetylation at the gene promoter of the NF-κB p65 subunit [61]. SIRT6 also prevents cardiomyocyte apoptosis by activating GATA4 transcription factor in a deacetylase-independent manner [62], and through the ADP-ribosylation of PARP [63], which increases its activity and, consequently, stimulates DNA double-strand break repair under oxidative stress. A recent study has also evidenced an anti-fibrotic role for SIRT6, since its deficiency resulted in the hyperactivation of transforming growth factor (TGF)β and subsequent deposition of collagen and other extracellular matrix proteins in the heart [64].

SIRT7: at the crossroads between epigenetics and disease

SIRT7, the last sirtuin discovered, is widely expressed throughout the body, but is significantly expressed in the heart and liver. It is the only sirtuin that predominantly localizes in the nucleoli, where it binds to RNA polymerase I and activates ribosomal rRNA-encoding DNA (rDNA) transcription [65]. Deletion of SIRT7 in mice has been shown to reduce the mean lifespan and yielded a multi-organ phenotype [66], but it has been reported to make mice resistant to HFD-induced fatty liver, obesity and glucose intolerance [67]. Interestingly, SIRT7-knockout mice have been observed to display AKT hyperphosphorylation and increased activity [68].

Sirtuins in diabetic cardiomyopathy

The expression and activity of sirtuins in the heart are significantly modified in animal models of diabetes, although the extent and direction of these changes depends on the species, gender, age, tissue and the model of diabetes analyzed, among other factors [3, 69-71]. As an example, the mRNA and protein levels of all sirtuins was reduced in the heart of the rat model of streptozotocin (STZ)-induced T1D, except for SIRT2, whose expression was increased [3]. By contrast, in the high-fructose diet-induced T2D model, SIRT1 and SIRT2 was reported to be reduced, and SIRT3 increased [3]. Strikingly, immunoblot analysis revealed increased acetylation of both cytoplasmic and nuclear proteins in the heart of the T1D model, but increased acetylation of only the nuclear proteins in the T2D model, suggesting the existence of a complex sirtuin signaling network. Sirtuins may modulate DCM by acting on oxidative stress, calcium homeostasis, metabolism, inflammation, fibrosis and apoptosis. In the following section, we will outline an overall framework of the intricate role of sirtuins in DCM.

Metabolism dysregulation and mitochondrial function

Cardiomyocytes have abundant mitochondria, and mitochondrial function notably impacts on heart physiology by regulating cell energy metabolism, redox signaling, apoptosis, calcium handling and cardiac contraction. Therefore, their deterioration or malfunction alters energy production, favoring oxidative stress and increasing cardiomyocyte apoptosis, thereby contributing to the pathogenesis of many cardiovascular diseases [11, 42]. Since sirtuins regulate mitochondrial function, their activity is unequivocally associated with the onset and progression of DCM. Mitochondrial sirtuins are responsible for most of the changes in lysine acetylation that are observed in diabetes, but the other sirtuins can also intervene.

Reduced SIRT3 activity has been linked to the development of diabetes in rodent models of T2D [72] and humans [31]. In agreement with this, a human genetic polymorphism in the human SIRT3 gene that reduces its activity was found to be associated with the metabolic syndrome [73]. Decreased SIRT3 expression in T1D cardiomyocytes impairs mitochondrial energetics and reduces ATP production [74]. Conflicting information is found regarding the acetylation status of the enzymes involved in FA oxidation, which might depend on the tissue, the specific enzyme and the different lysine residues that may be acetylated. However, at least in skeletal muscle and in the heart, hyperacetylation of mitochondrial proteins in SIRT3 knockout mice is associated with increased FA oxidation rates [75, 76]. Likewise, HFDs reduce SIRT3 expression in the heart, and this is accompanied by reduced glucose utilization, enhanced FA oxidation, increased ROS formation and impaired HIF-1α signaling, leading to impaired cardiac function [75, 77]. By contrast, SIRT3 activation represses HFD-induced obesity [29] and attenuates lipid accumulation in cardiomyocytes [34]. Overall, the data suggest that the increased FA oxidation in the heart in response to HFDs depends, at least in part, on the downregulation of SIRT3 activity and the resulting increased acetylation of mitochondrial β-oxidation enzymes [75]. A recent study demonstrated that treatment of cardiomyocytes with palmitate or feeding mice a diet rich in fat and sucrose downregulated the expression of SIRT3 and SIRT6, which contributed to obesity and the development of diabetes [70]. Systemic activation of SIRT6 in transgenic mice fed the same diet inhibited insulin resistance, reduced lipid accumulation and sustained cardiac mitochondrial function [70]. In accordance with this, SIRT6 knockdown in mice hampers the insulin-sensitizing action of the anti-diabetic rosiglitazone [78], with its cardiac-specific suppression resulting in mitochondrial degeneration and lipid accumulation in the heart [58]. The latter phenotype could arise from the blockade of IGF-AKT signaling. Kanwal *et al*. [70] elegantly demonstrated that SIRT3 and SIRT6 regulate each other to prevent the development of cardiomyopathy under diabetic conditions, with SIRT3 preventing a decline in SIRT6 expression by reducing oxidative stress and SIRT6 maintaining SIRT3 expression levels by inducing its nuclear factor erythroid-derived 2-like 2 (NFE2L2)-dependent transcription, a key player in the antioxidant defense.

