SIRT3 in Regulating Mitochondrial Function

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Mitochondrial function is finely regulated by post-translational modification of proteins and enzymes by reversible phosphorylation and acylation such as acetylation, malonylation and succinylation. SIRT3 is one of the sirtuin family enzymes that catalyzes the NAD+-dependent deacetylation of a myriad of mitochondrial proteins. SIRT3 deacetylates and activates polypeptides constituting respiratory enzyme complexes and several antioxidant enzymes. Deacetylation and activation of Foxo3a by SIRT3 increases the expression of Parkin and facilitates the mitochondrial quality control by mitophagy. SIRT3 regulates mitochondrial Ca2+ uptake and controls the opening of permeability transition pore, and thereby plays a role in maintenance of Ca2+ homeostasis. It has been demonstrated that deficiency of SIRT3 can result in a decrease of respiration and oxidative phosphorylation and knockout of SIRT3 can impair antioxidant defense by reduction of the activity of MnSOD. SIRT3 is involved in the upregulation of MnSOD and catalase through the transcriptional activation by Foxo3a. Moreover, SIRT3 targets and activates isocitrate dehydrogenase and increase the production of NADPH to promote antioxidant capacity of mammalian cells. On the other hand, oxidative stress can decrease the expression and activity of SIRT3 and impair the mitochondrial function. A deficiency of SIRT3 has been observed in the primary cultures of patients with mitochondrial disease such as CPEO and in mice with insulin resistance that are fed on the high-fat diet and diabetic rats, respectively. In summary, SIRT3 plays a pivotal role in the regulation of mitochondrial respiratory function and Ca2+ homeostasis and in the maintenance of redox homeostasis through transcriptional activation of Foxo3a to increase the expression of MnSOD and catalse and deacetylation of some of the antioxidant enzymes.

SIRT3 Mitochondria Respiratory enzyme Reactive oxygen species Oxidative stress Insulin resistance Diabetes Redox homeostasis Calcium ion Deacetylation

1. Definition

SIRT3 is a NAD^+-dependent deacetylase localized to mitochondria of mammalian cells. Several studies have demonstrated the roles of SIRT3 in the regulation of mitochondrial respiratory function, redox homeostasis, metabolic adaptation, insulin response and stem cell differentiation [1]. Polymorphisms, expression level and enzyme activity of SIRT3 have been related to longevity and metabolic syndrome in the human. SIRT3 deficiency has been implicated in the pathogenesis of aging and age-related metabolic diseases such as cardiovascular disease, fatty liver, insulin resistance and type 2 diabetes [1][2][3].

2. SIRT3 in Mitochondrial Function

SIRT3 promotes mitochondrial function by deacetylation and activation of respiratory enzymes. A deficiency of SIRT3 reduces intracellular ATP level in mouse embryonic fibroblasts, heart, kidney and liver of mice [4]. SIRT3 can directly deacetylate NDUFA9, a subunit of Complex I, via protein-protein interaction. Mitochondria from SIRT3-deficient mice displayed profound and selective defects in respiratory enzyme complexes and ATP synthase. Addition of exogenous SIRT3 could enhance mitochondrial respiration by increasing Complex I activity [4]. In addition, Cimen et al. identified the flavoprotein subunit A of succinate dehydrogenase (SDHA) in Complex II as a substrate of SIRT3 [5]. Comparison of acetylation profiles in mitochondrial proteins from wild-type and SIRT3 KO mice led to the identification of changes in acetylation on several lysine residues in SDHA. Based on the crystal structure of SDHA, acetylation of those lysine residues would block the substrate accessibility of succinate dehydrogenase (SDH). However, deacetylation by SIRT3 opens the pocket of the active site and increases enzymatic activity of SDH [5]. On the other hand, we have shown that SIRT3 interacts with oligomycin-sensitivity conferring protein (OSCP), a subunit of ATP synthase, and increases its activity [6]. The expression of SIRT3 is reduced in the primary culture of skin fibroblasts of patients with large-scale mtDNA deletion or chronic progressive external ophthalmoplegia (CPEO) syndrome. Oxidative stress from these pathogenic mtDNA mutations decreases SIRT3 expression, leading to a decline of respiratory enzyme function via post-translational modification [6].

Several studies have revealed that SIRT3 modulates the generation of intermediary metabolites to be used as a source of energy. SIRT3 can deacetylate and activate pyruvate dehydrogenase (PDH) to facilitate the conversion of pyruvate to acetyl-CoA [7]. That shifts glucose utilization from anaerobic glycolysis to aerobic metabolism. Additionally, SIRT3 increases the pool of acetyl-CoA from acetate by activating acetyl-CoA synthetase (AceCS2) in the prolonged starvation status [8][9]. Taken together, SIRT3 plays a role in the regulation of metabolic flexibility and coordinates the switch between different metabolic pathways in response to energy demand.

