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Review

Glutathione and thioredoxin dependent systems in neurodegenerative disease: What can be learned from reverse genetics in mice

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ABSTRACT

Oxidative stress is a major common hallmark of many neurodegenerative disease such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and stroke. Novel concepts in our understanding of oxidative stress indicate that a perturbed redox circuitry could be strongly linked with the onset of such diseases. In this respect, glutathione and thioredoxin dependent antioxidant enzymes play a central role as key regulators due to the fact that a slight dysfunction of any of these enzymes leads to sustained reactive oxygen species (ROS) production. Apart from their classical role as ROS scavengers, some of these enzymes are also able to control post-translational modifications. Therefore, efficient control of ROS production and reversibility of post-translational modifications are critical as improper control of such events may lead to the activation of pathological redox circuits that eventually culminate in neuronal cell death. To dissect the apparently opposing functions of ROS in cell physiology and pathophysiology, a proper working toolkit is mandatory. In vivo modeling is an absolute requirement due to the complexity of redox signaling systems that often contradict data obtained from in vitro approaches. Hence, inducible/conditional knockout mouse models for key redox enzymes are emerging as powerful tools to perturb redox circuitries in a temporal and spatial manner. In this review we address the basics of ROS generation, chemistry and detoxification as well as examples in where applications of mouse models of important enzymes have been successfully applied in the study of neurodegenerative processes. We also highlight the importance of new models to overcome present technical limitations in order to advance in the study of redox processes in the role of neurodegeneration.

1. Introduction

Increased cellular generation of reactive oxygen species (ROS) in the absence of a counteracting system leads to a state known as oxidative stress (Sies and Cadenas, 1985). Oxidative stress causes indiscriminate damage to biomolecules and results in tissue dysfunction that has long been believed to be a common denominator in a series of pathological conditions such as aging, cancer and neurodegenerative disorders (Lin and Beal, 2006; Reuter et al., 2010). Yet such a simplistic view has been challenged in the last few years, due to the recognition that ROS specifically impact on a broad range of signaling pathways (D'Autreaux and Toledano, 2007; Ostman et al., 2011). Furthermore, some of the early modifications induced by ROS, such as cysteine oxidation, are now recognized as reversible, making these modifications important in the context of altered protein function and signaling (Klomsiri et al., 2011). Thus, the challenge is to link chemistry with biology and place what might in some cases have been seen as phenomenology with strong mechanistic data (Winterbourn, 2008). Some modifications are well characterized for some proteins such as described for bacterial transcription factor OxyR; oxidation of its thiol group to a disulfide stabilizes the protein as a tetramer that is able to bind DNA (Choi et al., 2001; Toledano et al., 1994; Zheng et al., 1998). Another well-characterized protein modified by oxidation is SoxR, which consists of an [2Fe–2S] cluster that upon reaction with superoxide changes conformation and transactivates another transcription factor, SoxS (Pomposiello and Demple, 2001). The system SoxRS is able to induce the expression of approximately two dozens of genes that can be grouped in a broad class as oxidant-resistant dehydratase isozymes (*fum*C, *acn*A), enzymes involved in iron sulfur cluster repair (*ygg*X, *zwf*, *fpr*, *fld*A, *fld*B), drug efflux and resistance (*acr*AB, *tol*C, *mic*F, *mar*AB, *nfn*B, rimK) and other classes such as DNA repair (*nfo*), iron-uptake regulatory protein (*fur*) and antioxidation (*sod*A) (Imlay, 2008; Pomposiello and Demple, 2001).