Recent studies in mice with SIRT6 haploinsufficiency also report an important role for this sirtuin in the regulation in cardiomyocytes of glucose channeling into the TCA cycle [79]. According to these authors, SIRT6 transcriptionally represses pyruvate dehydrogenase kinase 4 (PDK4) in a FOXO1-dependent manner [79]. PDK4 is an essential enzyme for glucose oxidation and, therefore, SIRT6

deficiency results in cardiac lactate accumulation, compromised mitochondrial glucose oxidation and lesser ATP production [79].

SIRT5 might contribute to DCM and the progression of cardiac lipotoxicity through desuccinylation and the subsequent inhibition of SDH or by the activation of the hydroxyacyl-CoA dehydrogenase α subunit [47]. A lack of SIRT5 has been reported to impair FA metabolism in the hearts of mice during energy-demanding conditions due to the reduced activity of the enoyl-CoA hydratase α-subunit (ECHA) [49]. This leads to an accretion of long-chain acyl-CoAs and a decline in cardiomyocyte ATP levels [29], since ECHA desuccinylation by SIRT5 increases its activity and promotes the oxidation of long-chain acyl-CoAs.

Energy store depletion in diabetes increases intracellular NAD^+ levels, consequently activating AMPK. AMPK, in turn, activates SIRT1, which increases the AMP/ATP ratio and allows AMP to bind to the regulatory γ subunit of AMPK [80]. SIRT1 also deacetylates and activates LKB1, an upstream positive regulator of AMPK [15]. Thus, AMPK and SIRT1 regulate each other and share molecular targets that contribute to the maintenance of metabolic homeostasis in DCM. In fact, SIRT1 activation improves cardiac function in DCM by reducing insulin resistance, while its suppression in mice induces cardiac hypertrophy and dysfunction, insulin resistance and anomalous glucose metabolism [11]. AMPK-mediated phosphorylation of SIRT2 also plays a role in insulin signaling and the development of insulin resistance, since the activity of this sirtuin is required for optimal AKT activation [22].

SIRT1 favors mitochondrial dynamics and boosts ATP generation by deacetylating PGC-1α, which coactivates the mitochondrial regulatory transcription factors estrogen-related receptor (ERR)α, nuclear respiratory factor (NRF)1, NRF2, and mitochondrial transcription factor (TFAM) [11]. Treatment with resveratrol has been shown to increase the activity of TFAM, which is a downstream target of both SIRT1 and SIRT3 [11, 74], resulting in normalized mitochondrial function as well as reducing cardiomyocyte apoptosis, cardiac atrophy and fibrosis in a T1D rat model [74]. SIRT3 also contributes to the preservation of mitochondrial function in diabetes by removing damaged mitochondria in cardiomyocytes, probably through the stimulation of FOXO3-Parkin-mediated mitophagy [81]. In a similar way to SIRT1, SIRT6 is capable of restoring normal mitochondrial function and biogenesis during DCM through the activation of the AMPK-PGC-1α-AKT signaling pathway [82]. Finally, low SIRT7 activity has been linked to mitochondrial dysfunction and

cardiomyopathy, which probably arises from the deacetylation of distinct lysine residues in NRF2 [4, 83].

Inflammation and fibrosis

SIRT1 inhibits the activity of two important mediators of inflammation, p38 mitogen-activated protein kinase (MAPK) and NF-κB [84]. As a result, there is a reduction in the expression of pro-inflammatory cytokines, which attenuates cardiac inflammation and apoptosis.

The transcription factor activator protein-1 (AP-1), which is a heterodimer composed of proteins from the FOS, JUN and activating transcription factor (ATF) families, induces fibrosis of the interstitial substance and cardiomyocyte hypertrophy by increasing the deposition of collagen and the synthesis of endothelin-1, fibronectin and TGFβ. JUN deacetylation by SIRT1 reduces fibrosis by inhibiting the transcriptional activation of matrix metalloproteinase (MMP)9 [85]. Furthermore, in diabetes, hyperglycemia induces the transcriptional coactivator p300, which increases TGFβ levels. SIRT1 deacetylates and inhibits p300 and, thus, prevents fibrosis and heart failure in DCM [86]. Likewise, SIRT3 activation prevents collagen deposition and improves cardiac function in response to hypertrophic stimuli, in a process mediated by the inhibition of the TGFβ/Smad3 pathway [87]. Deacetylation by SIRT3 also activates GSK3β, which blocks TGFβ signaling [88]. In a mouse model of T1D, SIRT3 suppression was shown to enlarge the area of myocardial interstitial fibrosis and aggravate cardiac dysfunction [81]. These deleterious effects of SIRT3 deficiency were mediated, at least in part, by the activation of FOS transcription through specific histone H3 lysine acetylation at its promoter [30]. Similarly, a recent study demonstrated that SIRT3 deficiency, through NF-κB activation, stimulated the expression of monocyte chemoattractant protein 1 (MCP-1), a chemotactic factor that promoted the recruitment of macrophages into the myocardium [39]. These macrophages secreted pro-inflammatory cytokines (interleukin [IL]-6, tumor necrosis factor [TNF]-α and TGFβ) and augmented collagen deposition, with the resulting fibrosis disrupting contractile function and impairing both systolic and diastolic functions, thus hastening the progression of heart failure [39].

