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Review

SIRT3 regulation of mitochondrial oxidative stress

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ABSTRACT

Mitochondria play a central role in the production of reactive oxygen species as byproducts of metabolism and energy production. In order to protect cellular structures from oxidative stress-induced damage, cells have evolved elegant mechanisms for mitochondrial ROS detoxification. The mitochondrial sirtuin, SIRT3, is emerging as a pivotal regulator of oxidative stress by deacetylation of substrates involved in both ROS production and detoxification. This review will summarize recent findings on the regulation of mitochondrial ROS homeostasis by SIRT3.

1. Introduction

Mitochondria are the primary sources of reactive oxygen species (ROS), as there are many processes that generate superoxide (O_2^-) as a natural byproduct of mitochondrial metabolism, including leakage from electron transport chain (ETC) enzymes during oxidative phosphorylation and the tricarboxylic acid (TCA) cycle. ROS that are physiologically produced in the mitochondria participate in critical signaling pathways to mediate adaptive responses and regulate diverse biologies, including cell growth and differentiation (Finkel and Holbrook, 2000; Hamanaka and Chandel, 2010). However, exposure to excess ROS, which may arise as a consequence of an imbalance between production and detoxification pathways, leads to accumulated oxidative damage of critical macromolecules, such as DNA, RNA, proteins and lipids. Thus, creating an imbalance by lowering ROS below homeostatic levels might alter normal physiological functions, whereas increasing ROS may result in oxidative damage-induced decline of cellular function, and potentially accelerate aging and the onset of age-related diseases (Balaban et al., 2005; Finkel and Holbrook, 2000).

Sirtuins are a highly conserved family of proteins that share a conserved catalytic domain, which endows these enzymes with NAD^+-dependent protein deacetylase, deacylase and/or mono-ADP-ribosyltransferase activities (Du et al., 2011; Frye, 1999; Imai et al., 2000). Mammals have seven sirtuins (SIRT1-7) localized throughout the cell. Dependence on NAD^+ links sirtuin activity to the metabolic status of the cell, suggesting an important role as metabolic sensors for the regulation of cellular metabolism and mitochondrial function (Haigis and Sinclair, 2010). Three sirtuins, SIRT3, SIRT4 and SIRT5 are located in the mitochondria, highlighting a role in regulating metabolic and respiratory pathways in this organelle (Ahn et al., 2008; Haigis et al., 2006; Hallows et al., 2011; Hirschey et al., 2010; Nakagawa et al., 2009; Verdin et al., 2010).

SIRT3 resides primarily in the mitochondria and has been shown to bind and deacetylate several metabolic and respiratory enzymes that regulate important mitochondrial functions (Onyango et al., 2002). In fact, tissues from $SIRT3^{-/-}$ mice exhibit striking mitochondrial protein hyperacetylation, suggesting that SIRT3 functions as the major mitochondrial deacetylase (Lombard et al., 2007). Importantly, lysine acetylation has emerged as a key posttranslational modification in the regulation of mitochondrial function and metabolism, highlighting a role for SIRT3 in the modulation of these pathways (Zhao et al., 2010). Several studies in the past few years have shown that SIRT3 is induced during times of perceived energy deficiency, such as caloric restriction (CR) and fasting, to mediate adaptive responses (Hallows et al., 2011; Hirschey et al., 2010; Palacios

Abbreviations: ACO, aconitase; ATP, adenosine triphosphate; CoQ, ubiquinone; $CoQH_2$, ubiquinole; CR, caloric restriction; CsA, cyclosporine A; CypD, cyclophilin D; CytC, cytochrome C; ETC, electron transport chain; ETFDH, electron transferring flavoprotein dehydrogenase; FAD, flavin adenine dinucleotide; FeS, iron–sulfur; FMN, flavin mononucleotide; $FMNH_2$, reduced flavin mononucleotide; FoxO3a, forkhead box O3a; GPD2, glycerol 3-phosphate dehydrogenase; GPx, glutathione peroxidase; GSH, glutathione; GSR, glutathione reductase; GSSG, oxidized glutathione; HIF-1α, hypoxia-inducible factor-1α; HRE, hypoxia response element; IDH2, isocitrate dehydrogenase; KGDH, α-ketoglutarate dehydrogenase; ME, malic enzyme; MEF, mouse embryonic fibroblast; MPTP, mitochondrial permeability transition pore; NAC, N-acetylcysteine; NAM, nicotinamide; NNT, nicotinamide nucleotide transhydrogenase; PDH, pyruvate dehydrogenase; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; Prx3, peroxiredoxin; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SOD2, manganese superoxide dismutase; TCA, tricarboxylic acid; Trx2, thioredoxin; TrxR2, thioredoxin reductase.

