ROS systems are a new integrated network for sensing homeostasis and alarming stresses in organelle metabolic processes

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Abstract

Reactive oxygen species (ROS) are critical for the progression of cardiovascular diseases, inflammations and tumors. However, the mechanisms of how ROS sense metabolic stress, regulate metabolic pathways and initiate proliferation, inflammation and cell death responses remain poorly characterized. In this analytic review, we concluded that: 1) Based on different features and functions, eleven types of ROS can be classified into seven functional groups: metabolic stress-sensing, chemical connecting, organelle communication, stress branch-out, inflammasome-activating, dual functions and triple functions ROS. 2) Among the ROS generation systems, mitochondria consume the most amount of oxygen; and nine types of ROS are generated; thus, mitochondrial ROS systems serve as the central hub for connecting ROS with inflammasome activation, trained immunity and immunometabolic pathways. 3) Increased nuclear ROS production significantly promotes cell death in comparison to that in other organelles. Nuclear ROS systems serve as a convergent hub and decision-makers to connect unbearable and alarming metabolic stresses to inflammation and cell death. 4) Balanced ROS levels indicate physiological homeostasis of various metabolic processes in subcellular organelles and cytosol, while imbalanced ROS levels present alarms for pathological organelle stresses in metabolic processes. Based on these analyses, we propose a working model that ROS systems are a new integrated network for sensing homeostasis and alarming stress in metabolic processes in various subcellular organelles. Our model provides novel insights on the roles of the ROS systems in bridging metabolic stress to inflammation, cell death and tumorigenesis; and provide novel therapeutic targets for treating those diseases. (Word count: 246).

1. Introduction

Reactive oxygen species (ROS) are defined as oxygen-containing reactive species. It is well established that increased ROS play a vital role in promoting cardiovascular disease such as hypertension, aortic aneurysm, hypercholesterolemia, atherosclerosis, diabetic vascular complication, cardiac ischemia reperfusion injury, myocardial infarction, heart failure, and cardiac arrhythmias, chronic kidney disease, hyperhomocysteinemia, metabolic syndrome, induction of regulatory T cells (Treg) and T cell-mediated inflammation, cigarette smoking, metabolically healthy obesity and obesity, and tumorigenesis. Additionally, ROS have been studied as the therapeutic targets for these disease progression and complications. However, two important issues remain unknown, whether and how the ROS system senses danger associated molecular pattern (DAMP) and conditional DAMP signals in metabolic diseases and connects danger signals to proliferative, inflammatory and cell death-related responses.

ROS are generated by oxidant enzymes and scavenged by scavenging systems, which include enzymatic or non-enzymatic reactions. Imbalances between generation and scavenging ROS systems increase ROS levels and represent alarming stresses. ROS are generated in almost every subcellular organelle in cell, including plasma membrane, cytosol, mitochondria, nucleus, peroxisome.
endoplasmic reticulum (ER) [33], Golgi [29], and others. Of note, —LOX — Fig. 1. Y. Sun et al. human enzymes involved in generating H2O2 and ONOO– have the ability to cross lipid membrane and are classified as communication ROS. Three types of nitrogen-containing ROS are communication ROS.

Abbreviations: O2— superoxide, NO—nitric oxide radical, NO2—nitrogen dioxide, OH—hydroxyl radical, ROO—peroxy radical, R—alkyl radical, CO3—carbonate radical, H2O2—hydrogen peroxide, ONOO—peroxynitrite, 1O2—singlet oxygen, HOCl—hypochlorous acid, ONOO2 —nitrocarbonate, MitoETC: Mitochondrial electron transport chain, XO—xanthine oxidase, SOD—nitric oxide synthase, CYP—cytochrome P450, NOX—NADPH oxidase, LOX—lipooxygenase, SOD—superoxide dismutase, MPO—myeloperoxidase, MAO—monoamine oxidase. GPX—glutathione peroxidase, PRDX—peroxiredoxin, CAT—catalase. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

In this review, we analyzed ROS literature extensively regarding ROS production, localization, and functions. However, several important questions remain: 1) are they sensing and monitoring systems for each of 2801 metabolic pathways [49], in one-to-one basis, or an integrated sensing system for all the metabolic pathways compartmentalized in all the subcellular organelles; 2) it is non-evolutionarily economic and efficient to have one-to-one sensing system for each of 2801 metabolic pathways. An example of an evolutionally efficient sensing system is the humoral immune system, where only four groups of IgG subtypes [50] as backbone structures are needed in order to recognize millions of antigens. So, how can it be possible for danger-associated molecular pattern receptors (DAMP-receptors) [24, 27, 36] and conditional DAMP receptors [25], including plasma membrane-localized Toll-like receptors and cytosolically localized caspase-1 [4,5,51–57]/canonical and caspase-4 (humans)/caspase-11 (mice) non-canonical inflammasome pathways [58,59], to monitor the two cellular status, physiological homeostasis and pathologic abnormality of metabolic processes in all the organelles, in an evolutionarily efficient manner; and 3) is there any currently known sensing system capable of: i) localizing in all the subcellular organelles; ii) trafficking across the membranes of organelles; iii) utilizing a universal process shared by all the metabolic pathways, such as being sensitive to any abnormalities of electron donation and electron acceptance as elucidated in our previous reviews [37–40] and reports [41,42,60,61]; and iv) converging and activating inflammasomes [62]; and v) regulating gene expression, damaging DNAs and determining cell proliferation and cell death [44].

To address those significant issues, we analyzed recent progress in the ROS field. ROS levels are tired to generally every reaction via reception and donation of electrons and participate in various intra-organelle signaling pathways. Thus, ROS might act as an evolutionarily efficient monitoring system for cellular metabolic status. However, whether the functions of ROS in organelles other than mitochondrion are connected still needs to be elucidated. Superoxide (O2–) [30], nitric oxide radical (NO) [63] and hydrogen peroxide (H2O2) [54] are the most studied ROS subtypes, but other subtypes of ROS are not mentioned in many studies. Increased ROS are damaging to lipids, proteins and DNA; and some of these ROS can cross lipid membranes. However, the functional differences between ROS subtypes remain unknown. Furthermore, studies found that ROS are not only by-products of metabolic reactions, but also participate in physiological signaling [65–71], while increased ROS levels contribute to various metabolic outcomes via regulating metabolic processes and pathways [43,72–75]. Therefore, some antioxidant therapies fail because of the undesirable disruption of physiological functions caused by ROS. Therefore, the therapeutic window is important in order to maintain the ROS in normal levels. In addition to the regulatory mechanisms of gene transcription, mRNA splicing, mRNA stability with microRNA modulation, translation efficiency, and post-translation modifications, activations of different metabolic enzymes are also affected by metabolic processes. These enzymes alter the ROS production and scavenging, which qualify the ROS system for their metabolic sensing functions.

In this review, we analyzed ROS literature extensively regarding ROS generation, scavenging, subcellular localization, physiological and pathological signaling and outcomes. Since ROS are functionally
communicated each other between organelles, we propose a new working model that ROS is an integrated network for sensing homeostasis and alarming stress in metabolic processes in various subcellular organelles. Based on increased ROS levels, ROS participated in various working model that ROS is an integrated network for sensing homeostasis and alarming stress in metabolic processes in various subcellular organelles; and ROS scavengers also contribute to maintaining metabolic pathways (also see section 5) to initiate corresponded reactions such as proliferation, inflammation and cell death [76].

2. ROS serve as the new sensing network for homeostasis and stress of metabolic processes in various subcellular organelles

ROS are generated by oxidant enzymes localized in different subcellular organelles; and ROS scavengers also contribute to maintaining redox homeostasis through enzymatic or non-enzymatic mechanisms. Oxidative stress, as a concept formulated in 1985, is induced when ROS generation is increased, or ROS scavengers are dysregulated [77]. Since oxidative stress is strongly correlated with inflammation and cell death [74,78,79], the imbalance of ROS generation and scavenging systems is an alarming signal for cellular metabolisms. In addition, mitochondrial oxidative stress, as a concept formulated in 1985, is induced when ROS generation is increased, or ROS scavengers are dysregulated [77]. Since oxidative stress is strongly correlated with inflammation and cell death [74,78,79], the imbalance of ROS generation and scavenging systems is an alarming signal for cellular metabolisms. In addition, mitochondrial ROS convey information to the nucleus and modulate the expression of nuclear genes accordingly in both physiological [80] and pathological states [66]. Based on the studies we mentioned above, ROS work as a sensor of organelles’ metabolic homeostasis and alarming stress, additionally, ROS transduce signals among organelles and contribute to the determination of cellular responses [34,81]. To better understand the functions of ROS in physiological and pathological conditions, we classify eleven of ROS based on their chemical structures. However, only 41 specific generation enzymes for hydrogen peroxide and superoxide are also studied in Suppl. Table 1.

Table 1A
Reactive oxygen species (ROS) include seven radical- and four non-radical ROS; the generation enzymes and subcellular locations are presented. Among radical ROS, superoxide and nitric oxide are the dominant radicals which are generated by 10 enzymes and 4 enzymes, respectively. Among non-radical ROS, hydrogen peroxide, singlet oxygen and peroxynitrite are major ROS which are generated by 11 and 8 reactions, respectively.

| Category         | Name               | Formula | Classification | Generating system | Intracellular organelle | PMID             | Ref # |
|------------------|--------------------|---------|----------------|-------------------|-------------------------|-------------------|-------|
| Radicals         | Superoxide         | O2*     | ROS            | Complex I         | Mt                      | 24481843 [122]   |       |
| Radicals         | Nitric oxide       | NO*     | RONS           | LOX               | Cyto, Nuc               | 15475499 [30]    |       |
| Radicals         | Nitrogen dioxide   | NO2*    | RONS           | iNOS, nNOS, XO    | Px, SR                  | 24180388 [229]   |       |
| Radicals         | hydroxyl radical   | OH*     | ROS            | Haber-Weiss       | Mt, Cyto, Px             | 24987008 [31]    |       |
| Radicals         | peroxy radical     | ROC*    | ROS            | LOX               | Microsome, Mt, Px, PM   | 15728540 [96]    |       |
| Radicals         | alkoxyl radical    | RO*     | ROS            | LOX               | Microsome, Mt, Px, PM   | 15728540 [96]    |       |
| Radicals         | Carbonate radical  | CO*     | ROS            | SOD1              | Px                      | 17505962 [97]    |       |
| Nonradicals      | hydrogen peroxide  | H2O2+   | ROS            | NOX, SOD, MAO, XO | Px, Mt, Cyto, Px, Mt    | 27925481 [39]    |       |
| Nonradicals      | peroxynitrite      | ONOO−   | RONS           | Photooxygenation   | Mt, Cyto                | 22266568 [106]   |       |
| Nonradicals      | singlet oxygen     | ¹O₂     | ROS            | LOX               | Px                      | 169247 [104]     |       |
| Nonradicals      | hypochlorous acid  | HOCl    | ROS            | Cytochrome C, MPO | Px, Mt, Cyto            | 2076042 [108]    |       |

Abbreviations: XO—xanthine oxidase, NOS—nitric oxide synthase, CYP—cytochrome P450, NOX—NADPH oxidase, LOX—lipoxgenase, SOD—superoxide dismutase, eNOS—endothelial NOS, iNOS—inducible NOS, nNOS—neuronal NOS, MAO—monoamine oxidase, Mt—mitochondria, Px—peroxisome, Cyto-cytosol, PM—plasma membrane, ECM—extracellular matrix.

