Review

Reactive Oxygen Species: Stuck in the Middle of Neurodegeneration

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Abstract. Neuronal cell loss associated with neurodegeneration has recently been linked to mitochondrial dysfunction. Electron transport chain defects and reactive oxygen species (ROS) production are emerging as important players in the etiology of neurodegenerative diseases. Proper management of ROS and disposal of damaged cellular components are vital to the survival and function of neurons. Proteins involved in these pathways are often mutated in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease. In this review, we will discuss the roles of ROS in normal physiology, how changes in ROS production affect neuronal survival in neurodegenerative diseases, and the recent advances in mitochondrial antioxidants as potential therapeutics.

Keywords: Apoptosis, autophagy, mitochondria, neurodegeneration, reactive oxygen species

INTRODUCTION

Neurodegenerative diseases are characterized by the progressive loss of specific neuronal populations. Accumulation of protein aggregates occurs in the affected neuronal populations and has been suggested to be at the origin of their demise, at least under some circumstances. Accumulating evidence suggests, however, that mitochondrial dysfunction also plays an important role in the etiology of neurodegenerative diseases. The management of mitochondrial reactive oxygen species (ROS) and the damage they cause is emerging as a major contributor to neuronal loss in Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD). In this review, we will discuss the roles of ROS in normal physiology as well as the causes and consequences of mitochondrial-generated ROS in neurodegenerative diseases.

REACTIVE OXYGEN SPECIES

ROS are a group of small oxygen-containing free radicals that are extremely reactive due to their unpaired valence electrons. ROS are generally formed by the primary ROS superoxide (O_2^-), which is chiefly converted to hydrogen peroxide (H_2O_2) by superoxide dismutases (SOD) but may also be protonated to form hydroperoxyl radicals (HO_2^-). H_2O_2 can be transformed into a number of other ROS including hydroxyl radicals (OH), hydroxyl anions (HO-), singlet oxygen ($^1O_2$) and hypochlorite (ClO^-). There are many generators of cellular ROS including the mitochondrion, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (reviewed in [1]), xanthine oxidase [2], and uncoupled endothelial nitric oxide synthase (eNOS) [3]. In the majority of cell types, the mitochondrion is the major source of ROS. Superoxide production, due to inefficiencies in oxidative phosphorylation, accounts for

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up to 2% of the total oxygen consumed by mitochondria [4]. Mitochondrial-generated ROS (mtROS) are mainly produced at complex I and complex III of the electron transport chain, although a total of nine sites have been identified [5]. Complex I produces superoxide solely in the matrix while complex III generates superoxide in both the matrix and the intermembrane space [6].

One cannot discuss ROS without discussing its clearance. Cells possess a number of antioxidant defenses chiefly to protect the cell from oxidative stress, but are also involved in other functions including cellular signaling. As a charged molecule, superoxide is generally membrane impermeable and is a particularly damaging molecule. It is, however, rapidly converted to H_2O_2 by superoxide dismutases: copper/zinc superoxide dismutase (SOD1) in the cytoplasm and mitochondrial intermembrane space; and by manganese superoxide dismutase (SOD2) in the mitochondrial matrix [7]. The importance of superoxide detoxification is highlighted by the fact that SOD2 knockout mice live only weeks [8]. H_2O_2, however, is membrane permeable and is likely the main signaling molecule in ROS-mediated pathways. H_2O_2 is detoxified in cells by glutathione peroxidase (GPx), and in some cases by catalase, to form water [7]. Prolonged high levels of ROS that surpass the cell's antioxidant capacity result in oxidative stress.

ROS AS SIGNALING MOLECULES

Although high ROS concentrations are damaging to lipids, proteins, and DNA, there is a large body of evidence demonstrating that low and intermediary levels of ROS are physiologically important (reviewed in [9] and [10]). NADPH oxidase-derived ROS (noxROS), for instance, have been well characterized in the cardiovascular field for their role in cell signaling. NADPH oxidases are a group of enzyme complexes highly expressed in phagocytes and cardiovascular tissue, which catalyze the reduction of oxygen to form superoxide. In phagocytes, NADPH oxidase produces large bursts of ROS, killing foreign organisms as part of the immune defense system [11]. In cardiovascular tissue, however, NADPH oxidase produces relatively less ROS at a slow and sustained rate, serving as intracellular signaling molecules [12]. NADPH activity is regulated by growth factors, vasoactive agents, and cytokines: the signaling of which results in the assembly and activation of the complex. Increased noxROS result in the activation of key survival pathways, notably mitogen-activated protein kinase (MAPK) pathways and the phosphoinositide 3-kinase (PI3K)/Akt pathway [10], presumably through the oxidation of key cysteine residues. Depending on the stimulus, activation of these pathways may induce cell growth, apoptosis, proliferation, migration, or smooth muscle contraction [13]. Increased noxROS is involved in the development of cardiovascular disease through activation of these pro-survival pathways. For example, noxROS play a pivotal role in vascular remodeling implicated in angiotensin II (Ang II)-dependent hypertension (reviewed in [14]).