Although less known, there is also a potential role for other sirtuins in cardiac fibrosis. Suppression of SIRT5 [47, 49], SIRT6 [58] and SIRT7 [66, 83] in knockout mice has been reported to induce cardiac hypertrophy, inflammation, fibrosis, apoptosis, and downregulated cardiac performance compared to their wild-type littermates. Transgenic mice overexpressing SIRT6 display the exact opposite [58]. The repression of HIF-1α, AP-1 and NF-κB activities by SIRT6 might account for its favorable effects

[58, 61]. A recent study also demonstrated that SIRT6 deficiency resulted in the hyperactivation of TGFβ and the subsequent deposition of collagen and other extracellular matrix proteins in the heart [64]. Mechanistically, this was explained by SIRT6 binding to (and deacetylating) Smad3 and histone H3 at the promoter of the TGFβ gene, which repressed its transcription. SIRT6 also prevents fibrosis in the heart through the inhibition of the endothelial-to-mesenchymal transition, a key process in the conversion of cardiac microvascular endothelial cells to myofibroblasts, which are responsible for most of the extracellular matrix deposition and perivascular fibrosis in DCM [89].

The hyperacetylation and subsequent activation of the pro-hypertrophic AKT, GATA4 and p53 signaling pathways in the absence of SIRT7 activity might explain its effects in the heart [83]. SIRT7 might also regulate cardiac fibrosis by promoting the differentiation of fibroblasts into myofibroblasts, a highly active cell type that increases the deposition of extracellular matrix components [90].

Oxidative stress and apoptosis

Oxidative stress is a fundamental mechanism underlying DCM, since it induces cardiomyocyte hypertrophy, apoptosis and interstitial fibrosis. Activation of SIRT1 by caloric restriction in a mouse model of DCM was shown to improve mitochondrial function, alleviate oxidative stress and fibrosis, and blunt pro-inflammatory pathways, all of which contributed to the improvement of cardiomyopathy in these mice [14]. These effects were mediated by the activation of PGC-1α and the subsequent increase in SOD2 protein levels. Similar results have been reported after activation of SIRT1 by resveratrol. Thus, activation of SIRT1 by resveratrol treatment attenuated cardiac injury in rats with STZ-induced diabetes through the improvement of mitochondrial function and the reduction of oxidative stress, in a process which was partly mediated through the deacetylation of PGC-1α [91]. Likewise, SIRT1 activation by resveratrol ameliorated cardiac hypertrophy, electrocardiographic abnormalities and oxidative stress in the fructose-fed diabetic rat heart [92], although the latter study pointed to an additional mechanism entailing the deacetylation of NF-κB and histone H3 proteins, which led to the upregulation of SOD2. Another consequence of NF-κB inhibition was a reduction in the transcription of the NADPH oxidative subunits NOX1 and NOX2 [92]. SIRT1 activation also preserves endothelial nitric oxide synthase (eNOS or NOS3) activity by reducing its acetylation state, which contributes to its antioxidant effects, since increased NO production inhibits NADPH oxidase-dependent superoxide formation [93]. Fibroblast growth factor (FGF)21-induced expression of uncoupling proteins (UCP2 and UCP3) and SOD2 might account for some of the antioxidant activity of SIRT1, as FGF21 expression itself is, in turn, under the control of the SIRT1-PPARα pathway [94].

Activation of SIRT3, in a mouse model of angiotensin II-induced cardiac hypertrophy, was observed to restore mitochondrial function and reduce intracellular ROS levels through the upregulation of the SOD2 and catalase genes in a FOXO3-dependent manner [33], protecting cells from diabetes-induced oxidative stress. In a similar way, SIRT1 promotes the expression of the FOXO target genes involved in oxidative stress resistance and decreases the transcription of genes involved in apoptosis [95]. By contrast, SIRT4 overexpression promotes mitochondrial ROS generation, increases fibrosis, aggravates hypertrophy and worsens cardiac function in a mouse model of angiotensin II-induced cardiac hypertrophy [96]. Surprisingly, these harmful effects of SIRT4 involve the inhibition of SIRT3-mediated deacetylation of SOD2 [96]. Nevertheless, the antioxidant effects of sirtuins extend beyond SOD2 and catalase activation. For instance, studies in knockout mice suggest that SIRT3 may also attenuate oxidative stress by regulating the acetylation status of mitochondrial FA β-oxidation enzymes (β-hydroxyacyl-CoA dehydrogenase [HAD] and ACADL) [29, 32, 77]. Also, a recent study reported that SIRT3 expression negatively correlates with ROS production in human AC16 cardiomyocytes under hyperglycemia conditions, and this is due to the downregulation of JUN N-terminal kinase (JNK) phosphorylation [97]. The same study demonstrated that SIRT3 is a downstream target of PPARα, a fact which might account for the ability of the latter for maintaining antioxidant defense and oxidant equilibrium in cardiomyocytes [97].

ROS overproduction by mitochondria favors the release of cytochrome c and other pro-apoptotic proteins, which trigger caspase activation and apoptosis. Excess free FAs also trigger apoptosis in cardiac cells [98]. Thus, metabolism and oxidative stress are narrowly interrelated to apoptosis and, as a consequence, sirtuins are also involved in regulating cell death. For instance, an inverse relationship has been reported for SIRT1 and PARP activity in the hearts of T2D mice, which exhibit increased fibrosis, inflammation and oxidative stress [18]. Similarly, the saturated FA palmitate increases oxidative stress and induces apoptosis in cultured neonatal mouse cardiomyocytes, which depends on SIRT1 inhibition [98]. The anti-apoptotic properties of sirtuins may rely on several mechanisms. SIRT1 activation decreases cardiomyocyte apoptosis by preventing BCL2 downregulation and ROS production [98], positively regulating the transcription of the anti-apoptotic protein BCL2 like 1 protein (BCL2L1) [99], suppressing ER stress (see the following section) [10], and deacetylating and inactivating p53, thus preventing the recruitment of transcription cofactors to the promoter region of the PUMA and BAX pro-apoptotic genes [16, 99]. SIRT7 also has an anti-apoptotic role in the heart,

with some studies suggesting that this sirtuin might act synergistically with SIRT1 to prevent oxidative stress and apoptosis by regulating p53 [83].