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et al., 2009; Someya et al., 2010). Consequently, SIRT3 activation results in stimulation of oxidative metabolism via deacetylation of numerous mitochondrial enzymes (Finley et al., 2011b; Hirschey et al., 2010). SIRT3 has been shown to particularly regulate the production of ROS at the ETC, as well as the detoxification of ROS through activation of antioxidant enzymes (Ahn et al., 2008; Bell et al., 2011; Jacobs et al., 2008; Qiu et al., 2010; Someya et al., 2010; Sundaresan et al., 2009; Tao et al., 2010). Recent studies highlight the ability of SIRT3 to protect cells from oxidative damage, and implicate an important role of SIRT3 in regulating ROS homeostasis in response to CR (Qiu et al., 2010; Someya et al., 2010).

An intriguing picture is emerging whereby SIRT3 activation results in both a burst in mitochondrial oxidative pathways and ROS clearance. This review will discuss recent developments on the role of SIRT3 in controlling mitochondrial ROS homeostasis via the regulation of proteins that contribute to ROS production or detoxification.

2. Regulation of ROS production

Physiological levels of ROS can be generated from several enzymes. The complexes I, II and III of the ETC, glycerol 3-phosphate dehydrogenase (GPD2), and electron transferring flavoprotein dehydrogenase (ETFDH) can generate relatively high amounts of ROS. Additionally, the subunits of the α-ketoglutarate dehydrogenase (KGDH) and pyruvate dehydrogenase (PDH) complexes and aconitase (ACO) of the TCA cycle contribute low amounts of ROS (Brand, 2010).

The ETC is considered an important site of mitochondrial ROS production particularly because complexes I and III generate high rates of O_2^- during physiological conditions. The ETC functions to create a transmembrane proton gradient, known as the mitochondrial membrane potential ($\Delta\psi$), that is required for generation of adenosine triphosphate (ATP) via proton flow through ATP synthase. Electrons, derived from NADH and succinate, pass through a series of protein complexes (I–IV) that are embedded in the inner-mitochondrial membrane. The electrons are transferred to oxygen, generating water, and promote the transport of protons across the membrane to establish the $\Delta\psi$. Normal ETC flux results in a physiological level of electron leak and ROS production. Electrons leaking from the ETC react with oxygen (O_2) to form the free radical O_2^-. In aqueous solution O_2^- is in equilibrium with the hydroxyl radical (HO^-) and the hydroperoxyl radical (HO_2^-), of which the latter can act as a potent oxidant to initiate lipid peroxidation. Spontaneous dismutation of O_2^- results in the formation of hydrogen peroxide (H_2O_2) and HO^-, both capable of inducing damage to critical cellular structures. Dysregulated ETC function has serious consequences as electron leak and subsequent ROS production increase. When the cell cannot maintain ROS clearance, further damage to the ETC can occur via DNA mutation and protein oxidation, which can lead to more ROS generation. This highly damaging feed-forward cycle illustrates the necessity of proper regulation of ETC function and ROS homeostasis.

Likewise, the mitochondrial permeability transition pore (MPTP) is important for maintaining the $\Delta\psi$. Induction of the MPTP abolishes the $\Delta\psi$, resulting in decreased respiration efficiency and ATP production, and increases in ROS generation.

Several mitochondrial enzymes involved in the regulation of mitochondrial metabolic pathways, such as the TCA cycle and the ETC are acetylated (Choudhary et al., 2009; Kim et al., 2006; Schwer et al., 2009; Zhao et al., 2010). Interestingly, several subunits of mitochondrial ETC complexes and TCA cycle enzymes demonstrated increased acetylation in SIRT3$^{-/-}$ tissues, indicating that their activity may be regulated by SIRT3.

