

Mitochondrial Sirtuin 3 and Renal Diseases

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Key Words

Sirtuin 3 · Mitochondria · Renal diseases

Abstract

Mitochondria are dynamic organelles whose functions are tightly regulated at multiple levels to maintain proper cellular homeostasis. Mitochondrial Sirtuin 3 (SIRT3), which belongs to an evolutionary conserved family of NAD⁺-dependent deacetylases, is a key regulator of the mitochondrial respiratory chain, ATP production, and fatty acid β -oxidation, and it exerts an antioxidant activity. Changes in SIRT3 expression are critical in the pathophysiology of several diseases, such as metabolic syndrome, diabetes, cancer, and aging. In experimental acute kidney injury (AKI), impairment of renal function and development of tubular injury are associated with SIRT3 reduction and mitochondrial dysfunction in proximal tubuli. SIRT3-deficient mice are more susceptible to AKI and die. Pharmacological manipulations able to increase SIRT3 preserve mitochondrial integrity, markedly limit renal injury, and accelerate functional recovery. This review highlights all the selective rescue mechanisms that point to the key role of SIRT3 as a new therapeutic target for curing renal diseases.

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The kidney, along with the brain and heart, is one of the organs in the body that consumes the most ATP, and alterations in mitochondria have been recognized as a hallmark of the initiation and progression of kidney diseases [1]. Several attempts have been made to identify possible molecular mediators of organ damage with particular attention being paid to those involved in energy depletion.

Mitochondria were initially assumed to produce energy in the form of ATP, and are still commonly referred to as the ‘powerhouse of the cell’ because they provide more than 90% of energy to cells [2]. However, a growing body of evidence indicates that mitochondria are also actively involved in a multitude of cellular activities, including cell signaling, proliferation, and death.

Recent studies have highlighted the role of Sirtuin 3 (SIRT3) in regulating mitochondria homeostasis. SIRT3 is a nuclear DNA-encoded, 44 kDa NAD⁺-dependent deacetylase belonging to the Sirtuin family that comprises 7 proteins in mammals [3]. SIRT3 is localized in the mitochondrial matrix and controls a multitude of processes, including respiratory chain activity, tricarboxylic acid (TCA) cycle, fatty acid β -oxidation, and antioxidant pathway, which are fundamental for the functional integrity of organelles [4–6]. In recent years, our understanding of the roles of SIRT3 has extended from the description of a lysine deacetylase to a multifaceted global regulator of mitochondrial adaptive response to stress, indicating a new target for therapy aimed at improving end-organ damage and ultimately survival. The present

review briefly describes the advances in the understanding of SIRT3 and how it can be exploited therapeutically to obtain organ protection, in particular, focusing on renal diseases.

SIRT3 Regulates Mitochondrial Functions

Since its initial discovery, extensive research has identified several substrates for SIRT3 deacetylase activity that can affect different mitochondrial processes such as energy production and antioxidant defences, underlining the key role of SIRT3 in maintaining mitochondrial vitality.

Mitochondria are complex organelles containing 2 different membranes that allow peculiar metabolism compartmentalization [2]. The outer membrane acts as a permeability barrier to the cytosol and has a similar composition to the plasma membrane. It contains porins that make mitochondria permeable to metabolites and small peptides up to 3,000 Da, while larger proteins require specific outer membrane transporters (translocase of the outer/inner membrane) [7]. Conversely, the inner membrane serves as an electrical insulator through the electron transport chain (ETC) proteins anchored on the typical invaginations (cristae) responsible for ATP synthesis.

Energy production is a core functional element for cell survival and ATP depletion is a strong apoptotic signal. SIRT3 physically interacts with and regulates complexes I, II and V within the ETC [4–6]. Additionally, SIRT3 has been identified as a regulator of mitochondrial ribosomal protein 10, thus revealing SIRT3's role in the transcriptional modulation of respiratory complexes [8].

