ROS

RESEARCH HIGHLIGHTS

Blocking Reactive Oxygen Species Generation Inhibits Biogenesis in Mitochondrial Dysfunction

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ABSTRACT | While until recently reactive oxygen species (ROS) were thought to mainly act as agents of cell damage, there is growing information on the role of ROS as mediators of signaling to regulate cellular homeostasis. In an article published in Cell Metabolism (2018 November 28; 6:764–775. doi: 10.1016/j.cmet.2018.07.012), Dogan and colleagues reported a notable decline of the clinical and biochemical phenotype in a double mutant model of cytochrome c oxidase-defective mitochondrial myopathy and alternative oxidase (AOX). AOX directly oxidizes ubiquinone, preserving electron flow from carriers NADH and $FADH_2$, and abolishes the contribution of complexes III and IV to membrane potential in the mitochondrial respiratory chain. Although AOX can limit the generation of ROS and preserve redox homeostasis, thereby maintaining tricarboxylic acid cycle activity, the authors highlight that antioxidants can inhibit the homeostatic response to bioenergetic failure by modulating mitochondrial biogenesis. The result supports that interruption of ROS signaling might negatively impact the induction of mitochondrial biogenesis and antioxidant gene expression. This prevents cellular processes that are subject to redox regulation for oxidative damage chain, thereby leading to mitochondrial dysfunction.

KEYWORDS | Antioxidant; Mitochondrial biogenesis; Oxidative stress; Redox signaling

ABBREVIATIONS | AMPK, adenosine monophosphate-activated protein kinase; AOX, alternative oxidase; ATP, adenosine triphosphate; CoQ, coenzyme Q10; COX, cytochrome c oxidase; GPx1, glutathione peroxidase-1; H_2O_2, hydrogen peroxide; KO, knockout; mtDNA, mitochondrial DNA; OXPHOS, oxidative phosphorylation; PGC-1, peroxisome proliferator-activated receptor gamma coactivator 1; $\Delta\Psi$, membrane potential; RET, reverse electron transfer; ROS, reactive oxygen species; SOD, superoxide dismutase; Tfam, mitochondrial transcription factor A; UCP, uncoupling protein

CONTENTS

1. REACTIVE OXYGEN SPECIES AND MITOCHONDRIAL DYSFUNCTION

Reactive oxygen species (ROS) are generated as by-products of cellular metabolism, primarily in the mitochondria [1]. At low or moderate concentrations, ROS-mediated responses in normal cell function play an important role as regulatory intermediaries in signaling process [2–4]. However, under certain situations in which defects in the respiratory chain occur, there is inefficient adenosine triphosphate (ATP) generation and elevated ROS production, and the antioxidant response may not be enough to reset the system to the original level of redox homeostasis [5, 6]. This condition consists of an incomplete reduction of molecular oxygen in the respiratory chain resulting in the overproduction of oxygen-derived free radicals [7]. But, as a result, an adaptive response to oxidative stress due to mitochondrial dysfunction-elicited ROS modulates signaling pathways, such as metabolic reprogramming in affected cells [8, 9].

2. OXIDATIVE STATUS AFFECTS MITOCHONDRIAL BIOGENESIS

During energy transduction, a small number of electrons escape to oxygen prematurely, which has been implicated in the pathophysiology of various diseases [10]. Mitochondria appear to respond to this increased production of ROS by undergoing morphological and/or functional adaptations to improve ATP liberation within cells and/or to begin intramitochondrial signaling. In particular, studies have shown ROS-mediated mitochondrial biogenesis where redox signaling plays a major role in the physiological regulation of mitochondrial function [11–14].

The peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1) family is an example of a strong activator of mitochondrial function and a dominant regulator of oxidative metabolism in a variety of tissues [15]. In skeletal muscle, overexpression of PGC-1 increases mitochondrial content, mainly in the condition of muscle atrophy, decreasing overall protein degradation [16]. PGC-1 controls the induction of a set of proteins involved in the cellular response to mitochondrial oxidative stress, leading to the stimulation of certain nuclear-encoded mitochondrial genes [17]. The importance of PGC-1α in orchestrating mitochondrial biogenesis is associated with the ROS induction of many antioxidant-detoxifying enzymes, and proteins involved in extramitochondrial and intramitochondrial fatty acid oxidation (**Figure 1**).