SIRT3 modulates mitochondrial permeability transition pore (mPTP) via deacetylation of cyclophilin D (CypD), which is a regulatory protein of mPTP. Cardiomyocytes with SIRT3 deficiency is prone to open the mPTP and trigger apoptosis in response to oxidative stress [10]. It was found that mice with SIRT3 KO display accelerated age-dependent mitochondrial swelling and aortic constriction-induced heart failure or fibrosis [10]. Interestingly, the deacetylation site of CypD by SIRT3 is near the binding site of cyclosporine A, an inhibitor of CypD. Treatment with cyclosporine A could rescue deleterious phenotypes in SIR3-deficient cardiomyocytes [10]. In addition, SIRT3 is involved in the regulation of mitophagy in response to mitochondrial damage. Overexpression of SIRT3 recovers mitochondrial dysfunction and cardiomyocyte injury in a mouse model of diabetic cardiomyopathy [10]. However, SIRT3-mediated beneficial effects would be attenuated by the inhibition of mitophagy. The underlying mechanism is that deacetylation and activation of FoxO3A by SIRT3 upregulates *Parkin* expression to facilitate mitochondrial quality control by mitophagy [11].

Mitochondria are able to modulate influx and efflux of Ca^{2+} ions to alter both the amplitude and the spatiotemporal distribution pattern of the intracellular Ca^{2+} levels. Mitochondrial Ca^{2+} uniporter (MCU) machinery facilitates the entry of Ca^{2+} ions to the matrix. H^+/Ca^{2+} and Na^+/Ca^{2+} exchangers (NCX) efflux Ca^{2+} ions from the matrix to the cytosol. Tight regulation of these proteins is important to increase the Ca^{2+} level to activate mitochondrial enzymes and to prevent accumulation of Ca^{2+} ions and Ca^{2+} overload within the mitochondria [12]. It has been proven that

dysregulation of Ca^{2+} homeostasis is related to metabolic diseases such as obesity, insulin resistance and type 2 diabetes in the human and animals [13]. Higher intracellular Ca^{2+} level has been found in primary adipocytes isolated from obese human subjects with insulin resistance and diabetic rats [14][15]. It has been demonstrated that SIRT3 protects cortical neurons from oxidative stress-induced mitochondrial Ca^{2+} overload. Knockdown of SIRT3 exacerbates H_2O_2-induced cell death while overexpression of SIRT3 attenuates Ca^{2+} overload in mitochondria after H_2O_2 treatment [16]. In addition, SIRT3 inhibits the expression of *MCU* by reducing the level of H3K27ac on the promoter region of the MCU gene via the AMPK-dependent pathway. Downregulation of MCU by SIRT3 overexpression leads to the alleviation of the excess Ca^{2+} uptake into mitochondria and detrimental effects in brown adipocytes of mice treated with the high-fat diet [17]. Moreover, as above-mentioned, the role of SIRT3 in regulating mPTP opening may contribute to the modulation of mitochondrial Ca^{2+} homeostasis.

3. SIRT3 in Redox Homeostasis

Imbalance of redox homeostasis and accumulation of excess reactive oxygen species (ROS) are major causes of aging and metabolic disorders such as insulin resistance and diabetes. Emerging evidence has substantiated the notion that there is a link between SIRT3 and redox homeostasis regulation [1]. It has been proved that SIRT3 is required for the beneficial effects of caloric restriction to reduce the intracellular ROS levels in aging, obesity and diabetes [2][18].

Sirt3 has been shown to directly interact with 8-oxoguanine-DNA glycosylase 1 (OGG1), the enzyme responsible for the repair of the 8-hydroxy-2′-deoxyguanosine (8-OHdG) in DNA. SIRT3 deacetylates OGG1 and protects it from degradation and leads to promoting the repair of oxidative DNA damage, especially in mitochondrial DNA (mtDNA). Silencing SIRT3 causes more severe damage to mtDNA and nuclear DNA and triggers the cells to undergo apoptosis in response to oxidative stress challenge [19]. In the diabetic Zuker obese rat model, a decrease in SIRT3 activity and mitochondrial function was accompanied with a high level of 8-OHdG in the blood circulation and urine compared to non-diabetic lean rats [20]. Proximal tubular cells cultured in a high glucose medium revealed defective mitochondrial morphology, inactivation of SIRT3 and reduction of the antioxidant capacity. These results have substantiated the importance of SIRT3 in redox homeostasis, especially to combat the oxidative stress induced by the high glucose/lipid environment of diabetic tissues [20].

