Journal of Stem Cells Research, Reviews & Reports

Open Access

Austin Publishing Group

Mini Review

Tissue Regeneration and Healing by ROS-Mediated NOX2 and Ca^{2+} Ion Uptake

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Received: May 23, 2018; **Accepted:** June 11, 2018; **Published:** June 18, 2018

Abstract

Unrestrained generation of Reactive Oxygen Species (ROS) is thought to contribute to tissue damage and remodeling in mammalian tissues and cells. However, the locally specific production of ROS plays a critical role in the wound-healing response and in the repair of tissues. Recently, two articles reported the new and unexpected role of ROS generated by the NADPH oxidase 2 complexes in peripheral neural tissues, and mitochondrial ROS (mtROS) production by membrane injury-induced Ca^{2+} uptake, which triggers the activation of the Ras homolog family member A (RhoA) to facilitate actin remodeling in injured tissues. These data suggest caution regarding the use of indiscriminate antioxidation therapy after tissue or cell injury, as it may hinder oxidative regenerating signaling, therefore limiting the healing and regeneration of particular local tissues.

Keywords: Axonal regeneration; Mitochondrial redox signaling; NOX2 complex; Reactive oxygen species; Repair injured skeletal muscle cells

Introduction

Reactive Oxygen Species (ROS) included superoxide anions (O_2^-), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) are generated by reduced reaction of the oxygen [1]. O_2-regarded as the primary ROS, is generated abundantly in cells and is rapidly dismutated to H_2O_2, which then be converted to (•OH) (Figure 1). ROS are relatively unstable and exhibit a short half-life. The primary origin of intracellular O_2^- is four organelles such as (i) mitochondria, (ii) Endoplasmic Reticulum (ER), (iii) peroxisomes and (iv) phagosomes, which display localized generation of ROS [2]. In mitochondria Electron Transport System (ETC), ATP is synthesized coupling electron transfer of O_2 in both complex I and II of the ETC, which generates O_2^- [1]. In the ER, H_2O_2 is generated by posttranslational oxidative modification during the process of protein folding [2,3]. In peroxisomes, ROS are generated during the metabolic pathways, such as amino acid metabolism, α- and β-oxidation of long-side chain fatty acids and other reactions [4,5]. Another origin of ROS is the membrane-bound NADPH oxidase NOX family and Dual Oxidase (DUOX). These enzymes include seven family members [6] and localize in the membranes of organelle to generate extracellular ROS (eROS). They convert O_2 to O_2^- by using NADPH as an electron donor [7]. NOX proteins contain an intracellular flavin domain at carboxyl region, which exhibits the binding sites for NADPH and FAD (flavin adenine dinucleotide), and six transmembrane-α-helices domains, which include four histidine residues as heme-binding at amino-terminal portion. NOX proteins act as carrier to transport electrons across the membrane.

In cells, the balance between production of ROS and rescue by antioxidation is crucial for surviving. The antioxidation cascade is represented as enzymes such as (i) Superoxide Dismutase (SOD), (ii) catalases, (iii) Glutathione Peroxidases (GPxs) and (iv) Peroxiredoxins (PRxs) [3]. SOD family catalyzes the dismutation of reactive O_2-anions to H_2O_2 [6,7]. Antioxidant enzymes included catalases, GPxs, and PRxs are responsible to convert H_2O_2 to H_2O and O_2. The GPxs reduced H_2O_2 by oxidized glutathione to maintain the level of reduced glutathione for ROS homeostasis [8]. PRx-family has a redox-sensitive cysteine which is inactivated by oxidation *via* H_2O_2. Thioredoxin functions as an electron donor to refuel the level of reduced PRx [9].

ROS and Redox Signaling

It is widely believed that ROS are deleterious to the mammalian cells by causing oxidative stress *via* the indiscriminate damaging of nucleic acids, proteins, and lipids. The reactive O_2^- and •OH radicals can induce damages irreversibly and produce the cellular dysfunction. However, recent evidence has shown that ROS can also involve in signaling functions, known as "Redox signaling" [10]. For example, proteins that participate in a variety of processes, such as the phosphatases PTEN and PTP1B, MAPK kinases, and redox-dependent transcription factors like FOXO4, can be regulated by H_2O_2 through reversible cysteine oxidation [2,11]. H_2O_2 oxidizes the thiol of cysteine residue to produce reactive sulfenic acid (-SOH), which can form intra-and intermolecular disulfide (-S-S-) bonds or cyclic sulfonamide (-S-N) or can undergo hyperoxidation to produce sulfinic ($-SO_2H$) or sulfonic ($-SO_3H$) acids [12]. The reversible modifications may therefore alter the protein structure, function, or activation process of the target. Together with cysteine thiols, H_2O_2 can also oxidize several other amino acid residues, such as methionine, lysine, tyrosine, histidine, arginine, and proline [13].