It is also known that mitochondria have the ability to maintain ATP production by shifting from glycolytic metabolism to fatty acid, amino acid and acetate catabolism as an alternative source of energy for cell survival when the main energy source, pyruvate, is undersupplied [8]. This metabolic switch, also known as the Warburg effect, is regulated by SIRT3 at different levels [8]. All the above evidence indicates that SIRT3 is a global regulator of the ATP homeostasis.

As the site of oxidative phosphorylation, mitochondria are the main source of reactive oxygen species (ROS). Impairment of cellular metabolic function is often accompanied by exuberant ROS accumulation, and oxidative damage has been associated with multiple pathologies, including neurodegenerative diseases, diabetes, cancer, and premature aging [9]. Increased ROS production and decreased antioxidant defences make mitochondria

particularly susceptible to injury. For this reason, ROS levels are tightly regulated by several antioxidant enzymes, including superoxide dismutase 2 (SOD2), peroxiredoxins (PRX3 and PRX5) and glutathione peroxidases inside the organelles [10]. The role of SIRT3 in the regulation of antioxidant pathways is manifold, as SIRT3 has been shown to activate SOD2, favoring superoxide removal [10], and to interact with the TCA cycle enzyme isocitrate dehydrogenase 2, ultimately detoxifying cells from ROS [10].

SIRT3 Regulates Mitochondrial Structural Integrity

Mitochondria are also well acknowledged to play a key role in cellular calcium (Ca_2^+) homeostasis and are closely involved in the regulation of apoptosis [11]. Intrinsic apoptosis is primary, albeit not exclusively [12], dependent on the mitochondrial permeability transition pore (mPTP) opening that leads to mitochondrial membrane potential ($\Delta\Psi_m$) dissipation, cytochrome c release, and the activation of pro-apoptotic pathways [11]. In this regard, SIRT3 has been shown to prevent mPTP opening by deacetylating cyclophilin D in cardiomyocytes [13], thus preventing ROS generation, perturbation of Ca_2^+ homeostasis, and cell apoptosis.

In the last decades, extensive research in the field has provided compelling evidence that mitochondria are unlikely static organelles but their size, number, and location may vary significantly depending on the cell-specific energy demands. Mitochondria exist in dynamic networks that steadily divide and join through fission and fusion events [14]. Fission is needed to remove mitochondria from the network when the organelles become damaged, while fusion helps to ease physiological stress levels by mixing the contents of partially damaged mitochondria, thus diluting the accumulated oxidized proteins and ROS. Fission is mediated by cytosolic dynamin-related protein 1 (Drp1) which, upon activation, is recruited to mitochondria and forms a ring-like structure that constricts and ultimately severs the organelle [14]. Several Drp1-binding proteins have been identified on the outer mitochondrial membrane, including mitochondrial fission factor (Mff) and fission protein 1 [15]. Conversely, mitochondrial fusion is driven by the self-assembly of mitofusins (Mfn1 and Mfn2) and optic atrophy 1 (Opa1) that tether the outer and the inner membranes, respectively [15]. Mitochondrial dynamics are closely integrated with the quality control pathway of mitophagy [16], which segregates damaged mitochondria from healthy

networks. Mitophagy is mainly regulated by the Ser/Thr kinase PTEN-induced putative kinase 1 (PINK1) that in physiological condition is imported to mitochondria where it is steadily cleaved and degraded. Upon loss of $\Delta\Psi_m$, PINK1 accumulates on the outer mitochondrial membrane, where it recruits Parkin, a cytoplasmic E3 ubiquitin ligase, to target the damaged organelles for degradation [16]. The contribution of SIRT3 to regulating mitochondrial dynamics and mitophagy has been poorly explored. Recently, it has been shown that SIRT3 deacetylates Opa1, favoring mitochondrial fusion and cell survival in cardiomyocytes [17].

