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Chapter

Physiological Functions of Mitochondrial Reactive Oxygen Species

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Abstract

Mitochondria are the major energy producers within a cell in the form of adenosine triphosphate by oxidative phosphorylation. Normal mitochondrial metabolism inevitably generates reactive oxygen species (ROS), which have been considered to solely cause cellular damage. Increase of oxidative stress has been linked to various pathologies. Thus, mitochondrial ROS (mROS) were basically proposed as byproducts of oxidative metabolism, which undergo normalized by antioxidant enzymes. However, the mROS have extensively been esteemed to function as signalling molecules to regulate a wide variety of physiology. These phenomena are indeed dependent on mitochondrial redox status, which is dynamically altered under different physiological and pathological conditions. The oxidative stress is incurred by which the redox status is inclined to exceeded oxidation or reduction. Here, we attempt to integrate the recent advances in our understanding of the physiological functions of mROS.

Keywords: mitochondrial ROS, oxidative stress, oxidative metabolism, redox signaling, mitochondrial physiology

1. Introduction

Mitochondria are double-membrane-bound cellular organelles found in most eukaryotic organisms. The number of mitochondria in cell differs widely according to organisms, tissues and cell types, which is determined by the energy demand. Mitochondria occupy around 40% of the cytoplasm in heart muscle cells and 20–25% with ~2000 per cell in liver cells. Mitochondria, as the power plants of the cell, mainly generate energy in forms of adenosine triphosphates (ATPs) by oxidative phosphorylation (OXPHOS) during glucose metabolism [1, 2]. The OXPHOS is coupled with mitochondrial respiration in which mitochondrial transmembrane potential (MMP, $\Delta\Psi m$) is generated by pumping the protons via mitochondrial complexes I, III and IV of the electron transport chain (ETC) [3].

Molecular oxygen (O_2) is essential for the mitochondrial bioenergetic metabolism, which functions as the final electron acceptor for cytochrome *c* oxidase (complex IV) in the respiratory ETC that catalyses the four-electron reduction of O_2 to H_2O. Mitochondria are an important source of reactive oxygen species (ROS) within most mammalian cells [4, 5]; mitochondrial ROS (mROS) are basically produced as byproducts of this bioenergetic metabolism during the OXPHOS [6]. Electron leaks at complex I and III from ETC lead to forming partially reduced and highly

reactive metabolites of O_2, including superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), formed by one- and two-electron reductions of O_2, respectively [7]. In the presence of transition metal ions, the more reactive hydroxyl radical ($OH^{\cdot}$) is formed. The $O_2^{\cdot-}$ is rapidly dismutated to H_2O_2 by two dismutases including Cu/Zn-superoxide dismutase (Cu/ZnSOD) in mitochondrial intermembrane space and manganese-dependent superoxide dismutase (MnSOD) in mitochondrial matrix. Unless the dismutation of $O_2^{\cdot-}$ is catalyzed into H_2O_2, the radical oxidant promotes DNA damage, protein oxidation and lipid peroxidation in many types of cells. H_2O_2 is also cell damaging molecule to be degraded to water by catalase [8]. Although the $O_2^{\cdot-}$ generation by respiratory complexes is a well-established phenomenon, it is still poorly understood in mechanism [9].

Mitochondria have been implicated in the regulation of a number of physiological and pathological processes, including proliferation, differentiation, programmed cell death, innate immunity, autophagy, redox signalling, calcium homeostasis, hypoxic stress responses and stem cell reprogramming [10–16]. The mROS production contributes to mitochondrial damage in a range of pathologies, which is also is closely related to redox signalling in the cell [4, 17]. However, accumulating evidences show that mROS are not only deleterious molecules derived from the cellular metabolism but also indispensable participants in diverse cellular signalling and regulations [18–20].

In this chapter, we briefly summarize recent developments in our understanding of the involvement of mROS as signalling mediators in redox biology, rather than pathological stress, underlying physiological conditions.

2. Mitochondrial physiology and ROS production

Mitochondria, cellular organelles in cells of eukaryotic organisms, have a primarily role in the process of pyruvate breakdown and ATP synthesis, generating water and carbon dioxide (CO_2) as the end products via aerobic respiration [21]. Mitochondria turned into driving forces in biological evolution after the symbiotic engulfment of aerobic α-proteobacteria by a precursor of the eukaryotic cells around 2 billion years ago [22, 23]. Although mitochondria have preserved the double-membrane shape and ATP production characters of their ancestors, they have attained numerous additional functions in the cell, dramatically altering their structure and composition [24]. Most part of the bacterial genome was rapidly lost or transferred to the nuclear DNA [25]. Mammalian mitochondrial genome is transmitted solely through the female germ line [26]. Human mitochondrial DNA (mtDNA) is a double-stranded, circular molecule of 16,569 bp and contains 37 genes coding for two ribosomal RNAs (rRNAs), 22 transfer RNAs (tRNAs) and 13 proteins [22]. As the major power plants, mitochondria constantly produce reactive radical oxidants as byproducts during OXPHOS. Thus, in response to the metabolic or environmental stresses, mitochondria have accomplished antioxidant defence system [27]. Mitochondria are also highly dynamic to maintain the functions, which form a tubular network that continually changes by fission and fusion [28]. In this section, we concisely discuss overall mitochondrial biology and the ROS generation.

