MINI-REVIEW

Oxidative Stress and Antioxidants in Disease and Cancer: A Review

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Abstract

Reactive oxygen species (ROS), highly reactive molecules, are produced by living organisms as a result of normal cellular metabolism and environmental factors, and can damage nucleic acids and proteins, thereby altering their functions. The human body has several mechanisms to counteract oxidative stress by producing antioxidants. A shift in the balance between oxidants and antioxidants in favor of oxidants is termed as “oxidative stress”. Paradoxically, there is a large body of research demonstrating the general effect of oxidative stress on signaling pathways, less is known about the initial and direct regulation of signaling molecules by ROS, or what we term the “oxidative interface.” This review focuses on the molecular mechanisms through which ROS directly interact with critical signaling molecules to initiate signaling in a broad variety of cellular processes, such as proliferation and survival (MAP kinases and PI3 kinase), ROS homeostasis, and antioxidant gene regulation (Ref-1 and Nrf-2). This review also deals with classification as well as mechanisms of formation of free radicals, examining their beneficial and deleterious effects on cellular activities and focusing on the potential role of antioxidants in preventing and repairing damage caused by oxidative stress. A discussion of the role of phytochemical antioxidants in oxidative stress, disease and the epigenome is included.

Keywords: Reactive oxygen species (ROS) - DNA - proteins - MAPK - PI3K - antioxidants - phytochemicals

Reactive Oxygen Species (ROS)

In biological system, the oxidative stress refers to the physiological disturbance between the ROS such as H2O2 or O2- and the ability of the body to remove them. Oxidative stress can also be defined as the disordered redox signaling and control (Jones, 2006). A variety of ROS are produced throughout the body which are found to be the by-products of cellular aerobic metabolism, ongoing stress, and exposure to UV light or X-rays (Mittler et al., 2011); and play an important role in cell signaling and regulation of cytokine, growth factor and hormone action, transcription, ion transport, neuromodulation, immunomodulation, and apoptosis (Gloire et al., 2006; Mittler et al., 2011); and also play a fundamental role in normal functioning of immune system, proliferation of T cells, and immunological defence (Devadas et al., 2002; Hildeman, 2004).

Sources of Cellular ROS

There are many systems inside a cell that can generate ROS. Mitochondria are recognized as the major site for ROS production (Lenaz, 2012) and both complexes I and III have been established to be the specific sites for mitochondrial ROS generation (Drose and Brandt, 2012). Besides mitochondria, many enzymes are also capable of producing ROS. These include, but not limited to, NADPH oxidase (Bylund et al., 2010), xanthine oxidase (Agarwal et al., 2011), α-ketoglutarate dehydrogenase complex (Ambrus et al., 2011), dihydrolipoamide dehydrogenase (Zhang et al., 2011; Kareyeva et al., 2012).

Effects of Oxidative Stress on DNA

The multiple biochemical reactions in which oxygen is involved leads to the formation of reactive toxic intermediates that may cause DNA damage. Because oxidative damage to DNA can cause mutations; and mutations are known to cause cancer, much effort has been devoted to study the role in carcinogenesis of oxidative DNA damage (Friedberg and Meira, 2006; Gupta et al., 2012).
Effect of Oxidative Stress on Protein

Oxidative stress on protein can be both irreversible and reversible protein oxidative modifications. Irreversible modifications mainly include protein carbonylation and tyrosine nitration (Prokai et al., 2007; Rao and Moller, 2011) which are often associated with oxidative damage and have been used as biomarkers for assessment of oxidative stress in aging and diseases (Yan and Sohal, 1998; Stadtman, 2001; Prokai et al., 2007). While both carbonylation and nitration can have detrimental effects on the target proteins, evidence has also emerged that such modifications can also play positive roles in cellular function under stress conditions.

Reversible protein oxidative modifications include protein cysteine modifications (Cai and Yan, 2013). This should be particularly true for reversible cysteine oxidation, which not only reflects changes in cellular redox state, but can also protect the target proteins from further damage. Additionally, reversible cysteine oxidation is also involved in redox signaling cascades (Finkel, 2011; Chung et al., 2013) that can elicit positive stress responses to prevent unpredictable disastrous events such as stroke and heart attack.