41 specific generation enzymes for hydrogen peroxide and superoxide are also studied in Suppl. Table 1.
The subcellular organelles of ROS generation and scavenging system are organized as follows. Among the twelve subcellular localizations, mitochondria, peroxisome, plasma membrane, cytosol and nucleus cause more than five types of ROS.

### Table 1B

ROS scavengers and subcellular localization are indicated, and the scavengers include enzymatic- and non-enzymatic- scavengers. Among the 11 types of ROS, superoxide, peroxyl radical, carbonate radical, hydrogen peroxide and peroxynitrite can be scavenged by enzymes; and the rest six types of ROS are found to be scavenged by non-enzymatic scavengers but not enzymatic scavengers.

| Formula | Enzymatic scavenger | Localization (enzymatic-scavenger) | PMID | Ref # | Non-enzymatic scavenger | PMID | Ref # |
|---------|---------------------|-----------------------------------|------|-------|--------------------------|------|-------|
| O2\(^-\) | SOD                 | SOD1 (Cyto, Mt, Nuc, Px), SOD2 (Mt matrix), SOD3 (ECM) | 23442817 | [83] | Bilirubin                | 3140697 | [131] |
| NO\(^+\) |                    |                                   |      |       | Urate                    | 22101099 | [231] |
| NO2\(^-\) |                   |                                   |      |       | Ferrocyanide             | 22101099 | [231] |
| OH\(^-\) | GPX                 | Cyto, Nuc, Mt                     | 22178243 | [29] | PRDX1/2/3/4/5 Cyto, Nuc, Mt, Px | 3140697 | [131] |
| ROO\(^-\) | GPX                 | Cyto, Nuc, Mt                     | 22178243 | [29] | PRDX1/2/3/4/5 Cyto, Nuc, Mt, Px | 3140697 | [131] |
| H2O2    | CAT                 | Px, Mt, Cyto, Nuc                 | 17505962 | [97] | PRDX1/2/3/4/5 Cyto, Nuc, Mt, Px | 3140697 | [131] |
| ONOO\(^-\) | GPX                | Cyto, ECM, Mt, ER                 | 31819197 | [238] | PRDX1/2/3/4/5 Cyto, Nuc, Mt, Px | 3140697 | [131] |
| O2\(^2\) | GPX                 | Cyto, Nuc, Mt                     | 22178243 | [29] | PRDX5 Cyto, Nuc, Mt, Px    | 3140697 | [131] |
| HOCI    |                    |                                   |      |       | Urate                    | 22101099 | [231] |
|         |                    |                                   |      |       | Ferrocyanide             | 22101099 | [231] |

Abbreviations: GPX—glutathione peroxidase, PRDX—peroxiredoxin, CAT—catalase, NDGA—nordihydroguaiaretic acid, AMK—N\(^1\)-acetyl-5-methoxykynuramine, NAC—N-acetylcysteine, 5-HT/Serotonin—5-Hydroxytryptamine.

three types of ROS, including superoxide (O2\(^-\)), nitric oxide radical (NO\(^+\)) and hydrogen peroxide (H2O2), are mostly studied and characterized. In addition, even though the important signaling transducing roles of ROS have been found, the subcellular localizations and functions of different types of ROS are less studied and discussed.

As we mentioned above, eleven types of ROS are classified into two groups, radical ROS and non-radical ROS. Radical ROS include seven types such as superoxide (O2\(^-\)), nitric oxide radical (NO\(^+\)), nitric oxide di-oxide (NO\(^2\)), hydroxyl radical (OH\(^-\)), peroxyl radical (ROO\(^-\)), alkoxyl radical (RO•) and carbonate radical (CO3\(^-\)). Non-radical ROS includes four types such as hydrogen peroxide (H2O2), peroxynitrite (ONOO\(^-\)), singlet oxygen (O2\(^\bullet\)) and hypochlorous acid (HOCI). In addition, three other types of ROS are less studied, such as hydroperoxyl radical (H2O2\(^-\)), nitrocarbonate (ONOOCO\(^2\)), and ozone (O3\(^-\)), and are not discussed in this review. Reactive oxygen and nitrogen reactive species (RONS) are generally discussed as ROS in this review including NO\(^+\), NO2\(^-\) and ONOO\(^-\) [82]. As showed in Fig. 1, among the eleven types of ROS, four types of ROS such as NO\(^+\), NO2\(^-\), H2O2 and ONOO\(^-\) have the ability to cross lipid membrane and transduce signaling across organelles and cells, which we classify as organelle communication ROS in our new working model based on their functions in new integrated sensing and alarming system for metabolic stress. For the other types of ROS, they can hardly pass through lipid membrane due to limited half-life or its physical property, but they contribute to the production of other

### Table 1C

The subcellular organelles of ROS generation and scavenging system are organized as follows. Among the twelve subcellular localizations, mitochondria, peroxisome, plasma membrane, cytosol and nucleus cause more than five types of ROS.

| Organelle | Mt | Px | PM | Cyto | Nuc | Golgi | Caveolae | ER | Phagosome | Microsome | SR | ECM |
|-----------|----|----|----|------|-----|-------|----------|----|-----------|-----------|----|-----|
| O2\(^-\)  | +  | +  | +  | +    | +   | +     |          | +  |           |           | +  | -   |
| NO\(^+\)  | +  | +  | +  | +    | +   | +     |          | +  |           |           | +  | -   |
| NO2\(^-\) | +  | +  | +  | +    | +   | +     |          | +  |           |           | +  | -   |
| OH\(^-\)  | +  | +  | +  | +    | +   | +     |          | +  |           |           | +  | -   |
| ROO\(^-\) | +  | +  | +  | +    | +   | +     |          | +  |           |           | +  | -   |
| RO\(^•\)  | +  | +  | +  | +    | +   | +     |          | +  |           |           | +  | -   |
| CO\(^2\)  | +  | +  | +  | +    | +   | +     |          | +  |           |           | +  | -   |
| H2O2      | +  | +  | +  | +    | +   | +     |          | +  |           |           | +  | -   |
| ONOO\(^-\)| +  | +  | +  | +    | +   | +     |          | +  |           |           | +  | -   |
| O2\(^\bullet\)| + | +  | +  | +    | +   | +     |          | +  |           |           | +  | -   |
| HOCI      | +  | +  | +  | +    | +   | +     |          | +  |           |           | +  | -   |

1 Indicates relative ROS generation enzyme subcellular localization and - indicates relative ROS scavenging enzyme subcellular localization.
aggressive ROS or biological molecule damages [83], which contribute to activation of other stress pathways [44]. Nitrogen-containing ROS mostly are communication ROS. Lipid radicals are dominant in peroxisome. The ROS, which are generated by hydrogen and oxygen, such as HO• and H2O2, are connected with different types of ROS systems, and are functionally classified as the chemical connecting ROS in our new working model.

The enzymes generated and subcellular localization of eleven types of ROS are listed in Table 1A. The following results in Table 1A are highlighted: first, among seven radical ROS, O2•− and NO• are the dominant radicals, which are generated by 10 enzymes and 4 enzymes, respectively. Second, among non-radical ROS, H2O2 and O2 are major ROS, which are generated by 11 and 8 enzymes or reactions, respectively. Notably, cell type information related to those ROS are under-investigated. Enzymatic and non-enzymatic scavenging systems and organelle localization of eleven types of ROS are listed in Table 1B. Among the 11 types of ROS, five types of ROS such as O2•−, ROO•, CO•−, H2O2 and ONOO− can be scavenged by enzymes; and the rest are found to be scavenged by non-enzymatic scavengers but not enzymatic scavengers, suggesting that they are modulated indirectly by gene product regulations. We integrated the subcellular localization of each type of ROS in Table 1C, and the following results are easily found: 1) more than five types of ROS are found to be generated and functioned in the metabolic intensively active organelles, including mitochondria, peroxisome, plasma membrane, cytosol and nucleus. 2) the generation and scavenging enzymes of ROS are both found in some organelles. However, for some types of ROS only generation or scavenging system is found in the organelle. For example, O2•− was found to be generated in Golgi and ER, but the corresponded scavenging enzymes have not been reported in these organelles, indicating that O2•− related metabolic stress can either relay to other types of ROS or branch out to other stress pathways [84]. ROO• scavenging enzymes are found to be expressed in the nucleus, whereas it is currently unknown if ROO• is generated in the nucleus. Further studies are needed to determine the subcellular location of ROO• synthesis.

To clarify, the ROS generated by enzymes in the plasma membrane are released into the cytosol and are scavenged by intracellular systems. For example, ROO• is generated by enzymes located on the plasma membrane, and the scavenge enzymes are localized in the cytosol. To better illustrate the functions of these ROS, the biochemical methods and specific inhibitors are indicated in supplemental (S.) Tables 2 and 3. Based on the roles of different types of ROS in relaying the metabolic stress signals from 2801 metabolic pathways in organelles to a final end in activating inflammasomes, we classify various types of ROS into seven functional groups in our new working model: 1) metabolic stress-sensing (initiation), 2) chemical connecting (one type ROS converted/dis-mutated into another type of ROS), 3) organelle communication (organelle membrane crossing RNAs and capable of relaying metabolic stress to mitochondrial ROS central hub and/or nuclear ROS converging hub), 4) stress branch-out (non-inflammasome activating and crosstalk to other stress pathways (also see section 5) such as misfolded proteins for endoplasmic reticulum (ER) stress [84], DNA damage for nuclear stress [44], RNA damage for RNA degradation [85]), 5) inflammasome-activating (final stop for this sensing system), 6) dual functions, and 7) triple functions. We will explain the rationale for this functional categorization of each type of ROS in the following paragraphs.

2.1. Superoxide (O2•−)

Up to 1–4% of oxygen is reduced into O2•−, which is the first formed ROS. We classify O2•− as the dual function ROS with metabolic stress-sensing and chemical connecting. O2•− is generated in most organelles such as mitochondria, peroxisome, cytosol, plasma membrane, ER and nucleus. It could be generated by eight types of oxidant enzymes, including mitochondrial electron transport chain complex I, complex II, complex III, as well as xanthine oxidase (XO), NO synthases (NOS), cytochrome p 450 enzymes (CYP), NADPH oxidases (NOX) and lipooxygenase (LOX). O2•− is unstable in aqueous solutions because of its short half-life. O2•− can be scavenged or converted in three pathways: 1) O2•− is quickly dismutated to H2O2 after generation; 2) if the production level of O2•− is low (picomolar range), O2•− reacts with NO• to form ONOO−, which process is even faster than dismutation into H2O2 [30]; and 3) at high levels of O2•−, it can react with H2O2 to produce highly reactive radical OH• via protein iron-sulfur center reaction and iron release [30]. Of note, it has been reported that endogenously produced O2•− and H2O2 primarily contribute to NLRP3 inflammasome formation and activation [86] in mouse glomerulig result in glomerular injury or consequent sclerosis during hyperhomocysteinemia [87]. Therefore, O2•− and H2O2 are also functionally classified as the inflammasome activating ROS.