In addition to the activation of the aforementioned signaling pathways, ROS are also involved in activation of transcription factors that regulate cellular responses to ROS. One of the first responses to increased ROS is the upregulation of antioxidant defenses. Nrf2 is a major transcription factor activated by oxidative stress regulating expression of several important antioxidant proteins such as superoxide dismutases, peroxiredoxins, glutathione peroxidases, and heme oxygenases ([15] and reviewed in [16]). Nrf2 is normally kept inactive by interacting with Keap1 in the cytosol where it is targeted for ubiquitin-dependent proteasomal degradation. The interaction between Keap1 and Nrf2 is disrupted with increased ROS production, allowing Nrf2 to translocate to the nucleus and activate transcription [17]. Interestingly, the PD-related gene DJ-1 has been proposed to promote the dissociation of Nrf2 from Keap1, thereby increasing its activity [18].

While Nrf2 plays an important role in regulating antioxidant defenses in response to elevated ROS, other ROS-activated transcription factors, such as hypoxia-inducible factors (HIFs), control several aspects of cellular functions. HIFs are the master transcription factors responsible for the adaptation of cells to low oxygen (hypoxia). HIFs maintain oxygen and energy homeostasis by regulating genes involved in metabolism, proliferation, erythropoiesis, angiogenesis, and cell survival [19]. Active HIF is a heterodimer regulated primarily though degradation or stabilization of its oxygen sensitive α subunit, while its β subunit is constitutively expressed. Under normal oxygen conditions, HIFα is rapidly degraded by the proteasome; however, during hypoxic conditions HIFα is stabilized, dimerizes with HIFβ and activates transcription of its target genes. In addition to oxygen levels, HIFα is also regulated through mtROS [20–22]. Inhibition of mtROS through pharmacological and genetic inhibition of complex III, and through antioxidant treatments, indeed lead to increased HIFα stability [20–22]. mtROS stabilize HIFα by inhibiting HIF prolyl-hydroxylases (PHDs), key en-

zymes responsible for HIF degradation. PHD inhibition by ROS probably occurs via the Fenton reaction which oxidizes Fe^{2+} to Fe^{3+}, where Fe^{2+} is an essential co-factor [23]. Interestingly, recent evidence demonstrates that HIF activation plays a protective role in neurodegenerative diseases (reviewed in [24]). For instance, the PHD inhibitor 3,4-dihydroxybenzoate has recently been shown to protect neurons from the MPTP model of PD both *in vitro* and *in vivo* [25].

Nuclear factor-kappa B (NF-κB) is a second pro-survival transcription factor activated by ROS that regulates several important cellular defense mechanisms. NF-κB activates target genes involved in cellular survival, growth, differentiation, and inflammation. Normally, NF-κB is sequestered in the cytoplasm and held inactive by IκB (inhibitor of κB). Moderate ROS levels lead to phosphorylation and degradation of IκB and therefore the activation of NF-κB [26]. Once activated, NF-κB plays a pro-survival role through the transcriptional activation of anti-apoptotic proteins, such as XIAP and GADD45β [27], and genes involved in decreasing mtROS, most notably SOD2 [28,29]. In addition, NF-κB may play a pro-survival role by inhibiting the JNK and caspase cell death pathways [30]. While moderate ROS levels activate NF-κB, high levels inactivate NF-κB through oxidation of cysteine 62 of its p50 subunit, inhibiting its DNA binding [31].