Less is known about the role of SIRT3 on cardiac cell death, although a recent study indicated that it may regulate necroptosis, a programmed cell death pathway different from necrosis and apoptosis that is associated with the high inflammation state occurring in DCM [100]. The absence of SIRT3 in knockout mice is associated with an increase in the expression of the inflammasome-related protein NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3), which promotes pro-inflammatory cell recruitment, caspase 1 activation and pro-inflammatory cytokine secretion, ultimately exacerbating DCM in these mice [100]. Concerning the role of other mitochondrial sirtuins in apoptosis, SIRT4 exerts a cytoprotective effect against hypoxia-induced apoptosis of H9c2 cardiomyoblasts, mostly by suppressing mitochondrial BAX translocation (Figure 2) [42]. In a similar way, SIRT5 is inhibited in cardiomyocytes upon oxidative stress. Studies in knockout mice have demonstrated that SIRT5 can reduce oxidative stress-induced apoptosis in cardiomyocytes through its interaction with BCL2L1, which dampens the uncoupling of the mitochondrial respiratory chain, thereby decreasing superoxide levels in the mitochondria [55]. Moreover, SIRT5 suppression decreases the viability of H9c2 cardiomyoblasts by promoting caspase 3/7 activity and apoptosis [13].

Other pathophysiological mechanisms

The link between SIRT1 and ER stress in DCM is relevant. SIRT1 activation protects cardiac cells from the apoptosis induced with ethanol or under hyperglycemic conditions by preventing caspase 12 activation and ER stress [10]. The genetic suppression of SIRT1 significantly increases the expression of ER stress markers and inhibits its anti-apoptotic effect [10]. In fact, SIRT1 attenuates the ER stress pathways mediated by protein kinase R-like endoplasmic reticulum kinase (PERK)/eukaryotic translation initiation factor (eIF)2α, ATF6/CCAAT/enhancer-binding protein homologous protein (CHOP), and inositol-requiring enzyme 1α (IRE1α)/JUN N-terminal kinase (JNK) [10]. Another ER stress-related protein, the transcription factor X-box binding protein-1 (XBP1), which is activated by IRE1α, may also be inactivated directly by SIRT1 deacetylation [101]. The initiation of the unfolded protein response during ER stress, which involves the ATF6, IRE1α and PERK pathways, aims to protect cells by halting mRNA translation, enabling protein degradation, improving protein folding and potentiating autophagy. However, if ER stress is not mitigated, apoptosis occurs instead of autophagy in order to dispose of the damaged cells. This explains why reduced autophagy is often associated with DCM [81]. In mice with STZ-induced diabetes, SIRT1 activation increases autophagy

in the myocardium by inducing FOXO1-dependent Rab7 gene transcription, which contributes to the maturation of autophagosomes and their fusion with lysosomes [102]. Similar to SIRT1, SIRT3 overexpression in cultured cardiomyocytes prevents the suppression of the autophagy and mitophagy observed in mice with STZ-induced diabetes [81].

Sirtuins may also improve calcium homeostasis in cardiomyocytes. SIRT1 activation restores sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) protein levels in the heart of a mouse model of T1D, probably by activating specificity protein 1 (Sp1), which normalizes cardiac cell contraction and ventricular dysfunction [103]. Activation of SIRT1 in DCM also contributes to the improvement in Ca^{2+} homeostasis by increasing the expression of HOMER1a in a process that requires the phosphorylation and subsequent activation of extracellular signal-regulated kinase (ERK)1/2 [103]. HOMER1a is a scaffold protein that modulates the release of Ca^{2+} from the ER in cardiac myocytes and acts as a calcium-dependent endogenous ROS scavenger, thus presenting antioxidative properties. In DCM, there is an intracellular overload of Ca^{2+} and Na^{+} due to the overall increase in the levels of **advanced glycation end products** (**AGEs**), which impairs the activity of the Na^{+}/K^{+}-ATPase in the sarcolemma [80]. This worsens the energy transduction from the intracellular membrane [80] and can also lead to ROS generation and oxidative stress, which both correlate with the contractile dysfunction of the diabetic heart [80]. Interestingly, AMPK, by activating SIRT1, can restore Na^{+}/K^{+}-ATPase activity [80].

Finally, SIRT2 has a unique protective role in DCM in a model of STZ-induced T1D that involves the deacetylation of α-tubulin in microtubules [104]. Microtubules are cytoskeletal heterodimers containing α- and β-tubulin proteins that, in the myocardium, are involved in intracellular mRNA and protein transport and subcellular organization. Tubulin acetylation stabilizes microtubules and promotes cardiomyocyte hypertrophy and contractile impairment, thus contributing to the progression of DCM.