2.1 Mitochondrial structure and genome

A mitochondrion comprises four subcompartments, the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM), and the two soluble compartments intermembrane space (IMS) and matrix, which are architecturally and functionally distinct. The OMM encloses the organelle, which has a protein-to-phospholipid ratio similar to that of the plasma membrane [29]. It contains

large numbers of integral membrane protein, porin [30]. Voltage-dependent anion channel (VDAC) is a major trafficking protein that forms a beta barrel spanning the outer membrane, which transports nucleotides, ions and metabolites between cytosol and intermembrane space [31, 32]. The IMM is found inside of the OMM, which encloses mitochondrial matrix, extensively folded and compartmentalized [33]. The IMM is non-permeable to nucleotides, sugars and small ions; thus specific carrier proteins enable the molecules to transport across the membrane [34].

The mitochondrial respiratory complexes I–IV, in which electrochemical gradient is generated for OXPHOS to occur for ATP synthesis, are embedded in the IMM [22]. Mitochondria contain two aqueous compartments: the IMS and matrix. The IMS, existing between the OMM and IMM, relays molecular transport from cytosol to mitochondrial matrix or reversely [35]. The compositions of small molecules such as ions and sugars in IMS are chemically similar to those in cytosol, as OMM is selectively permeable to those molecules [36]. However, in case of large proteins, the specific signalling peptides are required to be transported across the OMM. Thus, the protein composition of the IMS is different to the protein composition of the cytosol (e.g. cytochrome c) [37]. The mitochondrial matrix, enclosed by IMM, contains mitochondrial DNA (mtDNA), RNA and proteins. Especially, a number of proteins in the matrix are involved in diverse biochemical processes such as tricarboxylic acid (TCA) cycle, fatty acid oxidation, amino acid degradation and mitochondrial dynamics (fission and fusion) [27, 38] (**Figure 1**).

2.2 Mitochondrial genome

Mitochondria contain their own genetic material (mtDNA), which is maternally inherited without DNA recombination and encodes 37 genes that participate in mitochondrial ATP synthesis. Thirteen genes of them are involved in OXPHOS, and the rest two rRNAs and 22 tRNAs. One human cell has hundreds to thousands of mtDNA copies [39, 40]. mtDNA has high rates of mutation and sequence evolution, and mutant and wild-type mtDNA are present in the cell at different proportions [41, 42]. The mtDNA mutations lead to abnormality in OXPHOS activity and ATP synthesis [43]. The mtDNA is exposed to OXPHOS-derived ROS without conventional histone proteins. Moreover, in the lacking repair mechanisms, mtDNA damage further amplifies during DNA replication [44]. Therefore, the mtDNA is susceptible to mutation

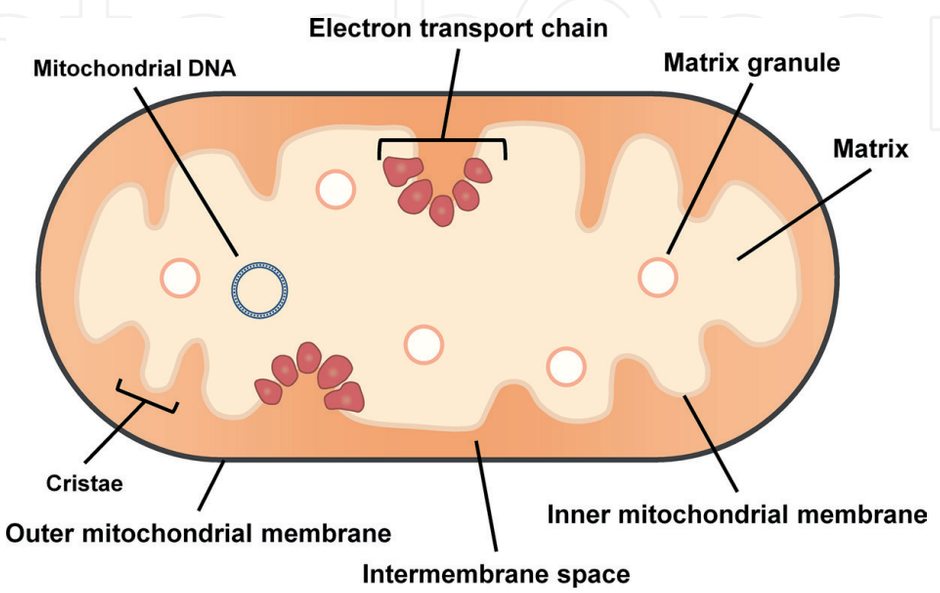

Figure 1.
Mitochondrial structure.

and damage. Therefore, mtDNA mutations and damage cause mitochondrial dysfunction, including ATP synthesis impediment, intracellular calcium level elevation, phospholipase activation and membrane phospholipids decomposition [45, 46].