The p53 tumor suppressor represents a different aspect of ROS-activated transcription factors, as it can promote both survival and death. Cellular damage activates p53, leading to the inhibition of cell cycle or initiation of apoptosis [32]. In addition to these classical roles, p53 also presents a pro-survival role in response to increased ROS levels by upregulating several antioxidants, including glutathione peroxidase [33, 34], SOD2 [34], and ALDH4 [35]. p53 also induces the pentose phosphate shunt through regulating TP53-induced glycolysis and apoptosis regulator (TIGAR) [36]. TIGAR inhibits glycolysis and directs glucose to the pentose phosphate pathway producing NADPH, which is required to reduce glutathione (GSH) and thus lower ROS levels [36]. This antioxidant function of p53 is activated during low cellular stress [37], while high stress and ROS concentrations results in p53-mediated apoptosis through activation of several pro-apoptotic genes such as BH3-only proteins [38] and a series of p53-induced genes (PIGs) [39]. Although the exact mechanism by which p53 senses ROS and responds via either pro-apoptotic or anti-apoptotic functions remains relatively unknown, these differing functions possibly depend on p53 levels, posttranslational modification, and cellular localization [40].

Taken together these studies suggest that while high ROS levels are damaging, low levels play an integral role in pro-survival pathways through both the activation of key signaling pathways and the activation of transcription factors (Fig. 1).

ROS IN NEURODEGENERATIVE DISEASES

Excessively high levels of ROS beyond the clearance capacity of the cell cause oxidative stress, mitochondrial dysfunction, cellular damage, and, in numerous cases, cell death. A range of data suggests that oxidative stress is at the center of various neurodegenerative diseases. Among these diseases, evidence of increased ROS has been reported in ALS [41], HD [42], PD [43, 44], and AD [45,46]. The most direct example is in cases of familial ALS (FALS) in which the antioxidant enzyme SOD1 is mutated [47,48]. Dysfunctional SOD1 causes an increase in oxidative stress, as shown in several animal models of ALS in which mutant human or mouse SOD1 are expressed. The toxicity of SOD1 mutants amounts to more than a disrupted enzymatic function, however, as the mutant protein forms toxic aggregates within mitochondria [49,50], impairing respiration and promoting mitochondrial dysfunction [51]. Increased ROS in FALS is therefore likely the result of a combination of loss of antioxidant function and accumulation of toxic SOD1 aggregates.

ROS generation in neurodegenerative diseases go beyond such a direct effect on antioxidant function, although mitochondrial damage is a recurrent theme. For example, decreased mitochondrial respiration [52], as well as increased ROS and oxidative DNA damage have been reported in HD transgenic mice and in the parietal cortex of human HD brains [42,53,54], while antioxidant treatment with Coenzyme Q_{10} promoted moderate improvement in disease progression and extended survival [55]. However, the best-characterized neurodegenerative disease where mitochondrial dysfunction is linked to ROS production is PD.

The first evidence for a role of ROS in PD came from the observation that human PD brains show signs of mitochondrial dysfunction and oxidative damage in degenerating areas including the substantia nigra [43,44]. This was further substantiated by the identification of several PD-related genes that are associated with mitochondrial function, namely the mitochondrial kinase PTEN-induced putative kinase 1 (PINK1), the E3 ligase Parkin, and the oxidative stress sensor DJ-1 [56]. Specifically, mutations in PINK1 cause mitochondrial
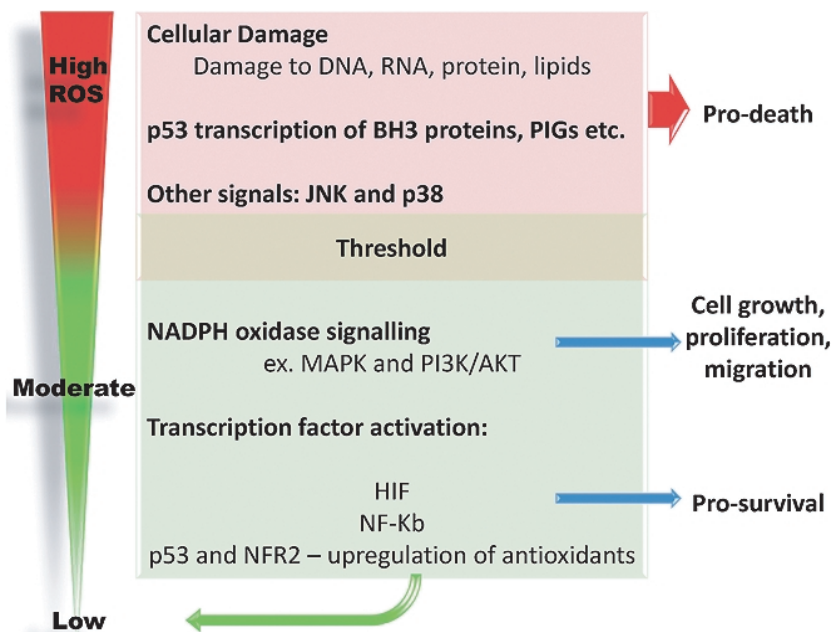
Fig. 1. Model of ROS levels moderating pro-survival and pro-death signaling. Moderate increases in ROS lead to activation of various cell signaling events that are generally pro-survival pathways. Moreover, activation of p53 and NRF2 are important to return the cell to a lower oxidative state by regulating the transcription of antioxidants. When ROS production exceeds a specific threshold (cell and stimulus specific), the cellular response shifts to promote cell death.