Reactive Oxygen Species (ROS) Homeostasis and Redox Regulation in Cellular Signaling

Oxidative stress results in macromolecular damage and is implicated in various disease states such as atherosclerosis, diabetes, cancer, neurodegeneration, and aging. Paradoxically, accumulating evidence indicates that ROS also serve as critical signaling molecules in cell proliferation and survival. While there is a large body of research demonstrating the general effect of oxidative stress on signaling pathways, less is known about the initial and direct regulation of signaling molecules by ROS, or what we term the “oxidative interface”.

Cellular ROS sensing and metabolism are tightly regulated by a variety of proteins involved in the redox (reduction/oxidation) mechanism. The molecular mechanisms through which ROS directly interact with critical signaling molecules to initiate signaling in a broad variety of cellular processes, include proliferation and survival (MAP kinases and PI3 kinase), ROS homeostasis, and antioxidant gene regulation (Ref-1 and Nrf-2) (Paul et al., 2012).

Regulation of MAPK signaling pathways by ROS

The function and regulation of the mitogen-activated protein kinase (MAPK) cascades have been comprehensively covered (Weston and Davis, 2007; Ramos, 2008). MAPK pathways are composed of a three-rung kinase tier: MAPK kinase kinases (MAPKKK) phosphorylate and activate MAPK kinases (MAPKK), which in turn phosphorylate and activate MAPKs. Among the members of the MAPK cascades, apoptosis signal-regulated kinase 1 (ASK1) is an upstream MAPKKK that regulates the JNK and p38 MAPK pathways leading to apoptosis through phosphorylation of MKK4, MKK3, and MKK6 MAPKKs (Ichijo et al., 1997). ASK1 is activated under various stress conditions including oxidative stress (Tobiume et al., 2001). ASK1-deficient mouse embryonic fibroblasts were shown to be less susceptible to TNF- or H$_2$O$_2$-induced cytotoxicity along with decreased JNK and p38 MAPK activation, suggesting that ASK1 plays a pivotal role in promoting cell death under oxidative stress (Tobiume et al., 2001). However, ROS activated ASK1 mediates p38 signaling pathway leading to non-apoptotic outcomes such as differentiation (Choi et al., 2011), thus reinforcing the role of ROS signaling in cellular homeostasis.

Regulation of PI3K signaling pathways by ROS

Another signaling pathway that plays a key role in cell proliferation and survival in response to growth factor, hormone, and cytokine stimulation is the phosphoinositide 3-kinase (PI3K) pathway. PI3K catalyzes the synthesis of the second messenger phosphatidylinositol 3, 4, 5-triphosphate (PIP3) from phosphatidylinositol 3, 4, 5-bisphosphate (PIP2), wherein the membrane bound PIP3 serves as a signaling molecule to recruit proteins containing the pleckstrin homology (PH) domain. These PH domain proteins, such as the phosphoinositide-dependent protein kinase (PDK) and protein kinase B (AKT) serine/threonine kinases are thus activated and mediate further downstream signaling events (Cantrell, 2001). The synthesis of PIP3 is negatively regulated primarily by the phosphatase and tensin homology (PTEN) phosphatase which dephosphorylates PIP3 back to PIP2 (Leslie and Downes, 2002). Through PTEN, the PI3K pathway is subjected to reversible redox regulation by ROS generated by growth factor stimulation (Lee et al., 2002; Kwon et al., 2004). It was also demonstrated that endogenously generated ROS following treatment with peptide growth factors such as insulin, EGF, or PDGF causes oxidation of PTEN leading to the activation of the PI3K pathway (See et al., 2005). PTEN oxidation is reversed by peroxiredoxin II, a cytoplasmic peroxiredoxin isoform, that eliminates H$_2$O$_2$ generated in response to growth factors (Kwon et al., 2004). Thus, the PI3K pathway is regulated by ROS in a similar manner as the MAPK pathways at the oxidative interface, where protein phosphatases are directly oxidized by ROS resulting in sustained activation of the signaling pathways. It is noteworthy that various oxidants and ROS-producing chemicals activate transcription of a battery of antioxidant genes through a PI3K-NFE2-like 2 (Nrf2)-antioxidant response element (ARE) mechanism, where PTEN knockdown enhances transcription of ARE-regulated antioxidant genes (Sakamoto et al., 2009); however, it is not known whether these oxidants induce PTEN oxidation and inhibition of phosphatase activity leading to gene activation.