Concluding Remarks and Future Perspectives

DCM is associated with high morbidity and mortality rates [105], making it very important to discover new targets for the development of more efficient drugs. Some of the main problems linked to DCM are: (1) its asymptomatic character, particularly at the early stages of the disease; (2) its atypical and diverse signs and symptoms that hamper its evaluation in clinical practice; and (3) its complex and

devious etiopathogenic mechanisms [1, 105]. Sirtuins mediate all these cell processes, thus making them potential targets for treating this disease (Box 2).

Data found in the literature indicate that all the sirtuins regulate one another in a complex network, coordinating cardiac physiology and preserving their proper function. However, it has not been completely elucidated yet how this interplay operates in DCM. SIRT1 activation seems to be a promising tool in the protection of the diabetic heart. However, despite its recognized cardioprotective effects, some studies indicate that SIRT1 could also behave as a pro-hypertrophic molecule [106-108]. For this reason, the selective activation of SIRT1 in the heart to treat DCM would be inadvisable. In fact, it is probable that its protective effects actually rely on the coactivation of other sirtuins. Unfortunately, much less is known about the other sirtuins in DCM. Mitochondrial sirtuins, which are regarded as the watchmen of mitochondrial function, deserve a special mention. Available studies on SIRT3 suggest that its activation might be useful in treating metabolic diseases that exert deleterious effects on the heart, as is the case with DCM [30]. However, more data are needed to unequivocally demonstrate that these effects arise from the selective modulation of SIRT3. Modulation of SIRT4 and SIRT5 has also emerged as an interesting strategy. Moreover, improving mitochondrial function would positively affect not only cardiac function, but also whole-body metabolic homeostasis in metabolic diseases.

Despite all this knowledge, the relative contribution of each sirtuin is yet to be completely elucidated. Many issues still remain far from resolved (see Outstanding Questions). It is important to know their specific interactome to deepen our knowledge on how their physical associations with other proteins regulate cardiac physiology and to comprehend how sirtuins regulate one another, since they share many overlapping functions. Many potential targets have been identified and, thus, it would be desirable to unequivocally elucidate the functional consequences of each posttranslational modification (e.g., acetylation, succinylation and malonylation) on a single target protein and in a context-specific manner to shed light on the functional significance of each sirtuin. Moreover, the functional consequences of posttranslational modifications on the same target protein may give rise to opposite effects, depending on the overall acylation pattern, the tissue or the cell environment [31, 75]. Of course, the development and validation of novel compounds that fine-tune and provide tissue-specific modulation of any sirtuin analog will also be very helpful. Even so, most of the results presented herein are based on preclinical data. Therefore, further preclinical studies and clinical trials are required before these therapeutic approaches reach clinical practice.

Acknowledgements

We apologize to the contributors to the field whose work is not cited here owing to space restrictions. We thank the Language Advisory Service of the University of Barcelona for their assistance. The authors have received funding from the Spanish Ministry of Economy and Competitiveness (RTI2018-093999-B-100), the "Fundació La Marató de TV3" and CIBER *de Diabetes y Enfermedades Metabólicas Asociadas* (CIBERDEM; CB07/08/0003) to M.V-C, and from the Instituto de Salud Carlos III (PI 18/00543 and INNVAL 18/20) to J.F.N. CIBERDEM is an initiative of the *Instituto de Salud Carlos III* (ISCIII) - Ministry of Economy and Competitiveness.

Disclaimer statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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857 **Glossary**

858 **Advanced glycation end products (AGE):** A diverse group of proteins and lipids that become
859 glycated and oxidized as a consequence of persistent exposure to hyperglycemia and are causatively
860 associated with the complications of diabetes because they are highly oxidative.

861 **Autophagy:** A homeostatic process that involves the degradation of unnecessary or dysfunctional
862 cytoplasmic components ranging from protein aggregates to whole organelles through the action of
863 lysosomes. Its main objective is to recycle cell components, removing damaged mitochondria and
864 other organelles to protect the tissue.

865 **Cardiac hypertrophy:** Abnormal thickening of the heart muscle that results from the enlargement of
866 cardiomyocytes and changes in the extracellular matrix, increasing ventricular dimensions and
867 myocardial dysfunction. Initially, it is an adaptive response to compensate for hemodynamic stress by
868 enhancing cardiac performance (physiological hypertrophy), but can evolve into pathological
869 hypertrophy in conditions of chronic stress.

870 **Cardiac remodeling:** A set of molecular, cellular and interstitial changes resulting from an imbalance
871 between pro- and anti-fibrotic factors that promotes the deposition of extracellular matrix proteins.
872 The resulting cardiac fibrosis impairs cardiomyocyte contractility and, ultimately, leads to cardiac
873 stiffness and heart failure.

874 **Diastolic dysfunction:** This occurs when ventricles do not properly relax and their filling is impaired,
875 thus resulting in a higher end-diastolic pressure for a given end-diastolic volume and causing blood
876 accumulation in other parts of the body.

877 **Endoplasmic reticulum (ER) stress:** The ER organelle is responsible for protein folding and
878 maturation. The ER found in myocytes, the so-called sarcoplasmic reticulum, stores calcium ions that
879 are crucial regulators of the excitation-contraction coupling process. Any disturbance that alters ER
880 function may result in the accumulation of unfolded or misfolded proteins, thus activating the unfolded
881 protein response (UPR). The UPR aims to promote cell survival by alleviating the adverse effects of
882 ER stress, which is attained by reducing general mRNA translation, increasing protein degradation
883 and inducing the synthesis of chaperones that are involved in protein folding. When ER homeostasis
884 is not re-established by the UPR activation, inflammation and apoptosis are induced.