dysfunction [57], while loss of Parkin in mouse models has been shown to result in oxidative stress and mitochondrial dysfunction [58]. Recently, loss of DJ-1 in mouse embryonic fibroblasts (MEFs) has been associated with decreased respiration, increased mtROS, and reduced mitochondrial membrane potential [59]. Interestingly, PINK1, Parkin, and DJ-1 potentially interact in a complex to stimulate proteasomal degradation of proteins likely to aggregate, effectively preventing the accumulation of these neurotoxins [60]. The beneficial role of PINK1 and Parkin may also be involved in clearance of damaged mitochondria resulting in additional reductions in ROS (discussed below).

ROS production has also been linked to another key feature of neurodegenerative diseases, namely the accumulation of protein aggregates, although this relationship is complex. For example, expression of PD mutant α-synuclein (a component of Lewy bodies in PD) in mice and neurons is toxic, induces mitochondrial dysfunction, and increases ROS and cell death [61–63]. A second example where accumulation of protein aggregates has been linked to mitochondrial dysfunction and ROS production is in AD. The presence of amyloid-β (Aβ) plaques is a characteristic feature of AD and its accumulation has been linked to oxidative stress, mitochondrial dysfunction, energy failure,

synaptic dysfunction, and ultimately neuronal loss [64–66]. Indeed, AD brains show signs of increased ROS including nuclear DNA and mitochondrial DNA (mtDNA) damage [45,46], while mitochondrial accumulation of Aβ reduces oxygen consumption, and decreases mitochondrial electron transport chain activity [64,67]. One other way in which Aβ may affect ROS production is through its effect on mitochondrial dynamics. Mitochondria exist as a highly dynamic network that constantly divide and fuse. Recent evidence suggests that Aβ disrupts this process, leading to mitochondrial fragmentation and increased ROS production [68–70].

The causative role of Aβ in AD, however, remains debated, and it is clear that other aspects of the disease, such as hyperphosphorylation and accumulation of tau, also play an important role in its progression. In that respect, it is interesting to note that while protein aggregates can promote ROS production and mitochondrial dysfunction, ROS may also cause the accumulation of these neurotoxic aggregates, including Aβ [71] and α-synuclein [57,72,73]. This, along with the observation that the increase in ROS production precedes the pathological appearance of Aβ plaques [64,65], suggests that mitochondria dysfunction can be an early event that precedes protein aggregation. It should also be kept in mind, however, that mitochondrial dysfunc-

tion might be secondary to alterations in other pathways that can affect both mitochondrial function and accumulation of toxic proteins. For example, deregulated calcium homeostasis in AD may also play a role in ROS generation, Aβ aggregation, and damage to mitochondria [74–76] with Aβ oligomers further promoting intracellular calcium entry in a deleterious positive feedback loop [77].

Oxidative stress is thus emerging as a common theme in neurodegeneration (Fig. 2), with decreased mitochondrial antioxidants, increased protein aggregation, and increased mitochondrial dysfunction all promoting increased ROS generation. While several pathways (described above) are activated to control ROS production, dysfunctional mitochondria need to be removed to prevent further damage. Recent work has highlighted a role for autophagy in clearance of damaged mitochondria.