SIRT3 and Age-Related Diseases

Due to its profound impact on the reprogramming of the mitochondrial protein acetylome, SIRT3 was first indicated as a central agent of adaptation to caloric restriction, capable of prolonging lifespan and delaying age-associated disorders [18]. SIRT3 maps on human chromosome 11p15.5 in a large cluster of genes that were associated with longevity. The discovery of single nucleotide polymorphisms in the *Sirt3* gene that resulted in longevity through increased SIRT3 expression provided evidence of a possible SIRT3-dependent regulation of human aging [19]. Considering that increased oxidative damage is a hallmark of aging, SIRT3 may likely impact longevity via its antioxidant activity. In this regard, we previously documented that mice deficient for angiotensin II type 1 receptor (AT_1R) outlived their wild-type littermates by 26% [20]. The longevity phenotype was associated with reduced oxidative damage, increased mitochondria number, and upregulation of the pro-survival genes nicotinamide phosphoribosyltransferase (NAMPT) and SIRT3 in the kidney [20]. In vitro studies in proximal tubular cells were allowed to establish that angiotensin II downregulated SIRT3 mRNA expression, an effect that was prevented by an AT_1 antagonist, indicating the biochemical link between angiotensin II and SIRT3 via AT_1R [20]. In line with this evidence, we recently documented that angiotensin II-induced insulin resistance, a key component of metabolic syndrome, is dependent on excessive ROS production, ultimately leading to SIRT3 downregulation in skeletal muscle [21]. Consistently, inhibition of ROS by the antioxidant acetyl-L-carnitine (ALCAR) was able to increase SIRT3 protein expression and ameliorated insulin resistance [21]. More recently, it has been shown that SIRT3 deacetylated and activated GSK3 β , thereby blocking TGF β 1 sig-

naling and tissue fibrosis [22]. Aged SIRT3-deficient mice developed more severe tissue fibrosis than their wild-type littermates in multiple organs, including the kidney, thus revealing a novel mechanism by which SIRT3 could regulate age-associated fibrosis [22]. Altogether these results indicate that SIRT3 plays a role in promoting longevity and attenuating age-related disease, such as metabolic syndrome and fibrosis, possibly through the attenuation of oxidative stress and the maintenance of mitochondrial integrity, and suggest that these pathways could be targeted to influence lifespan in mammals.

SIRT3 and Renal Injury

The biological relevance of SIRT3 as a protective agent against acute tissue damage was also offered by findings in acute kidney injury (AKI) [23]. AKI is a disease characterized by the rapid decrease of renal function, tubular injury, and a high mortality rate [24]. Due to the high energy demand, tubular cells are rich in mitochondria and alterations in these organelles have been recognized as a critical early event responsible for AKI progression [1]. In the experimental model of cisplatin-induced AKI, increased oxidative stress and mitochondrial damage were associated with an 80% reduction in the renal expression of SIRT3 [23]. With the aim of increasing SIRT3 for which agonists are not available, we thought of using AICAR, an activator of the SIRT3 upstream signal adenosine monophosphate-activated protein kinase (AMPK), or the antioxidant agent ALCAR. Both treatments markedly improved renal function and tubular damage. These renoprotective effects were coupled with the upregulation of proliferator-activated receptor coactivator-1 α , a crucial regulator of mitochondrial biogenesis capable of promoting SIRT3 gene expression, and NAMPT, the rate-limiting enzyme in the biosynthesis of NAD $^+$, ultimately leading to the restoration of SIRT3 expression/activity. At the ultrastructural level, AICAR treatment tipped mitochondrial dynamics toward fusion, as it counteracted cisplatin-induced fragmentation and reduced the expression of the mitochondrial fission mediator Drp1 in the organelles of AKI mice. That SIRT3 is not a dispensable regulator of mitochondrial functional integrity during tubular injury was further demonstrated in *Sirt3* $^{-/-}$ mice, which developed more severe disease and died prematurely after cisplatin injection compared to wild-type littermates. In addition, SIRT3-deficient animals did not benefit from the therapeutic effects of either AICAR or ALCAR, and