885 **Fibrosis:** Pathological wound healing resulting from undue accumulation of extracellular matrix
886 proteins, which arises in response to chronic tissue injury and inflammation. It may lead to tissue
887 remodeling and the formation of scar tissue that disrupts the organ architecture and normal function.

888 **Poly (ADP-ribose) polymerase (PARP)**: A NAD^+-dependent polymerase regulating DNA double-
889 strand break repair, chromatin remodeling, gene transcription and energy metabolism that is often

activated under conditions of oxidative stress and in diabetic cardiomyopathy. It also activates NF-κB and diverts glucose metabolism from its usual glycolytic pathway.

Box 1. Diabetic cardiomyopathy is a multi-faceted disease

The mechanisms underlying diabetic cardiomyopathy are multifactorial and comprise metabolic dysregulation, inflammation, oxidative stress, fibrosis and apoptosis.

Metabolic dysregulation

Despite free FAs being the preferred energy substrate in the adult heart, cardiac cells may use alternative fuel sources, including glucose, lactate or ketone bodies. In diabetes, due to the prevailing hyperglycemia and/or insulin resistance, there is a shift towards increased mitochondrial FA β-oxidation, at the expense of glucose, as the sole fuel source, which limits ATP production. Dysregulation of the transcriptional machinery controlled by the PPAR family of nuclear receptors is fundamental in this process. Thus, the activity of one of its target genes, the insulin-induced GLUT4 that controls the uptake of glucose in the heart, is downregulated, thereby contributing to the abovementioned substrate shift [2]. PGC-1α, a coactivator of PPARs and other transcription factor receptors (ERRα, NFE2L2 or NRF1 and NRF2), is a master regulator in controlling fuel utilization in the heart, since it regulates mitochondrial biogenesis, promotes FA oxidation and shuts down glucose oxidation [12, 16]. Regardless of the increased FA oxidation rate, intracellular lipid accretion and cardiac steatosis are hallmarks of the diabetic heart, resulting in lipotoxicity, since the accumulation of toxic lipid intermediates activates the pro-inflammatory transcription factor NF-κB and induces ER stress and mitochondrial dysfunction, which are all linked to cardiomyocyte apoptosis, myocardial fibrosis and contractile dysfunction [2].

The role of inflammation and fibrosis

Both elevated plasma levels of free FAs and hyperglycemia may trigger the cardiac transcriptional activity of NF-κB, thus increasing the secretion of cytokines and chemokines, which carry out numerous autocrine activities via the downstream activation of AP-1, NFAT and NF-κB itself [30]. All of them are involved in reducing cardiac contractility and the subsequent progression to heart failure in DCM, as well as in **cardiac hypertrophy** [2]. Inflammation harms myocardial tissues and causes **cardiac remodeling**, which is characterized by interstitial fibrosis, in a process regulated by AP-1 and NF-κB, among others. Hyperglycemia-induced formation of AGEs in cardiomyocytes also independently contributes to NF-κB activation [109], increasing interstitial fibrosis, myocardial stiffness, impaired cardiac relaxation and diastolic dysfunction [1].

Oxidative stress and apoptosis

The imbalance between glucose and FA oxidation in the heart causes the mitochondria to produce ROS, which accumulate in cardiomyocytes. The resulting oxidative stress stimulates pro-inflammatory transcription factors, promotes cell death, and elicits ER stress, which contribute to all

the stages of DCM. AGEs significantly aggravate intracellular oxidative stress. Apoptosis is hastened by hyperglycemia and ROS accumulation in the heart through the activation of MAPK, involving the pro-apoptotic JNK and p38, and the anti-apoptotic ERK1/2.

Other pathophysiological mechanisms

ER stress plays an important role in determining the fate of cardiomyocytes in DCM. If it persists, the activation of the NF-κB, p38 MAPK and JNK pathways will bring on ER stress-mediated cardiomyocyte apoptosis [10, 110]. AGEs inhibit cardiac SERCA2a expression and promote ER stress in cardiomyocytes [111]. When ER stress arises, cardiomyocyte calcium handling is also altered, thus aggravating diastolic dysfunction [1]. The ensuing acute rise in the intracellular calcium concentration results in mitochondrial calcium accumulation, which leads to ROS formation and apoptosis [112].

Clinician's Corner

1. Diabetic cardiomyopathy (DCM) is a chronic, prevalent (15% in T2D and 35% in T1D) and complex disease characterized by metabolic dysregulation, which is often accompanied by local inflammation, oxidative stress, mitochondrial dysfunction, cardiomyocyte apoptosis, extracellular matrix remodeling and fibrosis.
2. DCM is the leading cause of death among diabetic people, and affects both patients suffering from type 1 or type 2 diabetes, although with different pathogenesis and time course. Women with diabetes are at higher risk of developing DCM than their male counterparts.
3. The quality of glycaemic control is relevant for the development of DCM and heart failure. Each one percentual point increment in glycated haemoglobin (HbA1c) promotes parallel increases of 30%, for T1D patients, and 8%, for T2D patients, in the risk of developing heart failure.
4. The clinical picture of DCM is different in T1D and T2D. Patients with T1D develop myocardial remodeling with myocyte loss, interstitial fibrosis, LV chamber dilation, and reduced systolic function, featuring arrhythmias and anterograde HF symptoms such as fatigue. Symptoms in T2D patients appear rather insidiously, as pulmonary and systemic congestion, owing to diffuse myocardial fibrosis, LV concentric hypertrophy, and diastolic dysfunction with, at least initially, preserved ejection fraction (HFpEF).
5. The common final pathway for DCM, both in patients with T1D and T2D, is dilated cardiomyopathy with impaired systolic function (HFrEF) and chamber dilation, although this scenario appears later in the course of the disease in T2D patients.
6. New pharmacological approaches such as SGLT2 inhibition or treatment with GLP1 receptor agonists appear to tackle diabetes-induced metabolism disturbances and have been already shown to reduce cardiovascular mortality and incident heart failure hospitalizations in diabetic patients.
7. Sirtuins are a group of deacetylase enzymes that, according to preclinical studies, play an important role in regulating oxidative stress, calcium homeostasis, metabolism, inflammation, fibrosis and apoptosis, all of which are mechanisms involved in the pathogenesis of DCM. Therefore, they are promising molecular targets for the development of specific therapeutics for this life-limiting complication of diabetes mellitus.