2.2. Nitric oxide (NO•)/nitrogen dioxide (NO2•)

NO• is also the first formed ROS and the triple-function ROS with metabolic stress-sensing, chemical connecting and organelle communication, which is produced by a family of NO synthases (NOS), including neuronal NOS (NOS1, nNOS), inducible NOS (NOS2, iNOS) and endothelial NOS (NOS3, eNOS). In addition to NO•, studies found that NOS also produces O2•− when NOS is not coupled with its cofactors or substrate; and this process is called NOS uncoupling [88]. However, other publications also reported that NOS also generates O2•− in coupled conditions [88]. Besides, NO• is also generated by xanthine oxidase (XO) via reducing nitrates and nitrites [29]. Nitrites reacts with H2O2 to generate NO2• via the function of enzyme myeloperoxidase (MPO). NO2• also reacts with O2•− and produces ONOO− [89]. They both can cross the lipid membrane, which are responsible for communication between organelles and cells.

2.3. Hydroxyl radical (OH•)

As mentioned above, OH• is generated from H2O2 and O2•− through the Haber-Weiss reaction or Fe3+/mediated decomposition of H2O2 through Fenton reaction [31]. However, no enzymes have been identified for OH• generation [90]. In addition, there are no existing enzymatic systems to scavenge OH• [90]. These findings suggest that OH• generation and conversion overcome no energy barriers and do not require the enzymes. Furthermore, OH• has the capability to damage different cellular components via lipid peroxidation, protein damage, DNA bases damage, and membrane destruction [31]. Therefore, the generation and accumulation of OH• lead to cell death. However, based on recent understanding, the organelles that contain O2•− and H2O2 generation enzymes can produce OH•. Since OH• does not convert into other types of ROS, and does not activate nucleotide-binding domain, leucine-rich-repeat containing family, pyrin domain-containing 3 (NLRP3) inflammasome [87] but damage and “branch out oxidative stress” to other molecules and pathways, therefore, OH• is functionally classified as the stress-branch out ROS.

2.4. Peroxyl radical (ROO•)/Alkoxyl radical (RO•)

ROO• is generated as intermediates in the lipid peroxidation reactions [91]. Lipid peroxidation is a chain reaction process resulting in the generation of ROO•, which is characterized by repetitive hydrogen abstraction [92] and addition of oxygen to R•. 0.3% of cytosolic ROO• is in the protonated form as HOO•, which is the simplest form of ROO• [93]. RO• hardly attacks all major classes of biomolecules, mainly the polyunsaturated fatty acids (PUFA) of cell membranes [94] and DNA bases [95]. RO• is generated form ROO• via a tetroxide. RO• is a less aggressive ROS than HOO•, but more reactive than ROO• [29]. Studies reported that LOX contributes to lipid peroxidation and is localized in cytoplasm and nucleus [96], which indicating the subcellular
localization of \( \text{ROO}^- \). They are the most dominant ROS in peroxisome, which may have critical sensing functions in peroxisome. Therefore, \( \text{ROO}^- \) is functionally classified as the triple function ROS with metabolic stress sensing, chemical connecting and ROS-converting and stress-branch-out.

### 2.5. Carbonate radical (CO\(_3^–\))

\( \text{CO}_3^– \) is a negatively charged radical in physiological and pathological conditions, and is formed in the following four reactions: first, superoxide dismutase 1 (SOD1) peroxidase activity. Second, Xo-mediated oxidation of acetaldehyde, xanthine and hypoxanthine \([97]\). Third, hydrogen abstraction from bicarbonate by \( \text{OH}^- \). Fourth, nitricarbonate is converted into \( \text{NO}_2^- \) and \( \text{CO}_3^– \) \([97]\). \( \text{CO}_3^– \) is less oxidizing than \( \text{OH}^- \), but it has larger ranges of oxidative actions because of its longer half-life in comparison to \( \text{OH}^- \) \([97]\). \( \text{CO}_3^– \) could rapidly oxidizes DNA guanine residues and protein residues \([97]\). However, \( \text{CO}_3^– \) is unable to produce stable adducts, which makes it difficult to prove its production compared with other radicals. \( \text{CO}_3^– \) is functionally classified as the stress-branch-out ROS.

### 2.6. Hydrogen peroxide (\( \text{H}_2\text{O}_2 \))

\( \text{H}_2\text{O}_2 \) is generated via SOD-mediated dismutation of \( \text{O}_2^- \) \([31]\). There are three isoforms of SOD. First, \( \text{Cu}, \text{Zn}-\text{dependent SOD} \) (SOD1) converts \( \text{O}_2^- \) into \( \text{H}_2\text{O}_2 \) in mitochondrial intermembrane space (IMS) and cytosol. Second, \( \text{Mn}-\text{dependent SOD} \) (SOD2) converts \( \text{O}_2^- \) into \( \text{H}_2\text{O}_2 \) in the mitochondrial matrix (MM). Third, \( \text{Cu}, \text{Zn}-\text{dependent SOD} \) (SOD3) converts \( \text{O}_2^- \) into \( \text{H}_2\text{O}_2 \) in extracellular space \([83]\). Monoamineoxidase (MAO) resides in the outer mitochondrial membrane and generates \( \text{H}_2\text{O}_2 \) \([31,98]\). XO is one of the most studied ROS generation enzymes in the cytosol \([83,99]\) and mediates \( \text{H}_2\text{O}_2 \) production through the process of hypoxanthine and xanthine catalyze \([64]\). \( \text{H}_2\text{O}_2 \) is also generated in peroxisomes via various enzymes from the normal catalytic cycle \([100]\). These enzymes are mainly flavoproteins, including acyl-CoA oxidases, urate oxidase, \( \alpha \)-amino acid oxidase, \( \alpha \)-aspartate oxidase, \( \alpha \)-piperolic acid oxidase, \( \alpha \)-hydroxyacid oxidase, polyamine oxidase, and XO \([32]\). The adaptor and ROS-regulating protein P66shc translocates to the mitochondrion under oxidative stress via a protein kinase C (PKC)-dependent manner. After translocation, p66shc oxidizes cytochrome c, and generates \( \text{H}_2\text{O}_2 \) \([101]\). \( \text{H}_2\text{O}_2 \) is more stable than \( \text{O}_2^- \), and the concentrations of \( \text{H}_2\text{O}_2 \) are also 100 times higher than \( \text{O}_2^- \) in mitochondria. These properties render \( \text{H}_2\text{O}_2 \) as an ideal signaling molecule in cells \([83]\). \( \text{H}_2\text{O}_2 \) is released via aquaporin 8 formed channel from cell membrane. As discussed above, \( \text{H}_2\text{O}_2 \) is functionally classified as the dual function ROS with organelle communication and inflammasome activating.

To demonstrate the proof of principle that how ROS generating enzymes are connected to metabolic pathways, we also list the metabolic functions of 41H2O2-generating enzymes and \( \text{O}_2^- \)-generating enzymes, which suggests that many metabolic pathways contribute \( \text{H}_2\text{O}_2 \) generation and \( \text{O}_2^- \) generation, which are further connected to inflammasome activation and other cellular stress pathways \([102]\). The enzymes participate in various metabolic processes and pathways as shown in Supplemental Table 1. For example, by searching the metabolomics database (https://hmdb.ca/metabolites/HMDB0004062) using the method that we reported \([11]\), we can find that the enzyme aldehyde oxidase 1 is functional in amino acid valine, leucine and isoleucine degradation, tyrosine metabolism, tryptophan metabolism, vitamin B6 metabolism and nicotinate and nicotinamide metabolism.

### 2.7. Peroxynitrite (ONOO\(^–\))

As mentioned above, the powerful oxidant ONOO\(^–\) is produced from the rapid reaction between \( \text{O}_2^- \) and \( \text{NO}^- \) \([97]\). ONOO\(^–\) causes DNA single-strand breakage and activates poly-ADP-ribose polymerase (PARP) \([103]\). ONOO\(^–\) also induces nitration and S-nitrosylation of proteins and lipids \([103]\). It is capable to cross lipid membranes and thus has the potential to communicate cellular status between organelles. ONOO\(^–\) is functionally classified as the dual function ROS with organelle communication and stress branch-out.

### 2.8. Singlet oxygen (\( \text{O}_2\text{\(^\cdot\)\)})

\( \text{O}_2\text{\(^\cdot\)\}) \) is formed via photooxygenation and enzymatic reactions. The enzymatic reactions for \( \text{O}_2\text{\(^\cdot\)\}) \) production is mediated by several peroxides in lipid peroxidation, including lipooxygenase (LOX), dioxygenase and lactoperoxidase \([92,104]\). Oxidation of \( \text{H}_2\text{O}_2 \) and HOCI mediated by MPO \([105]\) contributes to the production of \( \text{O}_2\text{\(^\cdot\)\}) \) in a chemical system \([104,106]\). In addition, \( \text{O}_2\text{\(^\cdot\)\}) \) is also formed by CYP 2E1 and cytochrome c in the ER and mitochondria, respectively \([107]\). Fenton-type reactions may also give rise to the production in peroxisomes of \( \text{O}_2\text{\(^\cdot\)\}) \). Thus, \( \text{O}_2\text{\(^\cdot\)\}) \) could be a new player in the peroxisome-derived signaling network \([106]\). Furthermore, studies also reported that \( \text{O}_2\text{\(^\cdot\)\}) \) causes mitochondrial ETC proteins oxidation. In addition, studies found that \( \text{O}_2\text{\(^\cdot\)\}) \) generation after light stimulation significantly affects the activities of mitochondrial ETC complex I, complex III, complex IV but except complex II \([109]\). Studies found that the secondary generation of \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) are induced in \( \text{O}_2\text{\(^\cdot\)\}) \) caused dysfunctional mitochondrial respiration \([109,110]\). Thus, we classify \( \text{O}_2\text{\(^\cdot\)\}) \) as the triple function ROS with metabolic sensing, chemical connecting, and stress branch-out.

### 2.9. Hypochlorous acid (HOCI)

MPO catalyzes the formation of HOCI from \( \text{H}_2\text{O}_2 \) and chloride ion \([105]\). MPO is found to localize to the nucleus, mitochondria and cytoplasm, which may explain the subcellular localization of HOCI seen in neutrophil and monocyes \([111]\). However, it remains unclear whether HOCI roles in our integrated sensing and alarming system for metabolic stress.