Yet, in order to allow that these modifications are relevant in the context of cell signaling, they must be reversible and therefore can function as on/off switches under different conditions. In a physiological context, normal ROS production is generated to acti-

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vate certain circuits that are tightly regulated in a cellular and kinetic manner. Increasing levels of ROS, however, are able to saturate these specific targets and lead to activation of pathological circuits due to the loss of reversibility, such as cysteine over-oxidation (Wood et al., 2003a). The fact that ROS are implicated in the control and fine tuning of signaling modules places them as central players in the control of tissue and cell homeostasis. But the appreciation of ROS as second messengers in cells can be regarded as a double-edged sword because their efficient use relies mainly in their capacity to be generated at specific subcellular locations; however, it is their kinetics that defines and certifies their correct reaction with their downstream effectors.

In the context of neurodegenerative diseases, markers of oxidative stress such as protein carbonyls, lipid peroxidation adducts and end products, and DNA oxidation are found frequently in post-mortem brain tissue extracts from patients suffering for instance Alzheimer's (AD) and Parkinson's disease (PD) (von Bernhardi and Eugenin, 2012). Yet for the majority of disease cases it is not fully understood whether this is the cause or consequence of improper protein function and cellular processes. Impaired protein folding and degradation (e.g. Alzheimer's disease (Oresic et al., 2011), Huntington disease (Ribeiro et al., 2012)), intoxication and dysfunctional mitochondria (e.g. Parkinson's disease (Betarbet et al., 2000; Schapira et al., 1990)), mutations in antioxidant genes (amyotrophic lateral sclerosis, ALS (Rosen et al., 1993)), ischemia/reperfusion (e.g. stroke (van Leyen et al., 2006)), perturbed calcium homeostasis, excitotoxicity, neuroinflammation and glial cell dysfunction are all tightly associated with ROS activation. Thus, oxidative stress has been recognized a major component of these diseases, suggesting that hindering this critical downstream event might be highly useful to alleviating their progression (Lin and Beal, 2006).

The proper control of ROS by cellular antioxidant redox enzymes, in particular members of the glutathione- and thioredoxin-dependent systems, is thus of paramount importance not only to prevent oxidative stress-induced tissue damage, but also to allow for proper redox signaling by ROS as second messengers (D'Autreaux and Toledano, 2007). In order to introduce the reader to the field, this review will provide a brief overlook on the chemistry of ROS and how the cells are able to cope with such stress. Major focus will be on the role of thiol-dependent systems in the control and repair of oxidant-induced modification, focussing on selected processes with examples rather than comprehensive coverage of the subject. Strong emphasis is put on models that have been applied to address the role of these molecules in vivo. Finally, we discuss the importance of new models to overcome present technical limitations in order to advance the study of redox processes in the role of neurodegeneration.

2. ROS chemistry

In most areas of biomedical research and neurodegenerative diseases the term ROS has been used to describe the species formed by the reduction of O_2, namely superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\cdot}OH$). What we now term as ROS encompasses a wide array of molecules composed of free radicals and non-radical species. ROS comprise individual molecules such as hypochlorous acid (HOCl), singlet oxygen ($^1O_2$), lipid hydroperoxides (LOOH), ozone (O_3) and others. It is thus critical to cement the understanding that ROS are not a single chemical entity, rather the specific molecular identity of each ROS molecule is often critical in determining both its chemical reactivity and the biological response (Dickinson and Chang, 2011; Winterbourn, 2008; Winterbourn and Hampton, 2008). A single electron reduction of oxygen generates $O_2^{\cdot-}$, which in spite of its free radical, is relatively unreactive and undergoes spontaneous dismutation in aqueous solution at a rate constant of $k = 7.3 \times 10^5\ M^{-1}\ s^{-1}$. This reaction is catalyzed in the intracellular milieu by the enzymes Cu/Zn superoxide dismutase (SOD1) and Mn-SOD (SOD2) with rate constants of $2 \times 10^9\ M^{-1}\ s^{-1}$ s and $10^8\ M^{-1}\ s^{-1}$, respectively. This highly efficient removal of superoxide makes it very challenging for other molecules to compete with SOD for the reaction with $O_2^{\cdot-}$. The dismutation of $O_2^{\cdot-}$ generates H_2O_2, a molecule that presents preferential reactivity towards (seleno) cysteine residues in target proteins, including glutathione peroxidases, peroxiredoxins, phosphatases, caspases, and acetyltransferases (Dansen et al., 2009; Winterbourn and Hampton, 2008). Yet H_2O_2 can also interact with metal ions (with a very low $k < 10\ M^{-1}\ s^{-1}$), generating the strong oxidant $^{\cdot}OH$, that is able to react with almost every biomolecule at a diffusional rate constant. It is worth noting that the decomposition of peroxides by metal transitions ions is readily accelerated ($k \sim 10^3\ M^{-1}\ s^{-1}$) in the presence of specific ligands such as ATP or citrate (Rush and Koppenol, 1990).