2.3 Mitochondrial bioenergetics and dynamics

The most prominent roles of mitochondria are to produce the cellular energy in forms of ATPs via aerobic respiration and to regulate the cellular metabolism. Nutrients such as sugars (mostly glucose), lipids and amino acids are oxidized to primarily produce the energy [47]. Approximately 90% of cellular energy requirements are generated in mitochondria [48]. Glucoses and lipids are glycolysed into pyruvic and fatty acids, respectively, in the cytoplasm. Subsequently, these acids form acetyl coenzyme A (Acetyl CoA) via a series of catabolic reactions and then enter the TCA cycle in the mitochondrial matrix [49]. In the reaction of the TCA cycle, convert three equivalents of nicotinamide adenine dinucleotide (NAD^+) into three equivalents of reduced NAD^+ (NADH), one equivalent of flavin adenine dinucleotide (FAD) into one equivalent of $FADH_2$ and one equivalent each of guanosine diphosphate (GDP) and inorganic phosphate (P_i) into one equivalent of guanosine triphosphate (GTP). The NADH and $FADH_2$ are, in turn, used by the OXPHOS to generate ATPs. Thus, oxidation of nutrients provides electrons to the mitochondrial ETC in the form of NADH and $FADH_2$. The sequential transport of electrons from complex I or II to III and IV extrudes protons from the matrix to the IMS, generating an electrochemical gradient. In this process, the ETC requires two electron carriers: coenzyme Q_{10} (CoQ_{10}, also known as ubiquinone) and cytochrome c (Cytc) [50]. Along this electrochemical gradient, the protons flow through complex V (ATP synthase) on the IMM to return to the mitochondrial matrix. This reflux alters the conformation of complex V and drives the synthesis of ATP from ADP and P_i [47].

2.4 Mitochondrial dynamics (fission and fusion)

Mitochondria are highly dynamic and interacting organelles. Mitochondria are able to autonomously integrate (fusion) and divide (fission) by remodelling their morphology and moving along cytoskeletal tracks, in response to their metabolic or pathogenic conditions and cellular environment [29]. The mitochondrial lengths and networks are determined by the balance between fission and fusion rates [51]. Mitochondrial fission and fusion processes are mainly mediated by large guanosine triphosphatases (GTPases) in the dynamin family [51].

Mitochondrial fission requires the dividing of mitochondrial proteins and mtDNA thus that each daughter organelle normally functions without significant loss of soluble proteins from the mitochondrial matrix or intermembrane space [52]. Fission is required for the cellular distribution of mitochondria during cell division and embryonic growth [53]. Exceeded mitochondrial fission, not mutually balanced with fusion, leads to glucose oxidation, MMP decrease and hence the downregulation of ATP production [54]. The fission process is coordinated by a set of components in the cytosol, cytoskeleton, as well as mitochondria. Fission is mediated by a cytosolic dynamin family member, dynamin-related protein 1 (Drp1). Drp1 is recruited from the cytosol to form spirals around mitochondria and, subsequently, constricts the membranes at the fission site to split the mitochondrial cluster [29].

Mitochondrial fusion is mediated by a different set of dynamin-related GTPases. Mitochondrial outer membrane fusion is coordinated with inner membrane fusion. Three large GTPases are essential for mitochondrial fusion [55]. The mitofusins (Mfn1 and Mfn2) are transmembrane GTPases embedded in the OMM [56]. OPA1 is a dynamin-related GTPase associated with the IMM or IMS. Mitofusins and OPA1

physically interact to mechanistically mediate OMM and IMM fusion, respectively [29, 57, 58]. Mitochondrial fusion may increase to maximize the fidelity for OXPHOS in cellular energy demands [27].