### 3. Mitochondrial ROS systems serve as the central hub for connecting ROS systems with inflammasomes, trained immunity and immunometabolic pathways

Mitochondria consume the greatest amount of oxygen (80–95%) to allow oxidative phosphorylation (OXPHOS) for energy generation \([112]\). OXPHOS produces 95% of cell energy by coupling tricarboxylic acid (TCA) cycle and mitochondrial electron transport chain (ETC) in most cell types \([113]\), except for endothelial cells (both tip cells and non-tip cells) \([114]\), which mostly take use of glycolysis for energy generation \([115]\). During OXPHOS, mitochondrial ROS is generated at mitochondrial ETC \([38]\). In addition, studies found that mitochondrial ROS production \([38]\) accounts for 2% of total oxygen consumed in mitochondria in physiological condition, and fluctuates from 0.25% to 11% depending on the animal species and respiration rates in pathophysiological conditions \([31]\). Studies found that mitochondrial ROS (mtROS) are connected with ROS systems in other organelles, and initiated inflammatory \([78,116]\) or cell death signals \([117]\). Therefore, as the most oxygen consuming and ROS generation organelle, mitochondria serves as the central hub for connecting ROS systems in other organelles (upstream) with the downstream D Amp sensing pathways such as inflammasomes \([27]\), trained immunity \([42,48,118]\) and immunometabolic pathways \([119]\). At the central hub, the metabolic homeostasis, and alarming stress in each organelle are sensed and bridged to DAMP sensing pathways in the downstream into the cytosol (for example, inflammasomes).

mtROS are generated at mitochondrial ETC and by other mitochondrial oxidant enzyme systems. Mitochondrial ETC includes five complexes from complex I to complex V \([120]\). The complexes except complex II are assembled crossing both inner and outer mitochondrial membranes, while complex II expresses on mitochondrial inner
membrane. TCA cycle products such as nicotine adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2) donating electrons at membrane. TCA cycle products such as nicotine adenine dinucleotide synthase; ETC, Electron transport chain; H

\[ \text{Y. Sun et al.} \]

[101]. Additionally, complex II mediated O

\[ \text{to lipid, protein and DNA in mitochondria and cells (stress branch-out SOD1 in intermembrane space (IMS) (Table 1 B). Therefore, the most} \]

\[ \text{drogenase, Q oxidoreductase, pyruvate dehydrogenase, and 2-oxogluta} \]

\[ \text{proton, which is unlike complex I and III as mentioned above.} \]

\[ \text{mtROS generation of complex II draws less attention [31]. However,} \]

\[ \text{levels of complex II substrate} \]

\[ \text{eration as we reported [37, 39, 40, 60, 121]. Interestingly, due to the low} \]

\[ \text{complex I to complex IV is accelerated and uncoupled from ATP gen} \]

\[ \text{generation and promoted by proton leaks, which means the reactions between} \]

\[ \text{complex I and complex II, respectively, and during which process, O}^{2-} \]

\[ \text{is generated and released into mitochondrial matrix from complex I} \]

\[ \text{and complex II. The electrons then pass through complex III and ultimately} \]

\[ \text{reduce O}^{2-} \text{to water at complex IV (cytochrome C); and O}^{2-} \text{is} \]

\[ \text{also generated and released into both mitochondrial intermembrane space} \]

\[ \text{and mitochondrial matrix (MM) through this process at complex} \]

\[ \text{III. Meanwhile, positively charged protons (H}^{+} \text{) from complex I, III} \]

\[ \text{and IV, but not complex II, are actively being pumped from the mitochon} \]

\[ \text{drial matrix into the intermembrane space, and thus creating a mito} \]

\[ \text{ion ROS could transduce the signals to mitochondria and other organelles, imbalanced ROS participates and} \]

\[ \text{MtROS is connected to ROS systems of other organelles; and the} \]

\[ \text{signals are transduced between organelle. Studies found that extra-} \]

\[ \text{mitochondria ROS could transduce the signals to mitochondria and} \]

\[ \text{induce opening of inner membrane anion channel (IMAC) and depo} \]

\[ \text{lizarize Δψm and O}^{2-} \text{is produced in ETC} \]

\[ \text{the mtROS production via affecting ETC enzyme activities [132, 133] or mito} \]

\[ \text{chondrial permeability transition pore; NO, Nitric oxide; NOX NADPH oxidase; O}^{2-} \text{, Superoxide radical anion; O}^{4} \text{Oxidized low-density lipoprotein; PKC-ε, Protein kinase C-epsilon; ROS, Reactive oxygen species; XDH, Xanthine dehydrogenase.} \]

\[ \text{Abbreviation: Δψm, Mitochondrial membrane potential; BH4, Tetrahydrobiopterin; c-Src, Proto-oncogene tyrosine-protein kinase Src; eNOS, Endothelial nitric oxide synthase; ETC, Electron transport chain; H}^{2} \text{O}_{2}, Hydrogen peroxide; HOCl, Hypochlorous acid; IMAC, Inner membrane anion channel; Mt, Mitochondrion; Mt-K}^{+}, Mitochondrial adenosine triphosphate (ATP)-sensitive potassium channel; MPTP, Mitochondrial permeability transition pore; NO, Nitric oxide; NOX NADPH oxidase; O}^{2-} \text{, Superoxide radical anion; O}^{4} \text{Oxidized low-density lipoprotein; PKC-ε, Protein kinase C-epsilon; ROS, Reactive oxygen species; XDH, Xanthine dehydrogenase.} \]

**Table 2**

| Category | Triggering ROS | Pivotal proteins | Findings | PMID | Ref # |
|----------|----------------|-----------------|----------|------|-------|
| Extra-mt ROS induced Mt ROS | O^{2-} | IMAC | Extra-Mt O^{2-} induced opening of IMAC which depolarized Δψm; O^{2-} was produced in ETC complex III. | 12950841 | [128,129] |
|          | H_{2}O_{2}/NO | Mt-KATP | Extra-Mt H_{2}O_{2} activated Mt-KATP via PKCε leading to the increase of K^- influx and production of O^{2-} | 18568884 | [130,131] |
|          | HOCI | ETC enzymes | HOCI-oxLDL leads to Mt ROS generation via decreasing activities of ETC dehydrogenase and reductases | 19843872 | [123,133] |
|          | O^{2-}/H_{2}O | MPTP | Mt ROS was triggered by external ROS (possibly O^{2-}) and linked to MPTP opening and calcium sparks. | 18283575 | |
Fig. 2. A. Left panel (a): ROS induces ten types of cell functions (yellow) via various specific downstream pathways (grey). The ten cell functions include: 1) physiological signaling, 2) proliferation, 3) gene regulation, 4) cell cycle regulation, 5) epigenetic modification, 6) post-translational modification, 7) inflammation/innate immunity, 8) pyroptosis, 9) apoptosis, and 10) senescence. Right panel (b): The circus plot indicates that four major ROS generating and anti-ROS regulatory systems have both shared genes and unique genes. On the outside, each arc represents the identity of each gene list. On the inside, each arc represents a gene list, where each gene has a spot on the arc. Dark orange color represents the genes that appear in multiple lists and light orange color represents genes that are unique to that gene list. Purple lines link the different genes where they fall into the same ontology theme. (also see the detailed information in a supplemental table). Detailed information of Fig. 2A-b see supplemental Table 4.

Abbreviations: IRS1—insulin receptor substrate 1, DRP—dynamin-related protein, OPA1—optic atrophy 1, PTM—post-translational modification, H3K14Ac—histone 3 lysine 14 acetylation, PI3K—phosphatidylinositol-3-kinase, Akt—protein kinase B, mTOR—mammalian target of rapamycin, Ras—GTPase, MEK—MAPK kinase, ERK—extracellular signals-regulated kinase, NF-kB—nuclear factor kappa-lightchain-enhancer of activated B cells, MAPK—mitogen-activated protein kinases, JNK—MAPK-Jun N-terminal kinase, Nrf—Nuclear factor erythroid2-related factor, CDK5—cyclin dependent kinase 5, CKD2—cyclin dependent kinase 2, NLRP3—NOD-, LRR- and pyrin domain containing protein 3, GSDMD—gasdermin D, SOD—superoxide dismutase, LOX—lipoxygenase, NOX—NADPH oxidase.

B. The ladder indicates different levels of ROS contribute to different cellular functions. In redox homeostasis state, ROS contributes to physiological signaling. Low ROS increase contributes to proliferation and angiogenesis. Moderated ROS increase contributes to inflammatory response and innate immunity. High ROS increase contributes to various types of cell death. C. Seven types of stimulations promote ROS production, including cytokines, growth factors, hormone/neurotransmitters, hypoxia, high glucose, DAMPs and extracellular ROS.

Abbreviations: TNF—tumor necrosis factor-α, IFN—interferon gamma, IL-1—interleukin 1, IL-2—interleukin 2, IL-4—interleukin 4, IL-13—interleukin 13, IL-27—...
Furthermore, studies found that anti-inflammatory cytokine interleukin 35 (IL-35) inhibits endothelial cell activation via inhibiting the increase of mtROS [146]. In addition, we analyzed the target enzymes of various treatments in Fig. 4 and genes of their downstream pathways by circus plot (Fig. 2A and b; also see the Supplemental Table 4 for the details). Various stimuli have different ROS generation enzymes and downstream signaling, however, their signaling pathways crosstalk with each other.

Nucleotide-binding oligomerization domain (NOD)-like receptor-containing pyrin domain 3 (NLRP3) inflammasome is a multiprotein complex, which is composed of NLRP3, apoptosis-associated speck-like protein containing a caspase-recruitment domain (CARD) (ASC, PYCARD) and pro-caspase 1 [27]. Increased ROS levels contribute to NLRP3 inflammasome activation. Among various subtypes of ROS, ONOO⁻, O₂⁻, and H₂O₂ are reported to be involved in inflammasome activation [147]. Upon activation by ROS, NLRP3 inflammasome promotes secretion of pro-inflammatory cytokines such as IL-1β and IL-18 and an inflammatory form of cell death [4]. Inhibition of NOX activity, p22phox subunits and ONOO⁻ production significantly prevented caspase-1 activation and IL-1β production [147]. The structure of disulfide bond that connects the PYD domain and the nucleotide-binding site domain in NLRP3 is highly sensitive to ROS [147]. The inactive form of NLRP3 resides on ER and translocates on mitochondrial outer membrane upon activation [148–151]. NLRP3 located in mitochondria interacts with mitochondrial ASC to assemble inflammasome or with mitochondrial antiviral signaling protein (MAVS) [152]. MAVS is a mitochondrial adaptor protein which mediates the translocation and association of NLRP3 to mitochondria. MAVS facilitates NLRP3 binding with ASC and pro-caspase 1 [147,153]. In addition, ER and mitochondria associated membrane are found to be the potential site for NLRP3 assembly [154]. Reports of subcellular localization of ASC varies significantly [53], including mitochondria [155], cytosol [150] and nucleus [156]. Upon inflammasome activation, ASC is especially observed in cytosol, which is correlated with the subcellular localization of assembled NLRP3 inflammasome. Furthermore, ROS activate NLRP3 inflammasome via direct and indirect ways. Studies found that increased ROS level induced mitochondrial DNA damage is also an indirect pathway for NLRP3 inflammasome activation by ROS [152,154]. In high level of ROS, thioredoxin-interacting protein (TXNIP) is dissociated with TRX and activates NLRP3 inflammasome [157]. Therefore, mitochondrial ROS systems serve as the central hub for connecting ROS systems in other organelles (upstream) with the downstream DAMP sensing pathways such as inflammasomes, trained immunity, immunometabolic pathways, cell proliferation, migration and cell death.