Box 2. Pharmacological modulation of sirtuin activity

More than 3,500 SIRT1-activating compounds have been identified to date [12]. The most investigated is resveratrol (3,4′,5-trihydroxystilbene), a phenolic compound naturally found in the skin of grapes and berries. In a T1D model, resveratrol normalizes the protein levels and activities of SIRT1, SIRT2, SIRT3 and SIRT5, but not of SIRT4, SIRT6 and SIRT7 [3]. By contrast, the administration of resveratrol to a T2D rat model only normalizes SIRT1 levels and stimulates SIRT5 protein expression [3]. To further complicate the story, resveratrol promotes the deacetylase activity of SIRT5, but, at the same time, it inhibits its desuccinylase activity in a substrate-specific way [113]. Resveratrol ameliorates cardiac dysfunction in DCM through its antioxidant properties, as well as by alleviating metabolic dysregulation and the inflammatory response, improving calcium homeostasis, attenuating ER stress and the impaired autophagy, and reducing apoptosis [3, 10, 91, 92, 114-116]. The activation of SIRT3 by resveratrol could also contribute to the improvements in these processes [87, 117]. However, the beneficial effects of resveratrol and many other SIRT1-activating molecules (SRT1720, SRT2183 and SRT1460) on the heart also depend on their effects on other sirtuins and on sirtuin-independent activities that improve oxidative stress and inflammation [12, 118].

At the molecular level, resveratrol mostly acts through AMPK activation and by modulating NFE2L2 and the receptor for AGEs (RAGE) in T1D. In T2D, resveratrol mostly has anti-inflammatory effects [119]. Resveratrol restores mitochondrial function, increases glucose uptake and inhibits NF-κB activity [20, 91, 92, 120-122]. As a consequence, there are decreases in the expression of NADPH oxidase, the generation of superoxide, and the activity of inducible NOS (iNOS), thus reducing oxidative and nitrative stress [116]. Resveratrol also reduces inflammation through the regulation of the MAPK-dependent pathways (ERK1/2, p38) and high mobility group box 1 (HMGB1), a pro-inflammatory molecule released by immune cells in hyperglycemia [123, 124]. Its antioxidant activity is mostly dependent on NFE2L2, although reductions in FA oxidation, together with improved pyruvate dehydrogenase activity and decreased glucose oxidation, could also be involved [115, 121,

125]. Moreover, its anti-fibrotic and anti-apoptotic effects can occur through the suppression of the ERK1/2 [125], TGFβ/Smad3 [126] and FGF2 [127] signaling pathways, as well as via the activation of UCP2 [128].

Drugs that selectively inhibit some specific sirtuin activities could also be of interest. Honokiol, a natural lignan isolated from the bark and leaves of Magnolia trees, acts as a selective SIRT3 activator [88]. It displays prophylactic and therapeutic activities against cardiac hypertrophy and fibrosis in animal models [88]. Selective SIRT4 inhibitors, such as ZINC12421989, have been proposed to be suitable candidates for the treatment of cardiac hypertrophy and heart failure, or T2D [129]. Although several SIRT5 modulators have been described, they exhibit poor potency and/or low selectivity, which hinders their application [130].

Finally, some drugs belonging to currently approved clinical therapeutic groups may also exert some of their beneficial effects by modulating sirtuin activity. For instance, the anti-hypertensive losartan exerts anti-ischemic effects, at least in part, by normalizing SIRT3 activity in the heart [131], while the phosphodiesterase-5 inhibitor sildenafil and the strong natural antioxidant curcumin mediate antioxidant cardioprotective activities by activating SIRT1 [13].