2.5 Mitochondrial ROS production and antioxidant enzymes

Mitochondria are the major source of ROS generation [9]. In an organism, mitochondria utilize approximately 98% of the total amount of inhaled O_2, including 1–2% for ROS generation [59, 60]. Mitochondria actually produce ROS in a number of enzymatic reactions; the vast majority of the free radicals from the mitochondria are formed in the ETC during OXPHOS [61]. In the process of OXPHOS, electron leaks from the ETC combine with O_2 molecules to form ($O_2^{\cdot-}$). Mitochondrial $O_2^{\cdot-}$, primarily generated in complexes I and III, is catalysed by Cu/ZnSOD or Mn SOD to disproportionate into H_2O_2. Subsequently, H_2O_2 can be converted to $OH^{\cdot}$ by Fenton reaction. Mitochondrial $O_2^{\cdot-}$ can also bind with protons to form uncharged $HOO^{\cdot}$ radicals and subsequently react with unsaturated fatty acid of mitochondrial membrane lipids to produce lipid radicals. Mitochondrial nitric oxide (NO) interacts with $O_2^{\cdot-}$ to form reactive nitrogen oxide species (RNS) such as peroxynitrite ($ONOO^-$), which produce cellular dysfunction by S-nitrosylating proteins [62]. Mammalian cells have multiple enzymes to degrade H_2O_2, including peroxiredoxins (Prxs), glutathione peroxidases (Gpxs), thioredoxins (Trxs) and catalase. Mitochondrial H_2O_2 is primarily eliminated by the action of Gpx1, Gpx2 and Gpx4, Prx3 and Prx5 and Trx2 systems, which requires glutathione (GSH) [63–65]. Oxidized GSH (GSSG) is reduced to GSH by glutathione reductase (GR) activity [66]. Similarly, oxidized Trx2 is recycled by Trx reductase (TrxR). These H_2O_2 scavenging system ultimately depends on reduced nicotinamide adenine dinucleotide phosphate (NADPH) which is regenerated by three mitochondrial matrix-located enzymes: $NADP^+$-linked isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH)

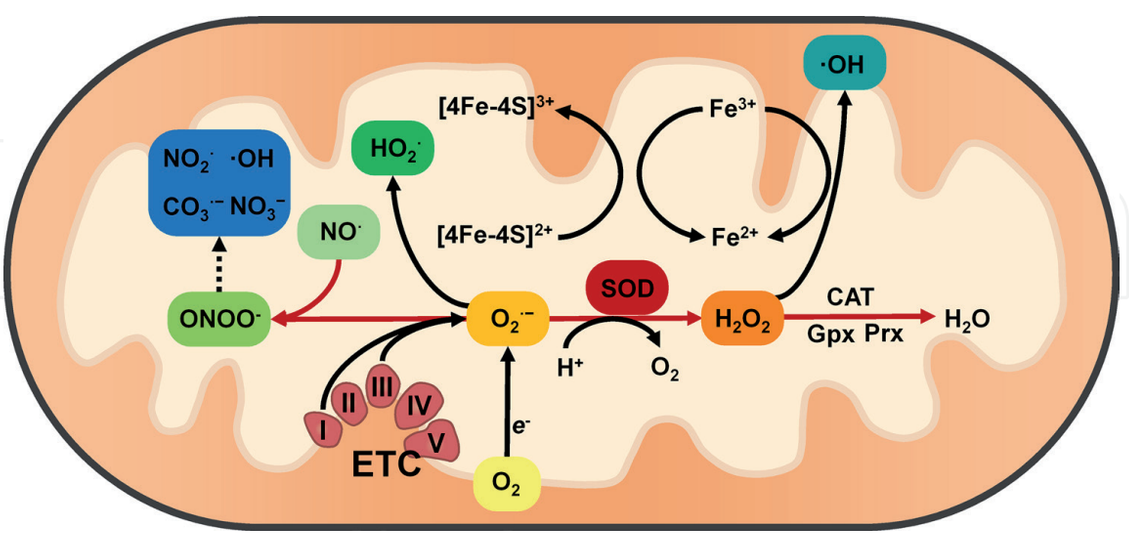

Figure 2.
Reactions and transformations of mitochondrial ROS. SOD enzymes catalyse the dismutation of superoxide ($O_2^{\cdot-}$), generating hydrogen peroxide (H_2O_2). The catalase (CAT), glutathione peroxidases (Gpxs) and peroxiredoxins (Prxs) convert H_2O_2 into water. H_2O_2 reacts with redox-active iron to generate the hydroxyl radical ($OH^{\cdot}$) through the Fenton reaction. The reaction between $O_2^{\cdot-}$ and nitric oxide ($NO^{\cdot}$) produces peroxynitrite ($ONOO^-$), whose decomposition in turn gives rise to some highly oxidizing intermediates including $NO_2^{\cdot}$, $OH^{\cdot}$, $CO_3^{\cdot}$ as well as, finally, stable NO_3^-. Thus, increased $O_2^{\cdot-}$ levels can also reduce $NO^{\cdot}$ and generate $ONOO^-$ toxicity. $O_2^{\cdot-}$ by itself can reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in iron-sulphur centres of proteins, leading to enzyme inactivation and concomitant loss of Fe^{2+} from the enzymes. The protonation of $O_2^{\cdot-}$ can form the more reactive hydroperoxyl radical ($HO_2^{\cdot}$).

and nicotinamide nucleotide transhydrogenase (NNT) [61]. Catalase catalyses the decomposition of hydrogen peroxide to water and oxygen, existing as a tetramer composed of four identical monomers, each of which contains a heme group at the active site. Catalase also requires NADPH as a reducing equivalent to prevent oxidative inactivation of the enzyme [67] (**Figure 2**).