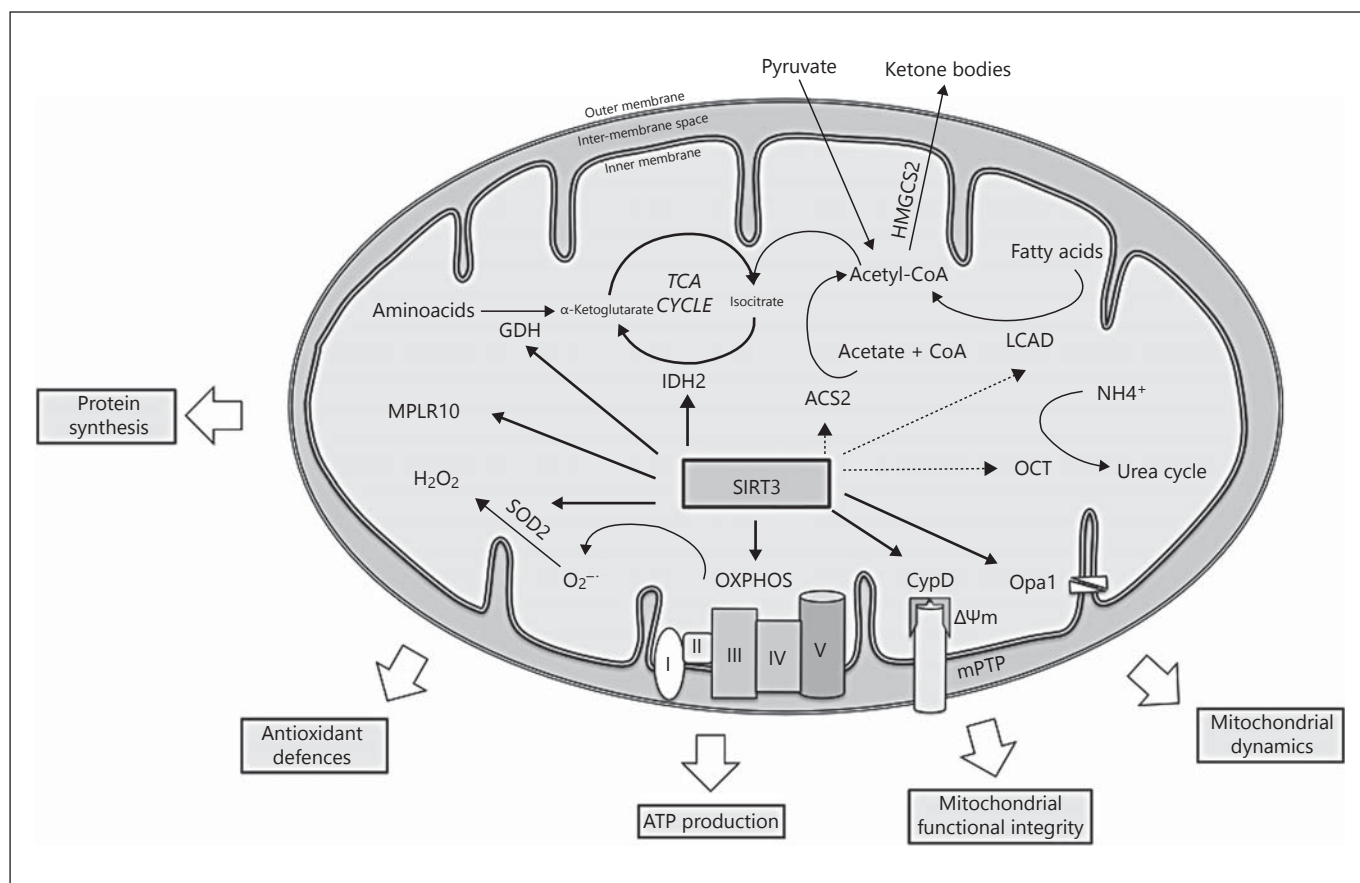


Fig. 1. Schematic representation of the SIRT3-regulated mitochondrial processes. ACS2 = Acetyl-CoA synthase 2; CypD = cyclophilin D; GDH = glutamate dehydrogenase; H_2O_2 = hydrogen peroxide; HMGCS2 = 3-hydroxy-3-methylglutaryl CoA; IDH2 =

isocitrate dehydrogenase 2; LCAD = long-chain acetyl coenzyme A dehydrogenase; MRPL10 = mitochondrial ribosomal protein; NH_4^+ = ammonia; $\text{O}_2^{\cdot -}$ = superoxide anion; OCT = ornithine transcarbamoylase; OXPHOS = oxidative phosphorylation.

these, conversely, had a consistently positive effect on SIRT3-competent mice.