Mice with SIRT3 deficiency displayed increased acetylation and reduced activity of superoxide dismutase 2 (SOD2), which is also known as MnSOD [21]. MnSOD is a mitochondrial antioxidant enzyme for converting the superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2), which can be further detoxified to H_2O by catalase. MnSOD serves as the first line O_2^-scavenger to cope with oxidative stress induced by electron leakage from mitochondrial respiratory chain. Two important lysine residues, K53 and K89, of MnSOD have been identified as direct target sites of SIRT3. Mutations of these two residues block deacetylation of MnSOD and lead to sensitizing ROS accumulation and mitochondrial dysfunction in the cells exposed to oxidative stress [22]. Moreover, Tao et al. also demonstrated that SIRT3 could deacetylate another two lysine residues, K68 and K122, on MnSOD and regulate its antioxidant activity [23]. Notably, in addition to its regulatory function on enzymatic activity, SIRT3 is involved in the induction of *MnSOD* and c*atalase* expression by the increase of FoxO3A transcriptional activity [24]. This has

been demonstrated in SIRT3-mediated amelioration of hypertrophy in primary cardiomyocytes [24]. Foxo3A-dependent upregulation of the protein levels of MnSOD and catalase leads to a decrease of the intracellular ROS levels and suppression of Ras/MAPK/ERK activation.

In addition to MnSOD, SIRT3 also targets and activates mitochondrial isocitrate dehydrogenase 2 (IDH2) via deacetylation [25]. IDH2 promotes antioxidant capacity in mammalian cells by increasing the NADPH level and the ratio of reduced GSH to oxidized GSSG. Under caloric restriction, it was found that SIRT3-mediated IDH2 activation was required for preventing cell death in aging [25]. The deacetylation of lysine 413 on IDH2 is regulated by SIRT3 to promote its antioxidant function. Genetic manipulation of IDH2 to mimic deacetylated lysine on 413 (K413R) dramatically increases the IDH2 activity and protects SIRT3 KO mouse embryonic fibroblasts from oxidative damage [26]. In contrast, incorporation of acetyl-lysine to IDH2 at position 413 inactivates the enzyme [26]. Moreover, glutamate dehydrogenase (GDH) is deacetylated and activated by SIRT3 and promotes the production of NAD(P)H by catalysis of the conversion of glutamate to α-ketoglutarate [27].

4. SIRT3 in Glucose Uptake, Insulin Sensitivity and Fatty Acid Metabolism

It has been demonstrated that SIRT3 is downregulated in skeletal muscle in both streptozotocin (STZ)-induced diabetic mice and high-fat diet-induced obese mice [28]. These results indicate a role of SIRT3 deficiency in the pathogenesis of insulin resistance and type 2 diabetes. Increasing evidence has shown that SIRT3-deficient mice displayed insulin resistance and glucose intolerance phenotypes upon feeding with the high-fat diet. Using hyperinsulinemic-euglycemic clamp, a gold standard method, Lantier et al. demonstrated that SIRT3 KO mice displayed a low glucose infusion rate upon exogenous insulin administration, which indicates the insensitivity of glucose uptake by peripheral tissues in response to insulin [29]. Mechanistically, the dramatic decrease of a glucose-dependent oxygen consumption rate is due to mitochondrial dysfunction caused by hyperacetylation of respiratory enzymes [29]. Loss of the SIRT3-mediated antioxidant defense also caused impairment of insulin signaling and glucose uptake in the peripheral tissues due to accumulation of ROS [29].

In addition to glucose metabolism, SIRT3-deficient mice display increased levels of fatty acid intermediates and accumulation of triglycerides resulted from reduced capacity of fatty acid oxidation in liver during fasting. Mass spectrometry analysis clearly identified the enzymes involving fatty acid oxidation as SIRT3 substrates, which include long-chain acyl-CoA dehydrogenase (LCAD), medium-chain acyl-CoA dehydrogenase (MCAD) and acyl-CoA dehydrogenase 9 (ACAD9). These findings suggest that SIRT3 also regulates the lipid utilization and contributes to the elimination of excess fatty acids upon feeding mice with a high-fat diet [30][31].

In addition to the diet-induced obese mouse model, SIRT3 also plays a role in the pathogenesis of streptozotocin (STZ)-induced diabetes. Absence of SIRT3 accelerates the progression of diabetes development and triggers other complications including cardiac problems and interstitial fibrosis [11]. Finley et al. demonstrated that SIRT3-targeted deacetylation and activation of SDH is impaired in the brown adipose tissues (BAT) of SIRT3 KO mice [32]. Considering the high ability in glucose/lipid uptake and metabolism of the BAT, its dysfunction has been strongly

correlated with the development of insulin resistance and type 2 diabetes [33]. A recent study has shown the importance of succinate-SDH pathway in promoting energy expenditure of BAT and its anti-obesity effect [34]. This further highlights the regulatory role of SIRT3 in the utilization of excess fuels to generate heat or energy and maintain metabolic homeostasis.

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