3. Physiological functions of mitochondrial ROS in diverse cellular processes

mROS generation is a ubiquitous phenomenon during life of eukaryotic cells [68]. mROS-induced oxidative stress is considered a main contributor to the aetiology of both normal senescence and severe pathologies. Under normal physiological conditions, mROS emission is accounted for ~2% of the total O_2 consumption, of which the decomposition is well-controlled [2]. Accumulation of mROS, which is an imbalance of neutralization, induces deleterious consequences such as neurodegenerative disease [69], cardiovascular disease [70] and cancers [71]. However, depending on the cellular environment, antioxidant machinery-regulated oxidative stress could initiate diverse cellular responses, involved in cell protection, initiating coordinated activation of mitochondrial fission and autophagy to carry out clearance of abnormal mitochondria and cells, which are to protect spreading the damage to the adjacent cells [72, 73]. H_2O_2 is the primary molecule of mROS utilized for intracellular signalling, which selectively reacts with cysteine residues in redox-sensitive proteins, altering activities or conformations of the proteins to regulate signal transduction [74–76]. Mechanistically, H_2O_2 oxidizes thiol groups (SH) on cysteine residues to form sulphenic acid (SOH), which react with GSH to become glutathionylated (GSSG), with neighbouring thiols to form a disulphide bond (S-S) or with amides to form a sulphenyl amide (S-N) [77, 78]. In this section, we introduce the physiological roles and regulations of mROS in diverse cellular processes such as proliferation, differentiation, autophagy, immunity and aging (**Figure 3**).

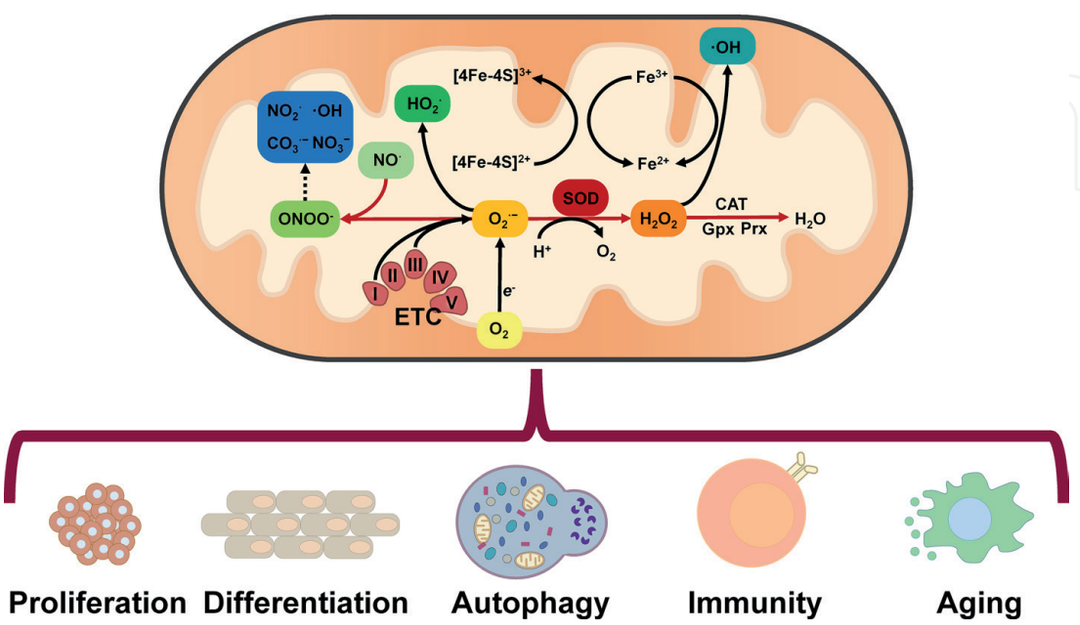

Figure 3.
Physiological regulation by mitochondrial ROS. mROS contribute to the various physiological cellular processes, including proliferation, differentiation autophagy, immunity and aging.

3.1 Proliferation

Accumulation of mitochondria-derived ROS enables to prompt cell proliferation inhibition and cellular senescence [79, 80]. However, the cells essentially utilize mROS for survival and growth via multiple mechanisms in diverse circumstances.

mROS regulate cell proliferation during hypoxia. Under the hypoxic condition (a low O_2 environment, generally 0.3–3% of O_2), the cells raise transcriptional and non-transcriptional responses to increase O_2 supply, simultaneously reducing O_2 consumption. These adaptations to hypoxia are enhanced by mROS. The hypoxia-inducible factors (HIFs) such as HIF1, HIF2 and HIF3 orchestrate the transcriptional response to the hypoxia, promoting erythropoietin (EPO) expression to increase erythropoiesis, vascular endothelial growth factor (VEGF) to promote blood vessel formation and glycolysis enzymes to retain ATP levels [81, 82]. HIFs are heterodimers consisting of two basic helix-loop-helix/PAS proteins: a stable β-subunit and one of three unstable labile α-subunits (HIF1α, HIF2α and HIF-3α) [83, 84]. Under normoxic conditions, prolyl hydroxylase domain protein 2 (PHD2) leads to hydroxylation of HIFα at two proline residues, which target via Von Hippel-Lindau (VHL) E3 ubiquitin ligase-dependent proteasomal degradation [85]. However, under the hypoxic condition, HIFα is stabilized, which is then dimerized with HIF-1β and binds HIF-response elements (HRE) to recruit gene transcription [86]. Moreover, mitochondrial DNA lacking ρ° cells are unable to stabilize HIFα proteins under hypoxic condition, which results from failure of mROS production by ETC deficiency. In contrast, MMP reconstitution restores mROS, which leads to HIFα and cell proliferation [87]. Chemical inhibition of mitochondrial ETC also attenuates mROS production in mitochondria-repleted cells, interrupting to stabilize HIFα under hypoxia [88]. Genetic loss of the complex III subunit Rieske iron-sulphur protein (RISP) or Cytc also inhibits mROS production and HIFα stabilization [89–91]. It is also indicated that mROS are requisite to activate HIFs by non-hypoxic stimulus [92].