PGC-1α is induced by stimuli such as thyroid hormone treatment and 5-aminoimidazole-4-carboxamide-1-beta-*d*-ribofuranoside-induced adenosine monophosphate-activated protein kinase (AMPK) activation [18, 19]. AMPK is a conserved serine/threonine kinase that participates essentially in maintaining cellular metabolic balance [20]. Of note, AMPK/ATP induces PGC-1α phosphorylation and antioxidant response, but cells lacking AMPK or PGC-1α signaling cannot respond to physiological mitochondrial ROS signal [21]. There is, therefore, a dynamic association between AMPK and mitochondrial ROS controlling in general cellular metabolic balance. This feedback mechanism mediated by the transcriptional co-activator PGC-1α attenuates ROS levels following oxidative stress.

3. A STUDY REGARDING OXPHOS DEFECT/ANTIOXIDANT TREATMENT/BIOGENESIS

The study by Dogan et al. [22] elegantly described an important picture for the specific inhibition of ROS generation resulting in the blockade of stimulant-dependent signaling (**Figure 2**). ROS are increasingly being recognized as important signaling molecules that regulate skeletal muscle function and are required for optimal cell functioning [7]. By studying ROS signaling, the authors examined a myopathic skeletal muscle-specific cytochrome c oxidase (COX) 15-knockout (KO) mouse by blocking ROS generation using an alternative oxidase (AOX).

Under stress conditions, including defects of the complex III or IV, that couple electron transport to the synthesis of ATP in the mitochondrial matrix, AOX can directly oxidize coenzyme Q10 (CoQ). This maintains the electron flow from NADH and $FADH_2$ but abolishes the contribution of complex III and complex IV to the formation of membrane potential ($\Delta\Psi$). Specifically, KO and AOX lines, generated in a KO-AOX double mutant, were used to examine whether AOX could alleviate the KO phenotype inhibiting ROS production. COX deficiency caused accumulation of the reduced form of CoQ

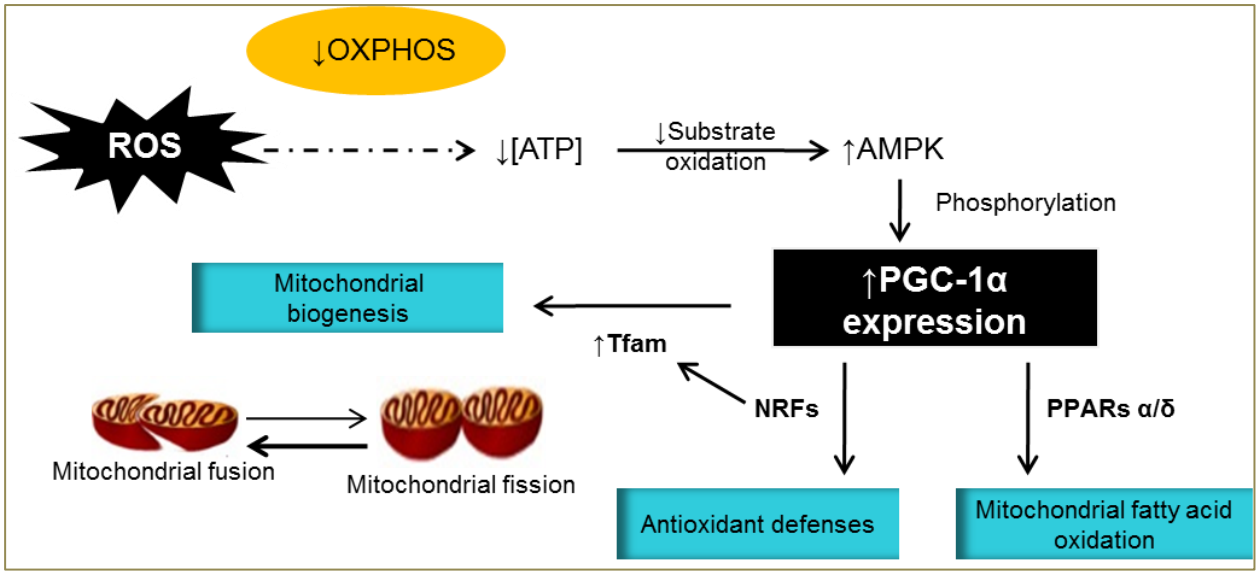

FIGURE 1. Regulation of PGC-1α by ROS stimulates mitochondrial biogenesis, antioxidant defenses, and fatty acid oxidation. See text for detailed description. NRFs denotes nuclear respiratory factors.