AUTOPHAGY AND APOPTOSIS

Macroautophagy (hereafter referred to as autophagy) is a conserved catabolic process allowing for recycling of nutrients during starvation. A basal level of autophagy is required to degrade damaged proteins and organelles. Autophagy is characterized by formation of double-membrane vesicles which deliver the cellular components to be recycled to lysosomes where they are degraded. Autophagosome formation is dependent on a series of conserved autophagy-related (ATG) genes (reviewed in [78]).

A role for autophagy in neurodegenerative diseases was first suggested by the phenotype of the ATG5 and ATG7 (two essential ATG genes) conditional knockouts in the central nervous system, where both models lead to an accumulation of ubiquitin-positive aggregates, neuronal loss, and death of the animals within several weeks of birth [79,80]. More recently, damaged mitochondria have been shown to be specifically removed by autophagy (mitophagy) in a process requiring PINK1 and Parkin, two aforementioned PD-related genes [81–84]. PINK1 is a labile mitochondrial outer membrane kinase that is stabilized on mitochondria that have lost their membrane potential [81,83, 84]. Following this increase in protein levels, PINK1 recruits the ubiquitin E3-ligase Parkin to the damaged mitochondria in a manner dependent on PINK1 kinase activation [81,83,84]. Once on the mitochondrial outer membrane, Parkin ubiquitinates substrates including VDAC1, leading to the recruitment of p62/SQSTM,

a ubiquitin binding protein that targets ubiquitinated substrates to autophagosomes for autophagy-dependent degradation [81]. PD-related mutations in either PINK1 or Parkin lead to the accumulation of damaged organelles, increases in dysfunctional mitochondria, and elevated ROS, further damaging the cell.