mROS are also involved in vascular smooth muscle cell (VSMC) proliferation. Angiotensin II (AngII) is a peptide hormone basically involved in sodium and water homeostasis and vascular contraction, which is also recognized to influence cell growth and proliferation [93]. AngII exerts physiological effects by signalling via interacting with angiotensin type 1 receptors (AT1Rs) [94]. In VSMCs, AngII signalling is required to activate a multitude of mitogenic signalling cascades via crosstalk with growth factor receptors such as epidermal growth factor receptor (EGFR), platelet-derived growth factor receptors (PDGFR) and insulin receptor (IR). Intracellular signalling of VSMC proliferation is stimulated by AngII signalling-triggered mROS production and subsequently induced via mitogenic serine/threonine kinases, including ERK1/2 and p38MAPK [95].

Despite the detrimental effects, mROS function as signal transduction molecules in regulation of stem cells [96]. Depletion of ataxia telangiectasia mutated (ATM) kinase or forkhead box O (FOXO) transcriptional factors increases mROS levels, which impairs hematopoietic stem cell (HSC) proliferation [97–99]. Although the increased mROS level impairs the differentiation of HSCs, a decreased mROS level also has negative effects for self-renewal in neural and spermatogonia stem cells (SCs) [100, 101].

3.2 Differentiation

mROS function as active signalling molecules for diverse cell differentiation. Stem cells (SCs, embryonic or adult) have potentials to self-renew for maintaining stem cell pool or differentiate to the multicellular organism and supply de novo

functional cells to tissues throughout the life of the organism. During differentiation of SCs, the mitochondrial oxidative metabolism is highly stimulated, and thus cellular respiration and mROS production increase [102–105].

In SCs, generally, mitochondria exhibit immature mitochondrial networks and primitive cristae [106]. As bone marrow-derived human mesenchymal stem cells (MSCs) differentiate to osteoblasts, mitochondrial biosynthesis increases by PGC-1α activation [102]. Mitochondrial mass and oxygen consumption increase during differentiation of human embryonic stem cells (ESCs) [107] or pluripotent stem cells (PSCs) [108]. Knockdown of the complex III protein RISP or mitochondrial-targeted antioxidants inhibited differentiation of human MSCs to adipocytes, indicating that mROS are required for differentiation of MSCs [109]. Furthermore, during differentiation of human PSCs, uncoupling protein 2 (UCP2) expression is repressed, which is required for metabolic transition from glycolysis to mitochondrial glucose oxidation. Knockdown of UCP2 expression facilitates mROS accumulation, which stimulate the PSC differentiation to cardiomyocytes. Ectopic UCP2 expression impairs the differentiation with retardation of mROS accumulation and embryonic body formation [110].

mROS, at least within physiological concentrations, have critical roles in processes of myogenic differentiation and muscle regeneration [111]. mROS could promote mitochondrial biogenesis, which is an essential molecule in myogenic differentiation, via peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1)-activated signalling pathway [112]. Myogenic cells are armed with antioxidant enzymes such as SODs, catalase, Gpxs, Prxs, γ-glutamylcysteine synthetase (γGCS) and heme oxygenase-1 (HO-1) [113–120]. These antioxidant enzymes could play as critical signalling molecules to maintain muscle homeostasis in company with primarily neutralizing excessive ROS [121]. mROS facilitate myoblast differentiation and hypertrophy via insulin growth factor 1 (IGF1) signalling pathway [111], which enhances phosphorylation of IGF1 receptor (IGF1R) [122]. Mitochondrial complex I-derived H_2O_2 acts as a signalling molecule to induce cardiac myogenic differentiation. Chemical inhibition of the complex I and treatment of mitochondrial-specific antioxidant exhibits reduction in mROS production and thus impairs the myoblast differentiation [123]. Moreover, mROS induced phosphatase and tensin homolog (PTEN) oxidative inactivation and thereby stimulated phosphoinositide 3-kinase (PI3K)-AKT signalling pathway to express myogenic genes during skeletal myoblast differentiation and muscle regeneration [124]. In differentiation of VSMCs, mROS production also elevates to activate p38 MAPK signalling pathway [125]. However, the complexity of mROS involvement still requires further investigation to elucidate the certain roles of oxidative stress in myogenic differentiation and muscle regeneration.