and excess of superoxide generation by reverse electron transfer (RET), but AOX normalized the CoQ pool and abolished the increases in RET and ROS production. On the other hand, surprisingly, KO-AOX showed a more severe phenotype upon AOX expression, exacerbating the phenotype of KO mice and decreasing their survival probability. The developmental abnormalities became apparent in KO-AOX with reduced cross-sectional area, number of centralized nuclei (an index of skeletal regeneration), and paired box protein-7 (a marker of resident myoblasts that indicates differentiation of satellite cells), thus impairing the capacity for the repair/regeneration of myofibers. Notably, the unexpected outcome observed in the double mutant model suggested possible mechanisms for an indirect effect consequent to decreased ROS generation.

Dogan et al. [22] showed the induction of mitochondrial biogenesis by the AMPK-dependent PGC-1α axis. Predominantly, interactions between ROS and AMPK activation can be responsible for maintaining the expression of PGC-1α [18]. In the absence of AMPK activation, there was reduction in basal PGC-1α mRNA expression and decline in ROS produced by *N*-acetylcysteine enhanced PGC-1 α mRNA decay. p-AMPK and PGC-1α were upregulated in KO mice, but there were within a normal range in KO-AOX animals. Moreover, citrate synthase, mitochondrial DNA (mtDNA), mitochondrial transcription factor A (Tfam), and subunits of the respiratory complexes were also increased only in KO. This supports the idea that ROS produced at least within skeletal muscle cells may be important for the maintenance of a number of transcription factors, potentiating their induction of mitochondrial bioenergetics-related genes.

Oxidative phosphorylation (OXPHOS) defects increase ROS, decrease ATP synthesis, alter trafficking metabolites, and cause abnormalities in the turnover/cell death by apoptosis/autophagy [23]. Nevertheless, no difference was detected in the ATP production rate, ATP levels, or the $\Delta\Psi$ between KO and KO-AOX muscle samples. AOX does not translocate protons across the mitochondrial inner membrane, which indicates that impaired bioenergetics is not the main reason for the more severe phenotype of KO-AOX mice. However, the array of data reported in this work demonstrated that AOX impaired ROS signaling in KO mice. Succinate-driven hydrogen peroxide (H_2O_2) production was significantly increased in KO mitochondria, but was lower in KO-AOX double mutants than in the control. In addition,