Failure to properly control ROS production and remove damaged organelles via mitophagy results in accumulation of damage and cell death. At least two types of cell death have been associated with ROS production: apoptosis and necrosis. High levels of ROS, associated with severe cellular damage can lead to necrotic cell death in a process that causes disruption of the cell membrane, causing further inflammation and tissue damage [85]. On the other hand, apoptosis is a tightly controlled process leading to complete removal of the damaged cells without eliciting an inflammatory response [85]. Apoptosis is regulated by a family of related proteins, BCL-2 homologues, that converge on mitochondria to regulate its outer membrane permeability and the release of several pro-apoptotic factors from the intermembrane space (reviewed in [86, 87]). Among these, Apoptosis Inducing Factor (AIF) and Endonuclease G (EndoG) translocate to the nucleus where they cause caspase-independent DNA degradation and cell death [88]. However, the major pathway activated in apoptotic cells relies on the release of cytochrome c [86,87]. Once in the cytosol, cytochrome c activates formation of the apoptosome, a protein complex comprised of cytochrome c, APAF-1 and caspase-9. The apoptosome activates effector caspases, the apoptotic proteases responsible for morphological changes leading to the dismantling of the cellular component. Cytochrome c release is dependent upon activation of two pro-apoptotic BCL-2 homologues, BAX and BAK [86,87]. Anti-apoptotic BCL-2 homologues (BCL-2, MCL-1, BCL-XL, and A1) inhibit apoptosis by blocking BAX/BAK function in healthy cells. The key molecules for BAX/BAK activation are the BH3-only proteins, a third class of BCL-2 homologues. BH3-only proteins induce BAX/BAK activation either through direct interaction (the so-called activator BH3 BID and BIM) or indirectly by inhibiting anti-apoptotic BCL-2 homologues [86,87]. While the exact contribution of each pathway is still debated, it is clear that BH3-only proteins are the upstream activators of cytochrome c release and apoptosis [86,87]. Activation of BH3-only proteins occurs through several mechanisms [86,87]. For example, BID activation is dependent on its cleavage by caspase-8 while BAD is regulated by phosphorylation. However, the main regulatory
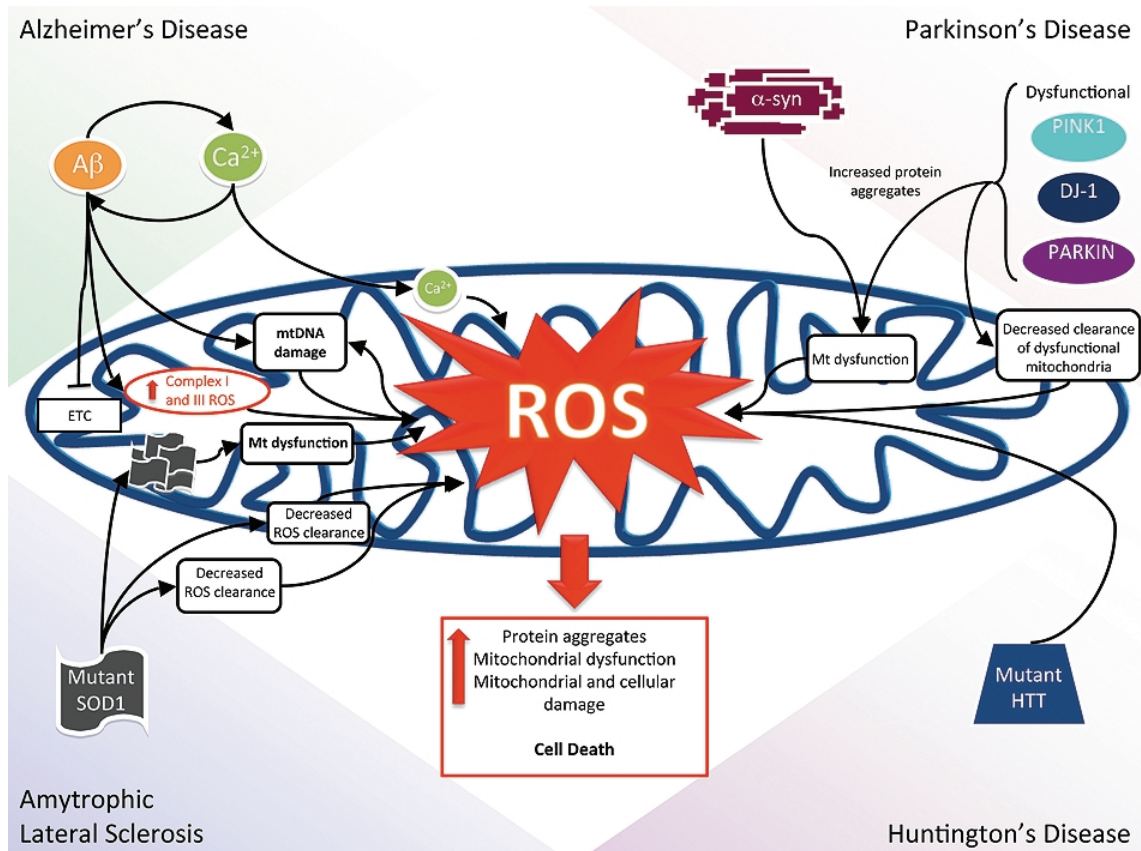
Fig. 2. Schematic of factors affecting mtROS in neurodegenerative diseases. Multiple pathways in neurodegenerative disease converge on the mitochondria to induce the production of mtROS. In the case of AD, Aβ accumulation is toxic to mitochondrial respiration and increases mtDNA damage and mtROS. In addition, deregulated Ca^{2+} levels are also detrimental to proper mitochondrial function. These two components are not mutually exclusive in that Aβ accumulation can deregulate Ca^{2+} levels and vice versa. Mutations in PD-related genes cause protein aggregation leading to mitochondrial dysfunction and oxidative stress. In addition, dysfunctional mitochondria are not appropriately disposed of by mitophagy, further increasing mtROS production. Aberrant levels of the mutant form of the protein huntingtin causes ROS generation in HD. Finally, mutations in SOD1 in FALS hinder ROS clearance and create toxic mitochondrial aggregates. Generation of ROS may in turn lead to further protein aggregation, mitochondrial dysfunction, cell damage, and cell death.

mechanism for several BH3-only proteins is transcriptional regulation. Following several types of cellular injury, including increased ROS, p53 is stabilized and activates expression of at least three BH3-only proteins, namely Noxa, Puma, and human BIK [38,89], providing a link between increased ROS production and induction of apoptosis. As p53 activation also promotes survival under some circumstances, other factors such as JNK and p38 are likely to participate in activation of BH3-only proteins following an increase in ROS. For example, JNK is activated by ROS and phosphorylates several apoptosis-related substrates such as MCL-1 and the BH3-only protein BIM [90,91]. In the case of MCL-1, this results in its proteasome-dependent degradation [91] while JNK-dependent phosphorylation of BIM increases its pro-apoptotic activity [90].