One mechanism aimed at explaining how target proteins are recruited during the oxidation process by H_2O_2. The colocalization of ROS and targets are occurred, and then the redox related molecules close to the ROS were also accumulated at the same sites [2]. For instance, NOX colocalizes with targets such as phosphatases or kinases, at the membranes, thereby affecting receptor-typed tyrosine kinase signaling [14]. Another mechanism suggests that H_2O_2 oxidizes

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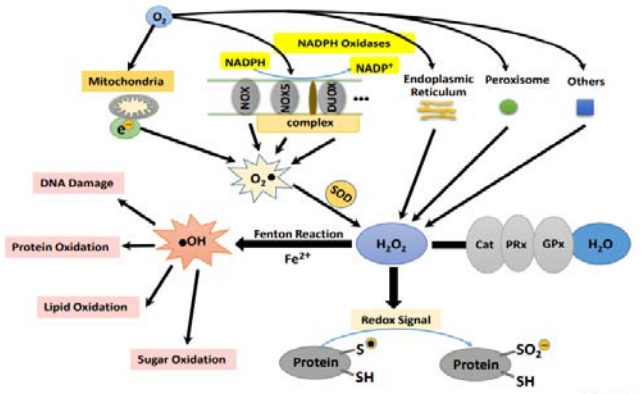

Figure 1: Schematic model of the ROS origin and enzymes involved in redox signaling.
Both generation of ROS and antioxidation system as redox signaling are presented.
P: Peroxidase domain; SOD: Superoxide dismutase; Cat: Catalase; GPxs: Glutathione peroxidases; PRxs: Peroxiredoxins.

scavenging enzymes like PRx or GPx, which transfer the oxygen atom to target proteins. This signaling is detected for the H_2O_2-dependent oxidation of the Apoptosis Signaling Kinase 1 (ASK1), and p38 associated phosphorylation, which is dependent on the formation of an ASK1-PRx1 disulfide intermediate [15]. A third mechanism is that the transient inactivation of scavenger by hyperoxidation or post-translational modification results in the accumulation of H_2O_2, thus allowing the oxidation reactions of target proteins [16]. Therefore, *via* the localized changes in the redox capacity, cells can regulate the ROS to select each signaling [17], Moreover, depending on the locus of Cys residues in protein, not all of these Cys residues are susceptible to H_2O_2-dependent oxidation equally [18]. Another system of ROS control involves in the H_2O_2 transport across membranes *via* aquaporin, which are integral proteins and are involved in the transport of the extracellular H_2O_2 produced by NADPH Oxidases (NOXs) across the membrane, to mediate intracellular signaling cascades as described [19]. Thus, H_2O_2 generates two different signaling networks for (i) regular deleterious functions and (ii) intracellular signaling cascades, for tissue repair.

ROS-Mediated Regeneration and Repair

Recently, two articles demonstrated the evidence of ROS merits for neurite regeneration and wound closure after tissue damages as the new connection. Hervera et al. [20] studied the regeneration of sensory neurons in the Dorsal Root Ganglion (DRG) that protrudes two axonal branches; the peripheral branches and the sensory Central Nervous System (CNS) branch (Figure 2A). A prior conditioning injury on peripheral branch can stimulate axonal regeneration after CSN damage, but the molecular mechanism remains to be elucidated.

Using ROS-sensing dyes, the authors showed that a regeneration-permissive Sciatic Nerve Crush (SNC) increased ROS production in the distant DRG but not a case of a permissive Dorsal Column Axotomy (DCA). They found neurite outgrowth on DRG from mice *ex vivo* after either a conditioning SNC or a local introduction of H_2O_2 to the nerve, indicating that ROS are critical for DRGs to grow the long axons. Furthermore, *in vivo* experiments by Sciatic Nerve Axotomy (SNA) and the conditioning lesion paradigm (DCA after SNC)