Non-enzymatic reactions in neuronal tissues involving $O_2^{\cdot-}$ involve its reaction with the free radical nitric oxide ($NO^{\cdot}$) also at a diffusional rate, generating the highly unstable peroxynitrite ($ONOO^-$). $ONOO^-$ presents a complex chemistry that is able to decompose and generate other oxidants. $ONOO^-$ has a low pKa (6.8) and is present as a mixture of the anion and its acidic form ($ONOO^-$/ONOOH) at physiological pH, and is able to decompose through two different pathways, either generating $^{\cdot}OH$ and nitrogen dioxide radical ($NO_2^{\cdot}$) or reacting with bicarbonate and generating the reactive carbonate radical ($CO_3^{\cdot-}$) and NO_2^- (Toledo and Augusto, 2012). The particularly high rate constant of the reaction of peroxynitrite with thiol proteins from the glutathione peroxidase/peroxiredoxin (Prx) family (10^5–$10^8\ M^{-1}\ s^{-1}$) suggests a role of these proteins in $ONOO^-$ detoxification (Ferrer-Sueta and Radi, 2009; Sies et al., 1997).

Another class of compounds important in signaling and disease is the class of fatty acids peroxides. Their generation occurs through enzymatic (described in more detail in the chapter 2) and non-enzymatic mechanisms. Non-specific lipid peroxidation proceeds through a chain reaction composed of three main steps: initiation, propagation and termination (Fig. 1). The oxidation of lipids and their decomposition is briefly reported here, for dissection of the mechanism we refer the reader to a recent review from Yin et al., 2011). In contrast to enzymatic lipid peroxidation and owing to the increased electron density around the double bonds, the allylic hydrogen atoms can be readily abstracted by oxidants such as ferryl radical, $ONOO^-$, hydroperoxyl radicals ($HO_2^{\cdot}$) and $^{\cdot}OH$. This results in the formation of lipid radicals which react with available oxygen. The resulting products are diverse and depend on the substrate oxidized (Higdon et al., 2012). At first, a radical is formed that is further stabilized by rearrangement of the molecules and subsequent fast addition of oxygen, leading to the formation of a peroxyl radical ($LOO^{\cdot}$). Once formed, the $LOO^{\cdot}$ is able to propagate lipid peroxidation by abstracting a hydrogen from an adjacent fatty acid molecule, thus forming a LOOH and a new $LOO^{\cdot}$. In addition, a reaction between two $LOO^{\cdot}$ can generate $^1O_2$ through the Russel mechanism that can further propagate lipid peroxidation (Miyamoto et al., 2006). LOOH can also undergo two electron reductions, generating a corresponding alcohol in what is believed to be a detoxification pathway. Single electron reduction leads to the formation of the alcoxyl radical that is able to undergo two competitive reactions, one involving cyclization ($k = 10^7\ M^{-1}\ s^{-1}$) and subsequent addition of oxygen, generating an epoxy–peroxyl radical that is probably the species involved in the propagation reaction (Marnett and Wilcox, 1995). Alternatively, this molecule can undergo β-cission ($k = 10^6\ M^{-1}$ - s^{-1}), yielding a series of small carbonyl compounds such as aldehydes and ketones (Fig. 1).