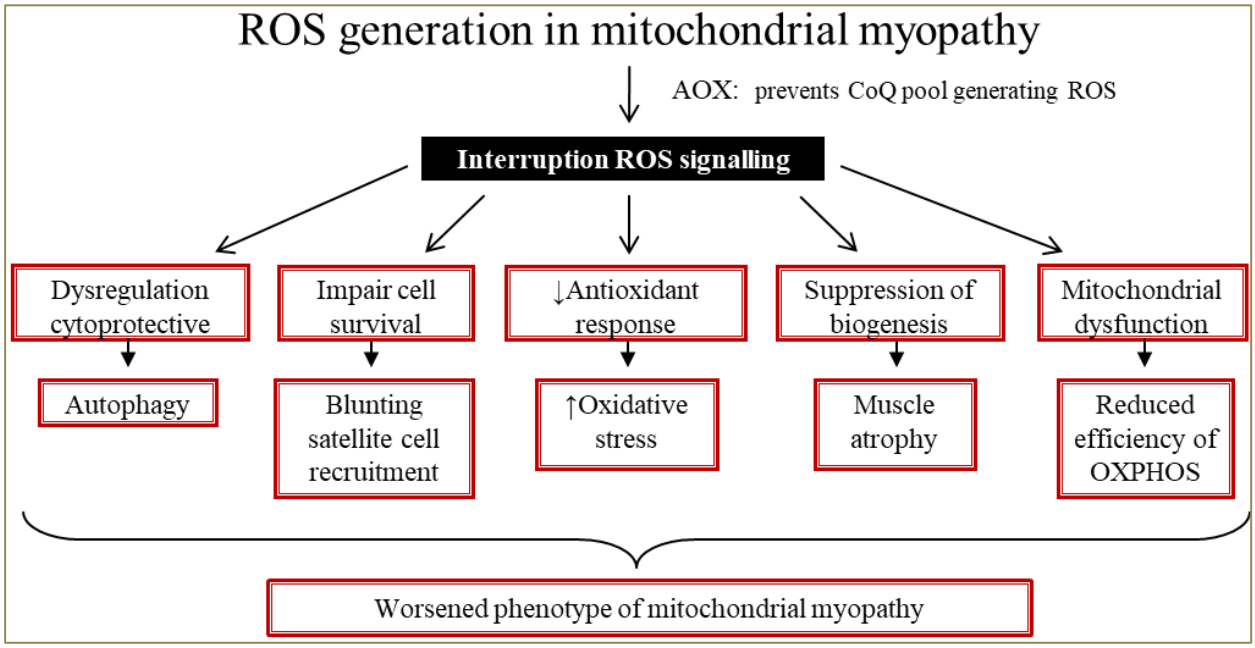

FIGURE 2. Schematic overview of the findings by Dogan et al. [22]. See text for detailed description.

a low level of mitochondrial aconitase activity detected in KO compared with control and AOX groups represented a balance between inactivation by superoxide and what H_2O_2 production was significantly increased. In contrast, KO-AOX samples showed normal values of aconitase activity, thus indicating susceptibility to direct attack by high superoxide levels, which raises H_2O_2 generation.

The expression of cytosolic enzymes involved in ROS metabolism was also examined and was found to be upregulated in KO but downregulated in KO-AOX. The mRNA expression level of cytochrome c, ATP synthase, uncoupling proteins (UCP2 and UCP3), as well as ROS-detoxifying enzymes like superoxide dismutase (SOD) and glutathione peroxidase-1 (GPx1) were all higher in KO than in KO-AOX. Indeed, superoxide is converted to H_2O_2 by the mitochondrial matrix enzyme Mn superoxide dismutase (MnSOD or SOD2) or by the Cu/ZnSOD (SOD1), which is present in both the mitochondrial inner membrane space and in the cytosol. H_2O_2 is more stable than superoxide and can diffuse out of the mitochondrion and the study showed that the antioxidant response was not restricted to the mitochondria when ROS were produced. GPx1, catalase, and SOD1 are all found largely in the non-mitochondrial cytoplasm and/or peroxisomes. Hence, H_2O_2 can be converted to water by mitochondrial and cytosolic GPx1 or by peroxisomal catalase only in KO group.

Reverse electron flow is well documented and is known to be highly dependent on $\Delta\Psi$ [24]. It disappears as the mitochondria are uncoupled and cannot occur if the electron transport chain is blocked by an inhibitor that abolishes $\Delta\Psi$ [25]. This suggests that uncoupling proteins may have a defensive function for mitochondria, mainly for molecules positioned in the matrix, to decrease the mitochondrial ROS production. In normal situations, $\Delta\Psi$ is largely maintained through a balance between transmembrane proton pumping to the intermembrane space mediated by electron transfer and proton translocation to the matrix for ATP synthesis by the ATPase complex. However, importantly, mitochondrial stress markers are increased in both KO and KO-AOX. This implies that although the cell is without energy production in both situations, the systems of antioxidant defense regulation in AOX group do not make it possible to restore the mechanisms of regulation for the recovery of mitochondria through biogenesis. Particularly,

protein synthesis and cell growth were reduced in both KO and KO-AOX muscles.

4. IMPLICATIONS FOR THERAPEUTIC INTERVENTIONS

It is established that excessive free radical production as the result of single electron leaks to oxygen during electron transport leads to macromolecule damage, but little is known about the evidence for mitochondrial dysfunction-elicited ROS producing adaptive responses. Mitochondrial defects can reduce mitochondrial energy production, and there are several insights into mechanisms underlying the interaction between mitochondria-derived ROS signaling and biogenesis. First, mitochondrial respiratory chain is a key site for cellular ROS production, which entails progressive damage of mitochondrial macromolecules including mtDNA. Second, the positive and negative effects of ROS have been attributed to mitochondria by the fact that these organelles are responsible for producing several of the cellular actions that support survival or death of cells. Third, ROS-mediated pathway can trigger PGC-1α induction in skeletal muscles to mitochondrial biogenesis, while cells that express PGC-1α show a higher survival rate. Lastly, PGC-1α expression in response to oxidative stress can have potential therapeutic importance. In this context, PGC-1α demonstrates dual actions by stimulating mitochondrial electron transport through ROS signaling and by suppressing elevated ROS levels. Overall, these regulations provide a clear mechanism whereby tissues such as skeletal muscles undergo cytotoxic protection from the ensuing increased ROS activation. Nevertheless, further studies should reveal exciting features regarding the function of ROS in biogenesis with opportunities for the development of novel therapeutics against mitochondrial diseases.

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