2.1. Complex I

Complex I, also known as NADH:ubiquinone reductase or NADH dehydrogenase, is the largest multiprotein complex of the ETC, which comprised 45 separate proteins. During forward electron transfer, NADH generated from the TCA cycle initially binds to NADH dehydrogenase, and transfers two electrons to the flavin mononucleotide (FMN) prosthetic group of complex I, creating reduced flavin mononucleotide ($FMNH_2$). $FMNH_2$ transfers the electrons via iron–sulfur (FeS) clusters to reduce ubiquinone (CoQ) to ubiquinole ($CoQH_2$). Complex I generates small amounts of O_2^- by transferring one electron from $FMNH_2$ to oxygen (O_2). Interestingly the rate of O_2^- production is determined by the ratio of NADH to NAD^+ (Kussmaul and Hirst, 2006). The best-known inhibitor of complex I is rotenone, which binds to the CoQ binding site, where it most likely disrupts electron transfer from the FeS clusters to CoQ. The CoQ binding site of complex I comprises a very potent source of O_2^- during reverse electron transfer, where electrons from the reduced $CoQH_2$ pool pass through complex I to reduce NAD^+ to NADH. While it is not precisely known which conditions trigger reverse-electron transfer in vivo, it might also play an important role in ROS production from complex I.

SIRT3 binds and regulates complex I acetylation level and activity (Ahn et al., 2008). Immunopurification of intact multisubunit ETC complexes from mouse liver protein lysates revealed that several complex I subunits in SIRT3$^{-/-}$ liver show increased acetylation. Consistently, general inhibition of sirtuin activity with nicotinamide (NAM) increases acetylation, while increased SIRT3 activity decreases acetylation of complex I. Accordingly, complex I activity as measured by rotenone-sensitive NADH oxidation, is inhibited in mitochondria from SIRT3$^{-/-}$ mice, and as a consequence basal levels of ATP are significantly reduced in mouse embryonic fibroblasts (MEFs) and in certain tissues including heart, kidney, and liver (Ahn et al., 2008). SIRT3 physically interacts with at least one of the known subunits of complex I, the 39-kDa protein NDUFA9, which has previously been identified to be acetylated at lysine 370 (Ahn et al., 2008; Kim et al., 2006). Immunoprecipitation of acetylated lysine from MEFs or mouse liver lysates confirmed increased acetylation of NDUFA9 in SIRT3$^{-/-}$ mice (Ahn et al., 2008). While the accessory subunit NDUFA9 is believed not to be involved in catalysis, a function in stabilization of the enzyme complex and regulation of its activity has been proposed, as well as a role in preventing ROS generation.

2.2. Complex II

Complex II, also known as succinate-coenzyme Q reductase or succinate dehydrogenase (SDH) is unique because it participates in both the TCA cycle and the ETC. It is composed of a flavoprotein subunit (SDHA), an FeS protein subunit (SDHB), and two hydrophobic membrane anchor subunits, SDHC and SDHD, which contain the binding site for CoQ. The flavoprotein SDHA subunit catalyzes the oxidation of succinate to fumarate and transfers electrons to FAD to form $FADH_2$ in the TCA cycle. The electrons are then transferred to FeS clusters in the SDHB subunit to CoQ which is reduced to $CoQH_2$. ROS production from complex II is proposed to occur at both the flavin and the CoQ sites (Quinlan et al., 2012). Mass spectrometry studies revealed that SDHA is acetylated at several conserved lysine residues (Schwer et al., 2009). Acetylated lysines were distributed throughout the amino acid sequence in all four domains of SDHA, and were found exclusively on the outer surface of the protein, accessible for acetylation after the complex II assembly. Evaluation of acetylated residues in SDHA crystal structure suggest that acetylation of the hydrophilic surface of SDHA may control the substrate entry to the active site of the protein and regulate the enzyme activity.

SIRT3 directly binds and deacetylates SDHA and regulates its enzymatic activity (Finley et al., 2011b). Hyperacetylated SDHA from SIRT3$^{-/-}$ mitochondrial lysates demonstrates decreased activity compared to wildtype tissues (Finley et al., 2011b). Treatment with kaempferol was found to induce SIRT3 expression in cells leading to decreased SDHA acetylation and increased complex II activity (Cimen

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