4. Nuclear ROS systems serve as a convergent hub and decision-makers to connect unbearable and alarming metabolic stresses from intracellular organelles to inflammation and cell death

Our previous reports showed that proatherogenic lipids (lysophosphatidylcholines, LPC)-induced mitochondrial ETC-generated ROS upregulate endothelial adhesion molecule intercellular adhesion molecule-1 (ICAM-1) expression and human aortic endothelial cell (HAEC) activation [42,60] via increasing histone 3 lysine 14 acetylation (H3K14ac) and increasing transcription factor AP-1 binding to ICAM-1 promoter in the nucleus [41]. In addition, we also found that LPC activates HAEC by increasing the enzyme expression of trained immunity (innate immune memory) [118]-related three metabolic pathways such as glycolysis, acetyl-CoA generation and mevalonate pathways via H3K14ac-mediated epigenetic innate immune memory mechanisms [48]. Our results have demonstrated that mitoROS and metabolic pathways are connected to an epigenetic histone modification and gene expression in the nucleus via so-called mito-nuclear communication [158], which is functional as a form of newly characterized innate immune memory (also termed trained immunity) [118,159]. Moreover, we
found that intracellular organelle-generated stress can be converged into the nucleus and regulate DNA damage and DNA repair pathways [44]; and presumably cytosol-located inflammasome-caspase-1 activation [5, 55, 56, 160] can regulate gene expression in caspase-1 processed IL-1β-, IL-18-, and sirtuin-1-, independent manners [52]. Taken together, our reports have demonstrated that in addition to mitochondria, cytosol stress and organelle stress are also converged into the nucleus in inflammasomes-dependent or independent manners [53].

The nucleus is responsible for epigenetic and genomic regulation of gene expression, cell differentiation, proliferation, senescence, and cell death [161]. Studies found that the nuclear membrane also contains cytochrome oxidases and electron transport systems, and may contribute to nuclear ROS production [162]. The basal levels of ROS in the nucleus are higher than those in the cytosol at resting state [163].

| Table 3 | Extracellular ROS induce intracellular ROS generation and promote different signaling and functions. |
|----------------------------------|--------------------------------------------------|
| **ROS** | **Target** | **Concentration** | **Function** | **PMID** | **Ref #** |
| ONOO- | Increase | 100μM | Angiogenesis | 18309287 | [192] |
| H2O2 | Increase | 1-4μM | Apoptosis | 9020024 | [218] |
| | Increase | 10 μM | Proliferation, Migration | 25315297 | [191] |
| | Increase | 10-20μM | Proliferation | 15109912 | [193] |
| | Increase | Cyto/Nuc ROS | 50μM | Nuclear Calcium signaling | 20095594 | [71] |
| | Increase | 100μM | | | 26461342 | [142] |
| | Increase | 200μM | Apoptosis | 29061494 | [187] |
| | Increase | 300μM | Tumor progression | 18801366 | [198] |
| | Increase | 1 mM | Caspase-independent apoptosis | 20084055 | [69] |

| Table 4A | Eight cytokines, four growth factors, three out of four hormone/neurotransmitters promote ROS production, and anti-inflammatory cytokines IL-10 and IL-35 inhibit ROS production. IGF-1, NE and Dopamin also decrease ROS production in certain condition. |
|----------------------------------|--------------------------------------------------|
| **ROS** | **Sensor** | **Target** | **Concentration** | **Function** | **PMID** | **Ref #** |
| TNF-α | Increase | TNF | 5-lipoxygenase | 10 ng/ml | Inflammation | 10934206 | [72] |
| | Increase | TNFRI | Complex II, ΔΨm | 20 ng/ml | Inflammation, Apoptosis | 17765224, 202 | [78], [117] |
| IFN-γ | Increase | IFN-γR | Complex II/NOX | 20 U/ml | Inflammation | 17765224 | [78] |
| TGF-β | Increase | TGFBR | NOX, Complex I | 10 ng/ml | Fibroblast secretion/EMT | 15677311 | [245] |
| IL-1 | Increase | IL1R | NOX4 | 50 ng/ml | Angiogenesis | 25315297 | [191] |
| IL-2 | Increase | IL2R | NOX4 | 100 U/ml | Angiogenesis | 18309287 | [192] |
| IL-4 | Increase | IL4R | NOX4 | 10 ng/ml | Inflammation, Innate immunity | 16249002, 1611680 | [194], [206] |
| IL-13 | Increase | IL13R | NOX4 | 10 ng/ml | Inflammation | 1611680 | [194] |
| IL-27 | Enhance | IL27R | NOX4 | 10 ng/ml | Cell death/damage | 19752325 | [175] |
| IL-10 | Decrease | IL10R | NOX4 | 200 ng/ml | Inflammation | 29371247, 31731100 | [146] |
| IL-35 | Decrease | IL35R | NOX4 | 10 ng/ml | Inflammation | 29371247, 31731100 | [146] |
| IL-17 | Increase | IL17R | NOX4 | 10 ng/ml | Cell death/damage | 19752325 | [175] |
| IL-27 | Increase | IL27R | NOX4 | 100 ng/ml | Inflammation | 29371247, 31731100 | [146] |
| IL-10 | Decrease | IL10R | NOX4 | 10 ng/ml | Inflammation | 1611680 | [194] |
| IL-35 | Decrease | IL35R | NOX4 | 10 ng/ml | Inflammation | 1611680 | [194] |
| IL-17 | Increase | IL17R | NOX4 | 10 ng/ml | Inflammation | 1611680 | [194] |
| IL-27 | Increase | IL27R | NOX4 | 10 ng/ml | Inflammation | 1611680 | [194] |
| EGF | Increase | EGRF | NOX4 | 10 ng/ml | Adhesion, Migration, Proliferation | 19635476 | [209] |
| | Increase | EGRF | NOX4 | 40 ng/ml | Angiogenesis | 17045920 | [194] |
| | Increase | EGRF | NOX4 | 100 ng/ml | Necrosis | 10854274 | [73] |
| PDGF | Increase | PDGRF | NOX4 | 15 ng/ml | Proliferation | 18972255 | [175] |
| | Increase | PDGRF | NOX4 | 20 ng/ml | Inflammation | 23774581 | [246] |
| VEGF | Increase | VEGFR | mtROS | 50 ng/ml | Migration | 31653897 | [143] |
| IGF-1 | Decrease | IGF-1R | mtROS | 50 ng/ml | Migration | 18567639 | [210] |
| NE | Decrease | IGF-1R | NOX4 | 100 ng/ml | Protect DNA damage | 26167254 | [186] |
| Insulin | Enhanced | IR | NOX4 | 100 μM | Protect DNA damage | 26167254 | [188] |
| Dopamine | Increase | MAO | NOX4 | 0-50 μM | Enhance insulin sensitivity | 19808019 | [183] |
| | Increase | D5R | NOX4 | 1 ng/ml | Physiological signaling | 20547771 | [188] |
| | Increase | D5R | NOX4 | 10 ng/ml | Physiological signaling | 23994527 | [189] |
| | Increase | D5R | NOX4 | 50 ng/ml | Physiological signaling | 23994527 | [189] |
| Serotonin | Decrease | 5-HT1BR | NOX4 | 1 μM | Protect from hypertension | 23425954 | [186] |
| AngII | Increase | AT1R | NOX4 | 1 μM | Protect from hypertension | 23425954 | [186] |
| | Decrease | AT1R | NOX4 | 1μM | Post-translational oxidative modification of protein, proliferation | 28473438 | [247] |
| | Increase | AT1R | NOX4 | 100 μM | Protect from hypertension | 23425954 | [186] |
| | Increase | AT1R | NOX4 | 10-100 μM | Protect from hypertension | 23425954 | [186] |
| | Increase | AT1R | NOX4 | 40 μM | Protect from hypertension | 23425954 | [186] |
| | Increase | AT1R | NOX4 | 100 μM | Protect from hypertension | 23425954 | [186] |
but the potential role of nuclear ROS have rarely been studied or discussed. On the other hand, an important question remains unknown whether nuclear ROS are generated in the nucleus or due to the trafficking from increased cytosolic ROS. In Table 1A, ROS generation enzymes are found to be expressed in the nucleus, which presents strong evidence for ROS production in the nucleus. In addition, comparing increased ROS levels in various organelles including cytosol, mitochondria, endosome, Golgi and nucleus [164], increased nuclear ROS significantly promote cell death compared to that of other organelles; in contrast, increased ROS in the ER does not always affect cell viability [164]. Therefore, nuclear ROS serves as a convergent hub and decision-makers to connect unbearable and alarming metabolic stresses from intracellular organelles to inflammation or cell death [165]. In addition, nucleus ROS connected with ROS systems from extracellular space or extra-nucleus also regulate inflammatory or cell death responses [76]. Theoretically, there are at least two means for microenvironmental ROS to affect cellular homeostasis via an impact on the cell nucleus [165]. For example, extracellular H2O2 (50 μmol/L) stimulation induces sustained increase of nuclear ROS and regulates nuclear calcium signaling (Table 3); and the antioxidant glutathione (GSH) [166] could reverse the increase of nuclear ROS [163]. In addition, the nuclear envelope membranes contain several G protein-coupled receptors, including prostaglandin E2 (EP3R) and endothelin-1 (ET-1) receptors. Activation of EP3R increases endothelial nitric oxide synthase (eNOS) RNA expression in nuclei. eNOS and inducible ROS (iNOS) are reported to also be present at the nuclear levels. Moreover, stimulation with NO donor sodium nitroprusside [167] results in an increase of intranuclear calcium that is dependent on guanylate cyclase activation, but independent of mitogen-activated protein kinases (MAPK) [168]. H2O2 and ET-1 increase both cytosolic and nuclear ROS in human endocardial endothelial cells and in human aortic vascular smooth muscle cells [163]. Furthermore, pro-hypertension hormone angiotensin II (Ang II) (1 nM) stimulation activates Ang II type 1 receptor (AT1R), which is highly expressed in nuclei, and increases ROS production via NADPH oxidase 4 (NOX4) activation, which has 65% expression in nuclei [169]. Based on these studies, nuclear ROS senses the status of cell conditions. The imbalanced ROS levels contribute to cell inflammation and cell death. Studies on nuclear ROS remain less than that of other organelles, and further studies are needed to clearly uncover the mechanisms underlying death signaling and nuclear ROS generation and scavenging. One of the reasons for this may be due to the fact there is not a simple, convenient and specific method for nuclear ROS detection except for ROS staining with fluorescent ROS probe carboxy H2DCFDA (DCF) and co-stained with live nucleic acid stain Syto-11 [163].

In our new model proposed here, we emphasize the mitochondria/organelle-to-nucleus retrograde communications [41]. Actually, recent progresses include nucleus-to-mitochondria (anterograde) and mitochondria-to-nucleus (retrograde) communication, mito-nuclear feedback signaling and proteostasis (pathways that control the biogenesis, folding, trafficking and degradation of proteins) regulation, the integrated stress response and non-cell-autonomous communication [170].