To further understand the mechanisms through which SIRT3 can regulate mitochondrial dynamics, we studied the effect of SIRT3 overexpression in an *in vitro* model of cisplatin-injured human renal proximal tubular epithelial cells. We documented that SIRT3 overexpression elicited mitochondrial fusion by limiting Drp1 recruitment through the downregulation of Mff on the mitochondrial outer membrane and by upregulating Opa1 in cultured tubular cells injured by cisplatin. Moreover, SIRT3 overexpression promoted mitochondrial integrity by counteracting the cisplatin-dependent mitochondrial depolarization, which translated into inhibition of PINK1 accumulation on the organelle outer membrane, thus preventing the disposal of mitochondria via mitophagy. Taken together, these results indicate that SIRT3 is a crucial regula-

tor of mitochondrial functional integrity in aging and a non-redundant player in tubular injury and repair in AKI.

The protective role of SIRT3 in the kidney has been further highlighted by previous findings showing that SIRT3 affected the activation of the mitogen-activated protein kinase-NF- κ B pathway during tubulointerstitial inflammation in proteinuric kidney disease [25]. SIRT3 overexpression suppressed the NF- κ B-dependent transcriptional activity of inflammatory genes, decreased the phosphorylation of ERK1/2 and p38, and reduced ROS levels, indicating a possible molecular mechanism underlying the SIRT3-mediated antioxidant and anti-inflammatory effects in proximal tubular cells [25].

Finally, *in vitro* evidence showed that NAD^+ -dependent activation of SIRT3 regulated hypertrophy in glomerular mesangial cells by enhancing the adenosine AMPK anti-hypertrophic signaling [26].

How to Increase SIRT3?

Given the key role of mitochondria disorders in mediating end-organ injury in several chronic diseases, such as cardiac dysfunction, cancer, and neurodegenerative disorders, along with the profound impact that SIRT3 has on mitochondrial adaptive stress responses, it is not surprising that SIRT3 has been identified as a potential site for therapeutic interventions [27–30]. The evidence that supplementation of NAD⁺ [31] and the administration of AICAR [23] protected from organ injury via SIRT3 offered the rationale for new therapeutic manipulation aimed at increasing SIRT3. Additionally, a recent work demonstrated that honokiol, a natural biphenol derived from the bark of the magnolia tree with antioxidant and anti-inflammatory activity, was capable of attenuating pre-existing cardiac hypertrophy by increasing SIRT3 [32], paving the way for the development of SIRT3 agonist to target organ protection, including the kidney.

Conclusions and Future Perspectives

A growing body of evidence points to SIRT3 as the master regulator of mitochondrial function by activating a wide range of targets involved in ATP production, en-

ergy metabolism, antioxidant pathway, and mitochondrial dynamics (fig. 1). The compelling effort to find out new therapies to arrest or slow age-associated organ injury has led to the demonstration that boosting SIRT3-dependent biological pathways might lend an important clue to obtain renal-protection in acute and chronic settings. The obvious translational consequence of the available studies is to identify specific SIRT3-activating compounds, which the current pharmacopeia is remarkably lacking. Natural SIRT3-activating compound, such as honokiol [32], could be the instrumental chemical template for developing synthetic SIRT3 agonists. The recent case of the Nobel Prize awarded for the discovery of artemisinin and its semi-synthetic derivatives for the treatment of malaria, would suggest it is correct to go in this direction with SIRT3.

Disclosure Statement

The authors have no conflicts of interest to declare.

Statement of Ethics

This study did not require informed consent nor review/approval by the appropriate ethics committee.

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