3.3 Autophagy

Autophagy is a conserved catabolic process that controls cellular degradation of unnecessary or dysfunctional cellular components in the lysosome [126]. Generally, the autophagy continuously occurs to recycle damaged proteins and organelles for cellular homeostasis under normal conditions [127]. The autophagy has at least three different types: (1) Macroautophagy (usually referred to as autophagy): cytosolic contents are delivered to the lysosome by autophagosomes. (2) Microautophagy: the contents are directly introduced into lysosomal membrane. (3) Chaperone-mediated autophagy: the target proteins contain a motif KFERQ, and then the chaperone (KFERQ)-protein complex binds lysosome-associated membrane protein 2A (LAMP2A) receptors on the lysosomal membrane [128]. Autophagy induction results in recruitment of autophagy-related proteins

(ATGs) to a punctate structure, phagophore assembly site (PAS), where proteins of the uncoordinated-51-like kinase 1 (ULK1) complex assemble to initiate autophagosome formation [129].

In autophagy signalling, mitochondria are considered as main source of ROS [130]. mROS, especially as H_2O_2, are required for autophagy induction in response to nutrient starvation and rapamycin, tumour necrosis factor α (TNFα) and nerve growth factor (NGF) deprivation [131–134]. H_2O_2 modulates the cysteine protease Atg4, which cleaves c-terminus of Atg8 (or light chain 3, LC3), and thus enables the addition of phosphatidylethanolamine (PE) to Atg8. Subsequently the active Atg8 is conjugated on the autophagosomal membrane, leading to the autophagosome formation [131]. H_2O_2 also disrupts the MMP to inhibit Akt/mammalian target of rapamycin (mTOR) signalling pathway for autophagy initiation [135, 136]. Furthermore, elevated H_2O_2 induces autophagy via activation of p38 MAPK signalling pathway in cardiac or skeletal muscle [137, 138].

In physiological energy metabolism, mitochondrial ATP production by OXPHOS induces mROS generation, resulting in a certain degree of constitutive mitochondrial damage and submitochondrial particles. The damaged mitochondria cause ATP depletion and Cytc release, which eventually leads to activation of caspases and then onset of apoptosis [139, 140]. To prevent cell death, the dysfunctional mitochondria are thus sequestered from the mitochondrial network and eliminated by selective autophagy, mitophagy, to properly maintain mitochondrial quantity and quality [130]. Therefore, mitophagy limits further mROS generation, which promotes turnover of mitochondria and avoids accumulation of dysfunctional mitochondria. Mitophagy is mainly controlled by the PTEN-induced kinase 1 (PINK1)-Parkin pathway, which is stimulated upon the MMP depolarization. PINK1 is a Ser/Thr kinase that translocates on the outer mitochondrial membrane, which is stabilized by low MMP, thereby sensing mitochondrial depolarization [141–143]. Then, PINK1 recruits Parkin that ubiquitylates OMM-located proteins such as VDAC1, resulting in recruitment of autophagic machinery and the selective sequestration of ubiquitylated mitochondria within autophagosomes [130]. Furthermore, the mitochondrial proteins, BCL2/adenovirus E1B 19-kDa-interacting protein 3 (Bnip3) and Bcl-2/adenovirus E1B 19-kDa-interacting protein 3 (Bnip3L/NIX), participate in mitophagy [144]. In response to oxidative stress after ischemia/reperfusion (I/R), Bnip3 is homodimerised, to be activated, resulting in induction of mitophagy [145]. NIX, an atypical BH3 protein, is required for mitophagy in erythrocyte development. It directly recognizes autophagosome-sited GABA receptor-associated protein (GABARAP) that is a functional homolog of LC3 and subsequently induces mitophagy [126, 146]. Bnip3 and NIX directly bind to the autophagy machinery components, differently to PINK1 or Parkin [147]. ULK1 also regulates mitophagy via translocation to mitochondria to phosphorylate FUN14 domain containing 1 (FUNDC1) protein, a mitochondrial outer membrane protein, which is a receptor for hypoxia-induced mitophagy [148].