5. Balanced ROS levels indicate a physiological homeostasis of various metabolic processes in subcellular organelles while imbalanced ROS levels present alarms for pathological organelle stresses in metabolic processes

ROS systems are found to play important roles in promoting the progression in metabolic diseases, such as cardiovascular disease (CVD), chronic kidney disease, inflammations, diabetes and tumors. However, the detailed mechanisms of how ROS play the critical roles remain poorly characterized. We propose that ROS system serves as the sensing network for cellular homeostasis and alarming stress in metabolic processes. Therefore, ROS levels are increased in metabolic disorders and participate in disease progression. Balanced ROS contribute to physiological signals and functions after sensing metabolic homeostasis, while imbalanced ROS participate in metabolic processes regulating proliferation, inflammation or cell death in pathological conditions. Studies found that lightly increased ROS (1.1 fold changes) contribute to cell proliferation, angiogenesis and metastasis, moderate increased ROS (1.1–20 fold change) reach the maximum inflammatory responses, and highly increased ROS (more than 20 fold change) promote apoptosis, necroptosis, autophagy, pyroptosis and ferroptosis [171,172] (Fig. 2B). However, cell types and stimuli cause variations for fold differences in ROS change effects. Thus, the criteria of increased ROS levels may be used in the same stimulation conditions and cell types, and it is not suitable for comparing across different experiments. Importantly, lightly increased ROS can be recognized as a complimentary signal to rescue the imbalanced ROS system and alarming stress and promote cell survival. Therefore, ROS system is a network connecting various DAMPs/conditional DAMPs, including cytokines, growth factors, hormones/neurotransmitters, hypoxia, and high glucose, and other disease risk factors, to the initiation of metabolic processes (Fig. 2C). In Table 4A, even though the fold changes do not match the criteria mentioned above, the response severity and scales are dose-dependently increased according to the stimulation strength, such as interleukin 1 (IL-1) [78,175], IL-13 [174,175] and Ang II [169,176–181]. Here, we attempted to collect the evidences to determine whether ROS systems are functionally qualified in sensing metabolic stress in organelles. One requirement for this function is the ROS system must have the capacity to sense the strengths of stress with different functional consequences to activate inflammagens and cross-talk to different stress pathways in inflammagens-dependent, or independent manners.

Balanced ROS levels are critical for cell signaling and homeostasis in physiological condition. In physiological homeostasis conditions, ROS initiate diverse cellular responses including triggering signaling pathways and regulating cell protection besides to activate inflammagens pathways. In addition, ROS participates in coordinating activation of mitochondrial fusion and mitophagy to optimize clearance of abnormal mitochondria and cell [31]. These process prevent the damage signals to spread to neighboring mitochondria or cells [31]. In addition, ROS regulate various metabolic processes in cell cycle and survival via calcium signaling in physiological conditions [67,68,177,182]. Moreover, lightly increased ROS complementarily rescue the imbalanced ROS system and alarming stress via regulating metabolic processes. For example, slightly increased ROS enhance insulin sensitivity via phosphatidylinositol-3-kinase (PI3K)-protein kinase B (Akt)-mammalian target of rapamycin (mTOR) pathway [183] and protein synthesis via Ras (a small GTPase)-MAPK kinase (MEK)-extracellular signals-regulated kinase (ERK) pathway [184], protect from DNA damage [185] and increase cell survival [69,186,187]. Additionally, ROS are slightly increased to induce cell proliferation [178,188–191] and angiogenesis [173,191,192] via regulating p38 MAPK-Jun N-terminal kinase (JNK) pathway [193], hypoxia induced factors alpha (HIF-α) stabilization [194] and signal transducer and activator of transcription 3 (Stat3) activation [195]. Studies found that NLRP3 inflammasome could be activated via PI3K-Akt pathway [196] and p38 MAPK pathway [197]. ROS serve as a bridge to connect metabolic stress and inflammagens activation and regulate metabolic pathways in a feedback mechanism. On the other hand, ROS contribute chronically to tumorigenesis or metastasis [189,190]. In addition to ROS regulation of certain signaling pathways above discussed, it remains poorly defined whether there are in physiological conditions regulate a functional role of vascular cell type-endothelial cells, which we recently classified as innate immune cells [36,200]. To address the issue, we examined human aortic endothelial cells (HAECs). Both mtROS and ATP are produced as a result of electron transport chain activity [38], but it remained enigmatic whether mitochondrial ROS (mtROS) could be generated independently from ATP synthesis. Our report shed light on this important question and found that, during endothelial cell (EC) activation, mtROS could be upregulated in a proton leak-coupled, but ATP
ling cell migration, and pathological inflammation is significant towards among mitochondrial metabolism, physiological EC activation, patrol thesis and endothelial damage or death. Mapping the connections mtROS production is uncovered for low grade EC activation, patrolling and consequently without causing mitochondrial damage and EC death. compromising mitochondrial membrane potential and ATP generation, dose mtROS production for physiological EC activation without

Table 4B

| Hypoxia | Increase | Sensor | Target | Concentration | Function | PMID Ref # |
|---------|----------|--------|--------|---------------|----------|-------------|
|         | NOX4     | 1% O2  |        | Angiogenesis, Migration | 26297045, [195], 29123322, [205] |
|         | SOD      | 5% O2  |        | Proliferation | 16624953, [189] |
| Hyperglycemia | Increase | GLUT    | H2O2  | 20 mM | Insulin secretion | 17409590, [70] |
| LPA | Increase | Edg | NOX | 33 mM | Angiogenesis | 23274656, [246] |
| LPAR5 | Increase | mtROS | 40μM | Trained immunity | 31153099, [48] |
| LPC | Increase | Galectin-3, CD63, SR-AI and RAGE | NOX | 400μg/ml | Liver fibrosis | 20133001, [249] |
| AOPPs | Increase | NOX4 | 50μM | Cytokine secretion | 31539804, [211] |
| HSA | Increase | P2X7 | Mt | 130nM | Cell death | 25713411, [74] |
| Zymosan | Increase | DOR1 | NOX | 200μM | Bacterial defense | 29237780, [253] |
| SIVA | Increase | NOX1 | 0.5μg/ml | Apoptosis | 32000869, [225] |
| Integrin | Increase | PRR (I2 integrin, Mac-1) | NOX2 | 20μg/ml | Apoptosis | 29569550, [255] |
| ET-1 | Increase | RTK | LOX, NOX | Cell adhesion | 12796479, [204] |
| Methionine | Increase | ET1R | eNOS | 1 nM | Nuclear Calcium homeostasis | 20393594, [71] |
| Homocysteine | Increase | ETAR | NOX | 10 nM | Protein synthesis | 15203192, [184] |
| PAMPs | Increase | MT | 500μM | Caspase-1 inflammation activation | 20706445, [259] |
| RSV | Increase | TLR2 | Mt | 500μM | Cytokine secretion | 22295065, [141] |
| DENV | Increase | TLR9 | Mt | 500μM | Innate immunity | 29880709, [116] |
| Shiga toxin | Increase | CD77 | Caspase-4/- | 200ng/ml | Pyroptosis | 30440047, [221] |
| Biophysical forces | Increase | TLR4 | NOX | 10ng/ml | Inflammation | 26508369, [213] |
| Hemodynamic strain | Increase | N/A | N/A | Average strain (12%) | Adhesion molecule expression, [214], 86986, [215] |
| Pulmonary stretch | Increase | Integrins | NOX, XO, eNOS | Uniaxial cyclic stretch (20%) | Membrane spreading | 23738008, [216] |

Abbreviations: EGF-Epidermal growth factor; PDGF-platelet-derived growth factor; VEGF-vascular epithelial growth factor; IGF-I-insulin-like growth factor; LPA-lysophosphatidic acid; LPC-lysophosphatidylcholine; AOPPs-advanced oxidation protein products; HAS-human serum albumin; eATP-extracellular ATP; RSV-respiratory syncytial virus; DENV-dengue RNA virus; dsDNA-double-stranded DNA; endothelin-1—ET1; RIRR-ROS-induced ROS release; TBE cell-Tracheobronchial epithelial cells; EC-Endothelial cell; EPC-Endothelial progenitor cell; HCC-hepatocellular carcinoma cell; PPP-pattern recognition receptors; EMT-epithelial mesenchymal transition; ΔYm, Mitochondrial membrane potential; NE-Norepinephrine; LPC-lysophosphatidylcholine.

synthesis-uncoupled manner [60]. As a result, EC could upregulate low dose mtROS production for physiological EC activation without compromising mitochondrial membrane potential and ATP generation, and consequently without causing mitochondrial damage and EC death. Thus, a novel pathophysiological role of proton leak [37,40] in driving mtROS production is uncovered for low grade EC activation, patrolling immunosurveillance cell (for example, non-classical monocytes [201]) trans-endothelial migration and other signaling events without compromising cellular survival. This new working model explains how mtROS could be increasingly generated independently from ATP synthesis and endothelial damage or death. Mapping the connections among mitochondrial metabolism, physiological EC activation, patrolling cell migration, and pathological inflammation is significant towards the development of novel therapies for inflammatory diseases and cancers [39].

Moderate increased ROS promote cytokine secretion [202], inflammatory cell adhesion [203,204] and migration [205]. Studies found that six proinflammatory cytokines cause increased cytosolic and mitochondrial ROS generation, including TNF-α [72,78,117], interferon gamma (IFN-γ) [78,174], interleukin 1 (IL-1) [78,173], IL-4 [174,206], IL-13 [174,175] and IL-27 [79] (Table 4A). The increased ROS generation promote inflammatory responses and innate immunity via regulating cytokine release and endothelial function [212, 213, 214]. Inhibition of cytokine production, IL-4 [146] and IL-35 [146,208], has revealed that anti-inflammatory cytokines, IL-10 [146] and IL-22 [150], are found to inhibit endothelial cell activation via decreasing mtROS production in the
presence of pro-inflammatory stimulation. Growth factors, epidermal growth factor (EGF) [209] and vascular endothelial growth factor (VEGF) [143, 210], induced cell migration via increasing ROS production and matrix metalloproteinases (MMPs) expression [143] (Table 4A). DAMPs stimulation, such as advanced oxidation protein products (AOPPs) [211], AGEs-advanced glycation end products (AGEs) [212], respiratory syncytial virus (RSV) [141], lipopolysaccharide (LPS) [213] and integrin [43], induce ROS generation via NOX activation, therefore, promote cytokine secretion and innate immunity (Table 4B). Furthermore, cells are exposed to several types of biophysical forces (hemodynamic strain and pulmonary stretch) in physiological and pathological condition [214–217]. Studies found that these biophysical forces also induce higher level of cytosolic and mitochondrial ROS which promote the inflammatory signaling and disease progression (Table 4B).

Highly increased ROS significantly contributes to different types of cell death, including apoptosis, pyroptosis (inflammatory cell death), necrosis and senescence [76]. TNF-α induced ROS generation by affecting mitochondrial membrane potential contributes to apoptosis [117]. IL-13 leads to cell death via NOX activation and ROS generation in microglia [175]. A high concentration of extracellular ROS [187], such as ONOO⁻ and H₂O₂, promotes apoptosis via DNA fragmentation [218], potentially NLRP3 inflammasome-DNA damage pathway [219], and caspase-independent pathway [69]. Epidermal growth factor (EGF) induced H₂O₂ generation promotes cell necrosis and senescence via regulating Ras-MEK-ERK pathway [73, 220]. In addition, Shiga toxin 2 increases ROS generation via caspase-4 activation/presumably noncanonical inflammasome pathway [59] and then promotes cell pyroptosis [221]. Moreover, mitochondrial membrane potential (MMP) collapse and ROS generation induce NLRP3 inflammasome activation. The elimination of ROS alleviates the cleavage of Gasdermin D (GSDMD) carried out by activated caspase-1/caspases-4/caspase-11 and non-canonical cytokine secretory pathways mediated by N-terminal Gasdermin D-protein pores on the plasma membrane [59]. Hydrogen peroxide treatment augments the cleavage of GSDMD by caspase-1. Four amino acid residues of GSDMD are oxidized under oxidative stress in macrophages, suggesting that GSDMD oxidation serves as a de novo mechanism, by which mitochondrial ROS promote NLRP3 inflammasome-dependent pyroptosis [222]. Other DAMPs, including AOPPs, serotonin and AGEs [223], accelerate cell death [75] via increased ER stress [74], p38 MAPK-JNK pathway [224, 225] and Gasdermin family [59] (Table 4B).