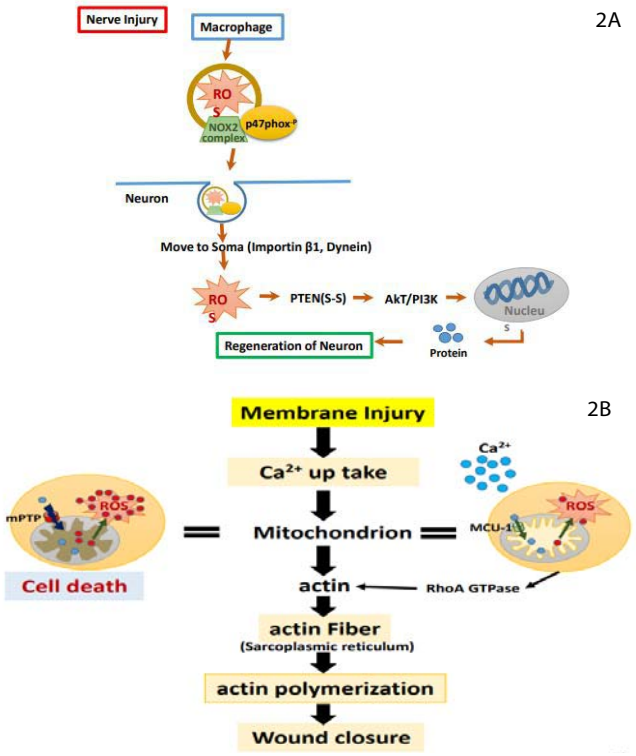

Figure 2: Merits of ROS signaling on nerve regeneration and Ca^{2+} uptake mediated repair of membrane injury.
(2A). Macrophages, which released exosomes carrying the ROS-producing NOX2 complex, were recruited into the site of nerve injury. At this site, NOX2 internalized and underwent retrograde transport into the endosomes *via* importin β1 and dynein. NOX2-ROS signaling mediated oxidation of PTEN and increased pAkt levels drove the axon to synthesis of the nerve regeneration at the injury site.
MT: Microtubules.
(2B). Membrane injury and Ca^{2+} -triggered mtROS signaling determined the survive-or-death decision making. Excess Ca^{2+} at the damaged site was scavenged by mitochondria and lysosomes that are accumulated, with SERCA pumping Ca^{2+} uptake into the sarcoplasmic reticulum. Cell death and surviving cascade were differentiated by the extent of the accumulating ROS; Cell death was decided by mitochondrial Permeability Transition Pore (mPTP). Cell survival through mtROS triggered RhoA activation to enhance cytoskeletal remodeling and to perform to wound closure. The mitochondrial Ca^{2+} uniporter MCU-1 mediated Ca^{2+} uptake into the mitochondrial matrix, leading to a local flare in mtROS production and RhoA activation to facilitate actin remodeling. These figures are modified based on the original figures reported from references [21,23]. Reprinted with permission from Springer Nature and AAAS.

support that redox signaling is needed and is sufficient for peripheral axonal regeneration. Importantly, when H_2O_2 conditioning preceded a spinal cord injury, the sensory motor recovery of the animals was significantly improved [20-21].

After SNC, the authors excluded the involvement of mitochondrial ROS. Instead, injury-induced recruitment of macrophages released exosome vesicles that contained NOX2 were taken up by DRGs. These exosome-borne NOX2 were transported to the soma, resulting in the oxidation and inactivation of Phosphatase and the Tensin homolog (PTEN), thus stimulating PI3K/Akt Kinase cascades and DRG outgrowth. The application of the ROS scavenger NAC (N-acetyl-cysteine) to the sciatic nerve limited DRG outgrowth after spinal cord injury, consistent with the results of direct H_2O_2

injection into the sciatic nerve SNA and conditioning H_2O_2 treatment induced prominent alterations in gene expression that were related to inflammatory signaling and to the pathways regulated by NOX signaling. After SNC injury, the phosphorylated p47phox and catalytic gp91phox in NOX subunits enriched along the axon and in the DRG. By which importin β1 and dynein were required for transporting NOX2 to the soma, leading to the commitment of ROS to cysteine oxidation, to inactivate PTEN and to activate PI3K/Akt signaling. In turn, this signal induces neurite outgrowth. Thus, the delivery of NOX2 oxidase from macrophages to injured neurons through exosomes stimulates ROS signaling and axon recovery.

In the second article, Horn et al. [22] reported the interplay between Ca^{2+} influx and mitochondria-generated ROS (mtROS) to stimulate actin-mediated closure of damaged membranes that was required for the survival of injured mouse muscle cells and human epithelial cells (Figure 2B). A membrane injury results in a steep influx of Ca^{2+} and excess Ca^{2+} which may trigger a cell death signaling, is scavenged by lysosomes and mitochondria, with the Sarcoplasmic Reticulum Calcium Adenosine Triphosphatase (SERCA) pumping Ca^{2+} into the sarcoplasmic reticulum. The Mitochondrial Calcium Uniporter (MCU-1) mediates Ca^{2+} uptake into the mitochondria leading to a local flare-up in mtROS production and activation of RhoA to facilitate actin remodeling for membrane repair. Membrane proximal lysosomes undergo Ca^{2+}-triggered exocytosis, which may also function to help exclude excess Ca^{2+}. Mitochondrial Ca^{2+} uptake provides an injury indicator that is capable of directing either cell survival through activation of mtROS-RhoA axis to enhance cytoskeletal remodeling for wound closure, or cell death cascades through induction of the mitochondrial permeability. Thus, quenching mtROS in mice muscles that had been exercised resulted in serious damage to myofibers and reduced muscle force [23].

Conclusion

The two articles reviewed here demonstrate that globally quenching ROS generation using antioxidants, which are a popular nutritional supplement, may have detrimental, context-specific effects that must be balanced against their potential merits.

Acknowledgement

We thank CC Ku and JB Pan for editing of this manuscript. This work was supported by the grants from the Ministry of Science and Technology (MOST 106-2320-B-037-001-MY3, MOST 106-2320-B-037-028; MOST 106-2314-B-037-017), the National Health Research Institutes (NHRI-Ex107-10720SI); and Kaohsiung Medical University (KMU-TP103A04, KMU-TP103G03, KMU-TP104E23, KMU-DT104001, KMU-M106001).

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