Figure legends

Figure 1. **Potential cardioprotective effects of SIRT1 in the diabetic heart.** SIRT1 improves metabolism by activating AKT, peroxisome proliferator-activated receptor (PPAR)α and protein tyrosine phosphatase (PTP)1B. Furthermore, it favors mitochondrial dynamics by deacetylating PPARγ coactivator-1α (PGC-1α). SIRT1 activates AMP-activated protein kinase (AMPK), either directly or through the activation of liver kinase B1 (LKB1). Interestingly, AMPK and SIRT1 regulate each other to maintain metabolic homeostasis. SIRT1 inhibits inflammation by reducing NF-κB activity and downregulating p38 mitogen-activated protein kinase (MAPK) activity. As a result, there is a reduction in the expression of pro-inflammatory cytokines and chemokines. It also blunts fibrosis through the deacetylation-dependent inhibition of AP-1, which reduces the transcription of matrix metalloproteinase (MMP)9 and p300, a transcriptional coactivator that regulates transforming growth factor (TGF)β expression. SIRT1 upregulates mitochondrial manganese-dependent superoxide dismutase (SOD2), thioredoxin 1 (TRX1) and catalase. SOD2 is induced through the activation of Forkhead box class O (FOXO)1, FOXO4, hypoxia-inducible factor (HIF)-2α and PGC-1α, and through the inhibition of nuclear factor-κB (NF-κB). The latter is also responsible for the reduction in the activity of NADPH oxidase. Regarding apoptosis, SIRT1 inhibits p53, caspases 3/12 and poly (ADP-ribose) polymerase (PARP) activities, reduces the protein levels of the pro-apoptotic BCL2-associated X (BAX) and B-cell lymphoma 2 (BCL2), and increases the expression of the anti-apoptotic BCL2 like 1 protein (BCL2L1). Finally, SIRT1 downregulates the endoplasmic reticulum (ER) stress pathways mediated by protein kinase R-like endoplasmic reticulum kinase (PERK), activating transcription factor (ATF)6 and inositol-requiring enzyme (IRE)1α/X-box binding protein-1 (XBP1).

Figure 2. **Potential cardioprotective effects of SIRT3 in the diabetic heart.** SIRT3 promotes fatty acid (FA) oxidation by deacetylating mitochondrial long-chain (ACADL), medium-chain (ACADM) and very long-chain (ACADVL) acyl-CoA dehydrogenases. It also promotes the tricarboxylic acid (TCA) cycle and activates the first enzyme of the pyruvate dehydrogenase complex, pyruvate

dehydrogenase E1α (PDHA1), which catalyzes the formation of acetyl-CoA that subsequently enters the TCA cycle. SIRT3 regulates glycolysis by activating lactate dehydrogenase A (LDHA) and promotes LKB1-mediated AMPK activation, thus upregulating glucose uptake. SIRT3 reduces both inflammation and fibrosis through the inhibition of NF-κB and the subsequent reduction in monocyte chemoattractant protein 1 (MCP-1) expression, which reduces macrophage recruitment. It also displays anti-fibrotic actions that depend on the: (1) direct blockade of the TGFβ/Smad3 pathway; (2) activation of glycogen synthase kinase (GSK)3β, an enzyme that blocks TGFβ signaling; and (3) inhibition AP-1 transcriptional activity. These effects result in a diminution in the secretion of cytokines and chemokines (interleukin [IL]-6, tumor necrosis factor [TNF]-α, MCP-1), collagen, matrix metalloproteinases (MMPs) and TGFβ. SIRT3 deacetylates FOXO3, thereby increasing the expression of SOD2 and catalase. SIRT3 can also directly deacetylate several lysine residues in SOD2 to increase its activity. SIRT3 indirectly reduces ROS production by increasing the efficiency of the electron transport chain (ETC), promoting the TCA cycle and regulating FA oxidation. SIRT3 prevents cardiomyocyte apoptosis by deacetylating cyclophilin D and, thus, inhibiting the mitochondrial permeability transition pore. SIRT3 also prevents necroptosis in a NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3)-dependent manner.

Figure 3. **Main effects of SIRT2, SIRT6 and SIRT7 with a potential role in the pathogenesis of the diabetic heart.** SIRT2 reduces myocardial fibrosis in a process that involves liver kinase B1 (LKB1) deacetylation and subsequent AMPK activation, and improves insulin signaling, since this sirtuin is required for optimal AKT activation. SIRT6 activates the AKT signaling pathway to prevent mitochondrial degeneration and lipid accumulation in the heart, while SIRT7 improves mitochondrial function by inducing NRF2. Overall, these effects result in an increase in FA and glucose oxidation, thus attenuating lipid accumulation in the heart. SIRT6 represses the activities of AP-1 and NF-κB, thus reducing the expression of cytokines and chemokines, and deacetylates Smad3 and histone H3 at the promoter of the TGFβ gene to repress its transcription. In contrast, SIRT7 induces the phosphorylation of extracellular signal-regulated kinase (ERK)1/2 and Smad2, which promote the differentiation of cardiac fibroblasts into myofibroblasts, the latter being a highly active cell type that increases the deposition of extracellular matrix components (collagen, fibronectin, matrix metalloproteinases [MMPs], and TGFβ) and, thus, promotes fibrosis. Finally, SIRT6 inhibits PARP and BCL2, and SIRT7 regulates p53 and PARP to prevent apoptosis.

Figure 4. **Main effects of SIRT4 and SIRT5 with a potential role in the pathogenesis of the diabetic heart.** SIRT5 promotes fatty acid (FA) oxidation by deacetylating mitochondrial very long-chain (ACADVL) acyl-CoA dehydrogenase and by positively regulating enoyl-CoA hydratase α-subunit (ECHA). It also demalonylates glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to promote the glycolytic flux, represses the activity of pyruvate dehydrogenase complex (PDC), and inhibits the activity of cardiac succinate dehydrogenase (SDH) within the TCA cycle. SIRT5 activates copper- and zinc-dependent superoxide dismutase (SOD1) as well as isocitrate dehydrogenase 2 (IDH2), and stimulates the expression of FOXO3-dependent antioxidant genes. SIRT4 prevents cardiomyocyte apoptosis by suppressing BAX translocation, whereas SIRT5 acts through BCL2L1, cytochrome c (CytC), peroxiredoxin (PRX) and caspases 3/7.