![Fig. 3. A novel working model: 1) ROS are a novel integrated network for sensing homeostasis and alarming stress in organelle- or cytosolic-metabolic stresses; and 2) ROS also serve as cellular communication signaling to increase neighboring cell ROS production. Abbreviations: O₂⁻—superoxide, NO—nitric oxide radical, NO₂—nitrogen dioxide, O₂H—hydroxyl radical, ROO—peroxyl radical, RO—alkoxyl radical, COS—carbonate radical, H₂O₂—hydrogen peroxide, ONOO—peroxynitrite, 1O₂—singlet oxygen, HOCl—hypochlorous acid, TCA: tricarboxylic acid cycle.](image-url)
processes and are participating in cellular signaling; second, the electron transfer process existed in most metabolic reactions is the most convenient cue, which could be sensed and monitored in the 2801 complex metabolic pathways. Indeed, we classify a few types of ROS that are functional in sensing metabolic stress; third, ROS systems make use of the most general chemical resources in cytosol and organelles—oxygen, which is also generally used in metabolic processes; Fourth, four types of ROS are our newly classified organelle communication ROS that are able to cross lipid membrane which is responsible for the communication of cellular metabolic status between organelles and cells, including NO•, NO², H₂O₂ and ONOO⁻ (Fig. 3). In addition, increased levels of ROS can activate inflammasome pathways in cytosol and are harmful to DNA, protein and lipid, that are newly classified stress branch-out (cross-talking) ROS. Moreover, some ROS can be converted into another ROS, which we classify as chemical converting ROS as mentioned in Section 2 in details. Fifth, after sensing the imbalance of metabolism in metabolic disease in the form of electron transfer imbalance, ROS activate inflammation initiation pathways such as capase-1 inflammasomes in the cytosol to initiate inflammation, which we classify as inflammasome-activating ROS. Furthermore, mitochondria consume 80–95% oxygen and generates mtROS, thus mitochondria are the ROS control hub for connecting cell status and metabolic processes. Increased nuclear ROS significantly increase cell death compared to other organelles. Therefore, the nucleus is the ROS signal converting hub in retrograde, which determines the functions of survival, tumorigenesis and various types of cell death. In homeostasis status, balanced ROS initiate physiological signaling, while imbalanced ROS in organelles as an integrated network promote pathological signals after sensing alarming stress [226]. The increased ROS cross organelle lipid membrane to share signals between organelles or plasma membrane to spread danger signals to neighboring cells via exosomes [227,228]. Our model provides novel insights on the roles of ROS system in bridging metabolic stress to inflammation, cell death and tumorigenesis; and provide novel therapeutic targets for treating those diseases.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2020.101696.

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Supplemental table 1

The 41 enzymes which are regulating superoxide and hydrogen peroxide are actively functioned and regulated in metabolic processes.

| Name                                                                 | Abbreviation | Function                                                                                   |
|----------------------------------------------------------------------|--------------|-------------------------------------------------------------------------------------------|
| Aldehyde oxidase                                                     | AOX1         | Valine, leucine and isoleucine degradation, Tyrosine metabolism, Tryptophan metabolism, Vitamin B6 metabolism and Nicotinate and nicotinamide metabolism |
| Amine oxidase (flavin-containing) A                                   | AOF-A        | Deamination of 5-hydroxytryptamine, norepinephrine and epinephrine.                       |
| Amine oxidase (flavin-containing) B                                   | AOF-B        | Deamination of benzylamine and phenyllethylamine                                           |
| α-Amino acid oxidase                                                 | OXDA         | Regulate the level of neuromodulator α-serine and dopamine synthesis                      |
| γ-Amino acid oxidase                                                 | OXLA         | Lysosomal antigen processing and presentation                                              |
| δ-Aspartate oxidase                                                  | OXDB         | Deamination of δ-aspartate and its N-methylated derivative, N-methyl δ-aspartate           |
| Aminolide-sensitive amino oxidase (copper-containing)                | AOC1         | Degradation of putrescine, histamine, spermine and spermidine                            |
| Cytochrome P450 3A4                                                  | CP3A4        | Metabolism of sterols, steroid hormones, retinoids and fatty acids                         |
| Cytochrome P450 2D6                                                  | CP2D6        | Metabolism of fatty acids, steroid and retinoids                                           |
| Cytochrome P450 2E1                                                  | CP2E1        | Metabolism of saturated fatty acids and xenobiotics                                        |
| Cytochrome P450 4A11                                                 | CP4A1        | Metabolism of fatty acids and their oxygenated derivatives (oxylipins)                     |
| ERO1-like protein-α                                                  | ERO1A        | Involved in disulfide bond formation in ER and reoxidizes P4HB/PDI                          |
| ERO1-like protein-β                                                  | ERO1B        | Involved in disulfide bond formation in ER and reoxidizes P4HB/PDI                          |
| FAD-linked sulfhydryl oxidase ALR                                    | ALR          | Regenerate the redox-active disulfide bonds in CHCHD4/MA40                               |
| Hydroxycytochrome oxidase 1                                          | HAOX1        | Active on 2-carbon substrate glycolate and 2-hydroxy fatty acids                           |
| Hydroxycytochrome oxidase 2                                          | HAOX2        | Oxidation of L-alpha-hydroxy acids and L-alpha-amino acids                                |
| Membrane primary amine oxidase                                      | AOC3         | Semicarbazide-sensitive (SSAO) monoamine oxidase activity                                 |
| Peroxisomal N1-acetylsperrmine/spermidine oxidase                   | PAOX         | Oxidation of N1-acetylsperrmine to spermidine and putrescine, and polynamine back-conversion |
| Peroxysomal acyl-CoA oxidase 1                                        | ACOX1        | Desaturation of acyl-CoAs to 2-trans-enoxy-CoAs                                           |
| Peroxysomal acyl-CoA oxidase 3                                        | ACOX3        | Oxidizes the CoA-esters of 2-methyl-branched fatty acids                                   |
| Peroxysomal sarcosine oxidase                                        | SOX          | Metabolizes sarcosine, i-pipeolic acid and i-proline                                      |
| Prenylcysteine oxidase 1                                             | PCVOX        | Degradation of prenylated proteins and cleavage of thioether bond of prenyl-cysteines     |
| Prenylcysteine oxidase-like                                          | PCVXL        | Prenylcysteine oxidase activity                                                           |
| Protein-lysine 6-oxidase                                             | LYOX         | Post-translational oxidative deamination of peptidyl lysine residues and regulate Ras expression |
| Pyridoxine 5'-phosphate oxidase                                      | PNP0         | Oxidation of pyridoxine 5'-phosphate and pyridoxamine 5'-phosphate                        |
| Retina-specific copper amine oxidase                                 | AOC2         | Oxidation of 2-phenylethylamine and tryptamine                                           |
| Spermine oxidase                                                     | SMOX         | Oxidation of spermine and metabolism of N1-acetylsperrmine and spermidine                 |
| Sulphydryl oxidase                                                   | QSOX1        | Oxidation of sulphydryl groups in peptide and protein thiols                             |

(continued on next page)
### Supplemental table 1 (continued)

| Name                  | Abbreviation | Function                                                                 |
|-----------------------|--------------|---------------------------------------------------------------------------|
| Sulfite oxidase, mitochondrial | SUOX         | Metabolism of sulfur                                                      |
| Xanthine dehydrogenase/oxidase | XDH         | Purine degradation; oxidation of hypoxanthine and xanthine                |
| NADPH oxidase 1       | NOX1         | Regulate cellular pH and mediate proton currents                           |
| NADPH oxidase 2       | NOX2 (CY24B) | Regulate cellular pH and mediate proton currents                           |
| NADPH oxidase 3       | NOX3         | Biogenesis of otoconia/otolith                                             |
| NADPH oxidase 4       | NOX4         | Inhibit phosphatase and regulate KCN-K/TASK-1 patatassium channel        |
| NADPH oxidase 5       | NOX5         | Calcium-dependent proton channel and may regulate redox-dependent processes in lymphocytes and spermatozoa |
| Dual oxidase 1         | DUOX1        | Synthesis thyroid hormone, contribute to thyroid peroxidase/TPO and lactorperoxidase/LPO activity |
| Dual oxidase 2         | DUOX2        | Synthesis thyroid hormone, contribute to thyroid peroxidase/TPO and lactorperoxidase/LPO activity |
| Superoxide dismutase [Cu-Zn] | SOD1        | Form fibrillar aggregates in the absence of intramolecular disulfide bond |
| Superoxide dismutase [Mn], mitochondrial | SOD2        | Transcription of RNA polymerase li and release of cytochrome C           |
| Extracelular superoxide dismutase | SOD3        | Response to copper ion and hypoxia                                         |

[1] The 41 enzymes are generated from PMID: 32231263 [102]. The function of enzymes are collected from Human metabolome database (https://hmdb.ca/) and Uniprot database (https://www.uniprot.org/).