Interestingly, several links exist between autophagy and apoptosis. For example, anti-apoptotic BCL-2 homologues can regulate the activation of autophagy through inhibition of Beclin-1, a protein required for the initiation of autophagosome formation [92,93]. In addition, JNK is activated by starvation (a classical autophagy inducer) and promotes autophagy through several mechanisms including inhibition of BCL-2 [94] and induction of p62 [95]. As discussed above, autophagy is a protective mechanism that promotes disposal of damaged cellular components. However, sustained or excessive autophagy can also lead to autophagic (type II) cell death [96]. Keeping a proper balance between the different aspects of ROS production and clearance (including ROS signaling and autophagic removal of damaged organelles) is therefore key to

maintaining survival of cells with high metabolic rates and long lifespan such as neurons.

MITOCHONDRIAL ANTIOXIDANTS

Since ROS and oxidative damage underlie a large number of human diseases, attempts have been made in administering large doses of antioxidants to patients. Unfortunately, these treatments have been largely unsuccessful, likely due to their limited cellular and mitochondrial uptake. In an attempt to increase uptake and efficacy, a large effort has commenced to develop mitochondrial targeted antioxidants (reviewed in [97]). Mitochondria-targeted ubiquinol (MitoQ), for example, was shown to prevent cell death caused by endogenous oxidative stress hundreds of times more potently than its untargeted homologue [98]. Of note here, MitoQ also inhibited HIF accumulation in hypoxia [22]. Furthermore, MitoQ gave promising results in the cardiovascular field where MitoQ treatment reduced cardiac hypertrophy and improved endothelial function of stroke-prone spontaneously hypertensive rats [99]. MitoQ entered into Phase II clinical trials for the treatment of both PD and hepatitis C in 2008. Results, however, remain to be presented.

Another example of a promising mitochondrial targeted antioxidant is SkQ1 [100], shown to reduce H_2O_2-induced apoptosis in human fibroblasts and HeLa cells [101]. SkQ1 has produced some extraordinary *in vivo* results including restoring vision to blind animals with retinopathy, decreasing lymphomas in $p53^{-/-}$ mice, and prolonging the lifespan of fungi, crustaceans, flies, and mice [101]. Further research will be needed to investigate whether or not SkQ1 can prevent neuronal cell death and if it could be used as a potential treatment in neurodegenerative diseases.

Szeto-Schiller (SS) peptides represent a second class of mitochondrial antioxidants that offer the advantage of localizing to mitochondria irrespective of mitochondrial membrane potential, which may improve their therapeutic potential [102]. SS peptides concentrate 1000-fold to mitochondria, reduce ROS in neuronal cells, and protect cells against neuronal cell death by tert-butyl hydroperoxide [103]. Additionally, in both mouse and human islet cells where mitochondrial dysfunction is at the center of cell death, SS peptides prevented mitochondrial depolarization and apoptosis [104]. *In vivo*, SS peptides are protective in a mouse model of ALS, increasing survival and motor performance, and decreasing cell loss [105]. SS peptides al-

so protect dopaminergic neurons against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity both in cell cultures and in the PD animal model [106].

The links between neurodegenerative diseases and ROS presented above, along with the results obtained using these new antioxidants, present compelling *in vitro* and *in vivo* evidence that ROS underlie neuronal death. These new mitochondrial ROS scavengers therefore represent a promising novel therapeutic approach to the treatment of these diseases.

CONCLUSION

As more efficient and better-tolerated mitochondrial antioxidants become available, there are a number of important matters to consider. First of all, mitochondrial antioxidant treatments may inhibit ROS-dependent apoptosis, but not the underlying mitochondrial defects and may therefore result in other non-ROS mediated cell death pathways. Secondly, since mtROS are involved in beneficial cell signaling, including some pro-survival pathways previously discussed, it will be important to ensure that these pathways are not blocked by these treatments. Perhaps moderate doses of mitochondrial antioxidants will be most efficient since the beneficial pro-survival pathways will remain relatively unaltered. And finally, since oxygen sensing through HIF requires mtROS [20], it would be interesting and important to investigate whether these treatments make mammals more susceptible to cell death following ischemic injury.

Regardless, mitochondrial antioxidants represent a promising avenue for neurodegenerative disease treatments. Research is still needed, however, to fully delineate the missing steps by which neurodegenerative diseases increase mtROS and how mtROS leads to cell death. These studies will potentially present new pharmacological targets upstream or downstream of ROS generation, allowing to target damaging ROS without interfering with physiological ROS signaling.

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