3.4 Immunity

In immune system, it is well known that ROS contribute to directly eliminate pathogens via the oxidative burst mediated by NADPH oxidases (NOXs) that are plasma membrane-bound enzyme complexes in phagosomes. However, intracellular redox state intervened by mROS has emerged to be essential for innate and adaptive immune responses [149, 150].

mROS are crucial for Toll-like receptor (TLR) signalling pathways [19]. Activation of cell surface TLRs such as TLR1, TLR2 and TLR4 increases in mROS production via TNF receptor-associated factor 6 (TRAF) and evolutionary

conserved signalling intermediate in Toll pathways (ECSIT) signalling pathway [151]. The TRAF6 or ECSIT depletion promotes reduction of mROS generation in macrophages and thus impairment of bacterial clearance [151]. Lipopolysaccharide (LPS)-induced pro-inflammatory cytokines such as TNFα and IL-6 are controlled by mROS generation [152]. Innate immune response enhancement in patients with TNF receptor-associated periodic syndrome (TRAPS) that is an autoinflammatory disorder is affected by missense mutations in the type 1 TNF receptor (TNFR1), which might be attributable to mitochondrial ROS generation [152].

mROS control pattern recognition receptors (PRRs) such as nuclear oligomerization domain (NOD)-like receptors (NLRs). NLRs form multisubunit protein complexes termed inflammasomes that activate caspase-1 resulting in proteolytic cleavage and pro-inflammatory cytokine IL-1β maturation [153, 154]. Pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) such as lipopolysaccharide (LPS), asbestos, ATP and uric acid activate NLR family pyrin domain containing 3 (NLRP3) inflammasome via mROS generation [155, 156]. Pharmacologic or genetic inhibition of autophagy elevates mROS concentration, which heightens inflammasome activation [157, 158]. Increase of mROS persuades lysosomal membrane permeabilization, which is required for NLRP3 activation [159]. Activation of NLRP3 inflammasome results in mitochondrial damage, interrupting mitophagic signalling [160]. Notably, calcium influx contributes to mitochondrial damage, which might increase mROS production and mtDNA release to amplify NLRP3 inflammasome activation [161, 162]. However, it remains to be further delineated how PAMPs and DAMPs increase mROS to properly activate NLRP3 inflammasome.

In adaptive immune responses, T cells are functionally crucial in response to the pathogens [150, 156]. In infectious condition, naïve T cells promptly proliferate and differentiate into effector T cells [163]. The activation of T cells requires increase in glycolysis and mitochondrial metabolism for synthesis of macromolecules in process of the proliferation and differentiation [156, 164, 165]. Elevated mROS concentration contributes to the T-cell activation; treatment of antioxidants inhibits cellular proliferation and interleukin-2 (IL-2) production [166]. Similarly, antioxidant administration to mice exhibits their reduced immunity after infection of the virus, suggesting that mROS are indispensable for the T-cell functions in vivo [167, 168]. The T-cell receptor (TCR) stimulation induces mROS production from complex I, which leads to activation of NF-κB and AP1 signalling, and in turn facilitates IL-2 and IL-4 productions that are imperative drivers in T-cell activation [169, 170].

3.5 Aging

Aging is a process that is concomitant with the accumulation of cellular damage over the time of all living organisms. In the 1950s, Denham Harman suggested the 'free radical theory of aging' as a molecular explanation for aging [171], in which free radicals, as byproducts of energy metabolism, develop cumulative cellular damage resulting in loss of organismal ability over time. The theory has been revised that the mitochondria-derived free radicals are causative of aging [172]. Mitochondrial dysfunction and consequent excessive ROS production result in inevitable cellular damage and subsequent cell death [173]. Oxidative damage to genomes, proteins and lipids has been associated with mitochondrial dysfunction and ultimately cellular senescence or death [174]. Consistently, overexpression of antioxidant enzymes reduces ROS production and subsequently protects DNA, which is interconnected to a prolonged life span in *Drosophila melanogaster* [175, 176].

Despite numerous evidences underpinning the detrimental roles of mROS in aging, the discoveries are questioning a direct correlation between oxidative stress

and the lifespan. A mitochondrial enzyme, doublecortin-like kinase 1 (MCLK1), reduction induces mitochondrial dysfunction that displays the regression of electron transport in mitochondrial respiratory chain and decline of TCA cycle activity [177]. In *Drosophila melanogaster*, mROS levels elevate along with age, but do not intervene with life span [178]. Furthermore, moderate ROS levels have been associated with an extension of longevity in *Drosophila melanogaster* and in young mice [179–181]. Therefore, physiologically controlled mROS might activate adaptive responses that are beneficial to the organism and extend life span.

4. Conclusion

Mitochondria are primary energy producers to generate ATPs via oxidative phosphorylation. For a long time, mROS have been considered as byproducts of biological energy metabolism during the ATP generation or by cellular redox system imbalance, which are highly aggressive and detrimental to the neighbouring cells and tissues. However, the roles of mROS have been extensively substantiated to understand normal physiology and pathology over the past decades. Mitochondria-derived H_2O_2 have been unequivocally recognized as essential molecules in a range of physiological processes in cells.

In this chapter, we have provided a brief discussion of current understanding of physiological roles of mROS by which mitochondria indeed contribute to the implementation of cellular proliferation, differentiation autophagy, innate and adaptive immunity and aging. In understanding the mechanisms regulating mitochondrial physiology and homeostasis, mROS production might provide a significant potential for the development of novel therapeutic strategy for the treatment of a wide range of human pathologies.

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Conflict of interest

The authors declare no conflict of interest.

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