### Supplemental table 2

Mitochondrial ROS and Amplex Red are used for mtROS detection in vitro; and mitoB is used for mtROS detection in vivo.

| Category      | Method                        | Reagent          | Off-target response | Comment                           | PMID         | Ref #  |
|---------------|-------------------------------|------------------|---------------------|-----------------------------------|--------------|--------|
| ROS, RNS      | Fluorescent probe             | ESR              |                     | Specific, sensitive, expensive    | 24862270     |        |
| Extracellular O$_2^-$ | Fluorescent probe         | HPr+             | Membrane impermeable |                                    | 29790367     |        |
| General ROS   | Fluorescent probe             | DCFH-DA          |                     |                                   | 24862270     |        |
| General ROS   | Fluorescent probe             | H$_2$DCF         |                     |                                   | 23665586     |        |
| General ROS   | Fluorescent probe             | CM-H$_2$DCFDA    |                     |                                   | 24862270     |        |
| O$_2^-$      | Fluorescent probe             | HE/DHE           | O$_2$               | Cell permeable, intensity quantifiable, low specificity | 24862270     |        |
| O$_2^-$      | Fluorescent probe             | DPF              |                     |                                   | 16297980     |        |
| O$_2^-$      | Fluorescent probe             | 2-(2-Pyridil)-benzothiazoline |                  |                                   | 16297980     |        |
| O$_2^-$      | Chemiluminescent probe        | L-012            |                     |                                   | 24862270     |        |
| O$_2^-$      | Chemiluminescent probe        | Lucigenin        | ONOO-               | Cell permeable, low selectivity/sensitivity | 24862270     |        |
| O$_2^-$      | Spectrophotometry             | NBT              |                     |                                   | 24862270, 20387054 | 260, 266 |
| O$_2^-$      | Fluorescent probe             | cpYFP            |                     |                                   | 24862270, 27288722 | 260, 267 |
| O$_2^-$      | Fluorescent probe             | CHD              |                     |                                   | 16297980, 27909341 | 260, 263 |
| O$_2^-$      | Fluorescent probe             | 3-CCA            |                     |                                   | 16297980, 26516887 | 260, 268 |
| O$_2^-$      | Fluorescent probe             | APF              | HOCI                |                                   | 16297980, 26516887 | 260, 268 |
| O$_2^-$      | Fluorescent probe             | HPP              | HOCI                |                                   | 16297980, 16168507 | 260, 269 |
| O$_2^-$      | Fluorescent probe             | FL               |                     |                                   | 16297980, 27909341 | 260, 263 |
| ROO•         | Fluorescent probe             | C11-BODIPY$_{581:591}$ | H$_2$O$_2$, ROO•, ONOO• | Lipid peroxidation sensor           | 16297980, 21396693 | 260, 270 |
| ROO•         | Fluorescent probe             | DPPP             | Lipid peroxidation sensor |                                   | 16297980     |        |
| H$_2$O$_2$   | Histochemical staining        | DAB              | In Situ imaging of ROS |                                   | 24862270, 20387054 | 260, 266 |
| H$_2$O$_2$   | Fluorescent probe             | DCFH             | COO•, N02•, H0•     |                                   | 16297980, 27909341 | 260, 263 |
| H$_2$O$_2$   | Fluorescent probe             | HyPer            |                     |                                   | 24862270, 27302663 | 260, 265 |
| OONO•       | Fluorescent probe             | DHR              | HOCI                |                                   | 16297980     |        |
| 1O$_2$       | Fluorescent probe             | DMA              |                     |                                   | 16297980     |        |
| 1O$_2$       | Fluorescent probe             | DPAX             |                     |                                   | 16297980, 19794197 | 260, 271 |
| 1O$_2$       | Fluorescent probe             | DMAX             |                     |                                   | 16297980, 19794197 | 260, 271 |
| HOCI         | Fluorescent probe             | BODIPY$_{581}$  |                     |                                   | 26043093     |        |
| Mt O$_2^-$   | Fluorescent probe             | MitoHE           |                     |                                   | 23668959     |        |
| Mt O$_2^-$   | Fluorescent probe             | MitoSox Red      |                     |                                   | 24862270     |        |
| Mt H$_2$O$_2$ | Fluorescent probe             | MitoB            | Analyzed by mass spectrometry ex vivo |                                   | 23726990     |        |
| O$_2^+$, H$_2$O$_2$ | Fluorescent probe         | Amplex Red       | High sensitivity, low background fluorescence |                                   | 24862270, 16297980, 27909341 | 260, 262, 263 |

(continued on next page)
### Supplemental table 2 (continued)

| Category                  | Method          | Reagent             | Off-target response                      | Comment                                                                 | PMID     | Ref # |
|---------------------------|-----------------|---------------------|------------------------------------------|-------------------------------------------------------------------------|----------|-------|
| Redox status changes      | Fluorescent probe | roGFP               | Slow in reaction, non-sensitive, real-time, cell friendly but restriction in receptor cells |                                                                         | 24862270, 27306519 | [260]  |
| Redox status changes      | Fluorescent probe | rxYFPs              |                                                                         |                                                                         | 24862270, 27306519 | [260]  |
| Redox status changes      | Fluorescent probe | rxRFP               |                                                                         |                                                                         | 24862270, 27208426 | [260]  |
|                           |                 |                     |                                                                         |                                                                         |          |       |

ESR: electron spin resonance; DCFH-DA: 2,7-Dichlorodihydrofluorescein diacetate; H$_2$DCF: 2',7'-dichloro-dihydrofluorescein; DHE/HE: dihydroethidium; DPBB: 1,3-Diphenylisobenzofuran; L-012: luminol analogue 8-amino-5-chloro-2,3-dihydro-7-phenylpyrido; NBT: Nitroblue tetrazolium; CHD: 1,3-Cyclohexanedione; 3-CCA: Coumarin, coumarin-3-carboxylic acid; SECCA:N-succinimidyl ester of coumarin-3-carboxylic acid; HPF: 2-[6-(4'-Hydroxy)phe-noxy-3H-xanthen-3-on-9-y1]benzoic acid; APF: 2-[6-(4'-aminophenoxy-3H-xanthen-3-on-9-y1] benzoic acid; Fl: Fluorescein; DDA: 1,3-Cyclohexanedione; DPAXs: 9-[2-(3-Carboxy-9,10-diphenyl)anthryl]-6-hydroxy-3H-xanthen-3-ones; DMAX: [2-(3-Carboxy-9,10-dimethyl)anthryl]-6-hydroxy-3H-xanthen-3-one; CM-H2DCFDA: 5-(and 6)-chloromethyl-2',7'-dichlorohydrofluorescein diacetate; scopoletin: 7-hydroxy-6-methoxy-coumarin; HVA: Homovanillic acid (4-hydroxy-3-methoxy-phenylacetic acid; DHR: Dihydrorhodamine 123; Amplex Red:10-acetyl-3,7-dihydroxyphenoxazineDAB: Diaminobenzidine; C11-BODIPY581/591: 4,4-Difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid; DPPP: Diphenyl-1-pyrenylphosphate; mitoHE: mito-hydroethidine; HPr$^+$: hydropropidine.

### Supplemental table 3

ROS generation Inhibitors.

| Target                  | Drug          | Cell type         | Concentration | PMID     | Ref # |
|-------------------------|---------------|-------------------|---------------|----------|-------|
| General ROS             | NAC           | RPE cell          | 1 mM          | 17765224 | [78]  |
|                         | NAC           | Epithelial cell   | 30 mM         | 15109912 | [193] |
|                         | NAC           | Fibroblast        | 20 mM         | 16081426 | [220] |
|                         | NAC           | INS-1 cell        | 0.4 mM        | 17400930 | [70]  |
|                         | PDTC          | RPE cell          | 50uM          | 17765224 | [78]  |
|                         | Vitamin C     | Endothelial cell  | 10uM          | 10682488 | [278] |
|                         | Vitamin E     | Endothelial cell  | 10uM          | 10682488 | [278] |
| Mitochondrial ROS       | complex I rotenone | RPE cell          | 2.5uM         | 17765224 | [78]  |
|                         | complex II TTFA | RPE cell          | 10uM          | 17765224 | [78]  |
|                         | complex III Malonate | Heart Mt          | 25-100uM     | 18433712 | [280] |
|                         | Compiled IV Sodium azide | Motoneuron      | 2 mM          | 14607007 | [282] |
|                         | ATPase        | Oligomycin        |               | 20107899 | [283] |
|                         | Non-selective Mito-Tempo | HAEC             | 1 mM          | 27127201 | [60]  |
|                         | Non-selective Mito-Vit-E | HUVEC           | 1uM           | 31638979 | [143] |
| NOS                     | Non-selective i-NNAME | L6 myotube       | 1 mM          | 16092620 | [181] |
|                         | nNOS          | NOSIP             |               | 19298861 | [89]  |
|                         | eNOS          | NMMA              |               | 28904082 | [284] |
| NOX                     | Non-selective apocynin | SMC              | 50uM          | 29472601 | [177] |
|                         | DFI           | VSMC              | 100uM         | 23775814 | [246] |
|                         | DPI           | RPE cell          | 5uM           | 17765224 | [78]  |
|                         | DPI           | Endothelial cell  | 15uM          | 16249002 | [206] |
|                         | DPI           | HT-29 cell        | 10uM          | 25122478 | [188] |
|                         | DPI           | Fibroblast        | 20uM          | 16081426 | [220] |
|                         | DPI           | NIH-3T3 fibroblast| 5uM           | 12796479 | [204] |
|                         | NOX1 NOX1/4  | AEBSF             | 0.9uM         | 23251382 | [285] |
|                         | NOX1 NOX1/4  | ML171             | 1uM           | 29472601 | [177] |
|                         | NOX1 NOX1/4  | GKT136901         |               | 24319690 | [286] |
| LOX                     | NDGA          | Epithelial cell   | 10uM          | 12976479 | [204] |
| MPO                     | ABAH          |                   |               | 23251382 | [285] |
| MAO                     | Selegeline    | Astrocyte         | 20uM          | 20547771 | [67]  |

NAC: N-acetyl-l-cysteine, PDTC: pyrrolidene-dithiocarbamate, MnTBAP: Manganese III tetrakis, TFFA: 2-thienyltrifluoroacetone, i-NNAME—N omega-Nitro-arginine methyl ester hydrochloride, NMMA—NG-nomomethyl-arginine, NOSIP—nitric oxide synthase interacting protein, DPI—diphenylene iodonium, SEBSF—4-(2-aminoethoxy)-benzenesulfonyl fluoride, NDGA—ndihydroguaiaretic acid, ABAH—4-aminobenzoic acid hydrazide.
The targets in various treatments in Figure 4 (MitoETC and membrane potential, NOX, SOD and LOX) and genes of their downstream pathways are analyzed by circus Supplemental Table 4

| Target                        | Mitochondria ETC and membrane potential | NOX | SOD | LOX |
|-------------------------------|-----------------------------------------|-----|-----|-----|
| Downstream signaling genes    |                                         |     |     |     |
| ROMO1                         | CDR5                                    | MAPK3 | PTK2 |
| BCL2L1                        | MAPK1                                   | MAPK1 | PLA2G4A |
| CASP3                        | MAPK2                                   | MAPK2 |    |
| CASP8                        | MAPK3                                   | MAPK3 |    |
| NFKB1                        | NFKB1                                   | NFKB1 |    |
| MAPK1                        | PIESCB                                  | AKT1 |    |
| MAPK2                        | HMOX1                                   | HMOX1 |    |
| MAPK3                        | NFKB1                                   | NFKB1 |    |
| RAC1                          | MMP2                                    | MMP2 |    |
| AKAP1                        | MMP9                                    | MMP9 |    |
| UTRN                         | PRRT2                                   | PRRT2 |    |
| OPA1                         | CDKN2A                                  | CDKN2A |    |
| NLRP3                        | IRS1                                    | IRS1 |    |
| PYCARD                      | NFE2L2                                  | NFE2L2 |    |
| CASP1                        | MAP1LC3A                                | MAP1LC3A |    |
|                              | PTK2                                    | PTK2 |    |
|                              | STAT3                                   | STAT3 |    |
|                              | VEGFA                                   | VEGFA |    |
|                              | MAPK3                                   | MAPK3 |    |
|                              | MAPK1                                   | MAPK1 |    |
|                              | CTNNB1                                  | CTNNB1 |    |
|                              | CDK2                                    | CDK2 |    |
|                              | MAPK8                                   | MAPK8 |    |
|                              | MAPK2K                                  | MAPK2K |    |
|                              | VCAM1                                   | VCAM1 |    |

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