Antioxidants

Oxygen is an element indispensable for life (Bucci, 2009). When cells use oxygen to generate energy, free radicals are created as a consequence of ATP (adenosine triphosphate) production by the mitochondria. These by-products are generally reactive oxygen species (ROS) that
result from the cellular redox process. These species play a dual role as both toxic and beneficial compounds. Thus, the delicate balance between these two antagonistic effects is clearly an important aspect of life. At low or moderate levels, ROS exert beneficial effects on cellular responses and immune function; whereas at high concentrations, they generate oxidative stress, a deleterious process, that can damage all cell structures (Genestra, 2007).

Oxidant/antioxidant balance has been suggested as an important factor for initiation and progression of cancer (Gupta et al., 2012). The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ (endogenous) or externally supplied through foods and/or supplements (exogenous). Endogenous and exogenous antioxidants act as “free radical scavengers” by preventing and repairing damages caused by ROS; and therefore can enhance the immune defence and lower the risk of disease and cancer (Valko et al., 2006; Chatterjee et al., 2007).

Antioxidant Classification

Endogenous antioxidant compounds in cells can be classified as enzymatic antioxidants and non-enzymatic (metabolic and nutrient) antioxidants.

The major enzymatic antioxidants directly involved in the neutralization of ROS are: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GRx) (Pacher et al., 2007; Halliwell, 2007). SOD, the first line of defence against free radicals, catalyzes the dismutation of superoxide anion radical (O2·−) into hydrogen peroxide (H2O2) by reduction. The oxidant formed (H2O2) is transformed into water and oxygen (O2) by catalase (CAT) or glutathione peroxidase (GPx). The selenoprotein GPx enzyme removes H2O2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power. Besides hydrogen peroxide, GPx also reduces lipid or nonlipid hydroperoxides while oxidizing glutathione (GSH) (Halliwell, 2007).

The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants, belonging to endogenous antioxidants, are produced by metabolism in the body, such as lipoid acid, glutathione, L-arginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin, etc (Willcox et al., 2004); while, nutrient antioxidants, belonging to exogenous antioxidants, are compounds which cannot be produced in the body and must be provided through foods or supplements, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids, etc.

Nutrient antioxidants have been shown to be involved in detoxification of the reactive oxygen species (ROS) (Gupta and Singh, 2013) and play an important role in helping endogenous antioxidants for the neutralization of oxidative stress (Donaldson, 2004). The nutrient antioxidant deficiency is one of the causes of numerous chronic and degenerative pathologies and cancer. Each nutrient is unique in terms of its structure and antioxidant function (Willcox et al., 2004).

Antioxidant Process

When an antioxidant destroys a free radical, this antioxidant itself becomes oxidized. Therefore, the antioxidant resources must be constantly restored in the body. Thus, while in one particular system, an antioxidant is effective against free radicals; in other systems, the same antioxidant could become ineffective. Also, in certain circumstances, an antioxidant may even act as a pro-oxidant, for example, it can generate toxic ROS (Young and Woodside, 2001). The antioxidant process can function in one of two ways: chain-breaking or prevention. For the chain-breaking process, when a radical releases or steals an electron, a second radical is formed. The last one exerts the same action on another molecule and continues until either the free radical formed is stabilized by a chain-breaking antioxidant (vitamin C, E, carotenoids, etc) or it simply disintegrates into an inoffensive product. The classic example of such a chain-breaking reaction is lipid peroxidation. For the preventive way, an antioxidant enzyme like superoxide dismutase, catalase, and glutathione peroxidase can prevent oxidation by reducing the rate of chain initiation, for example, either by scavenging initiating free radicals or by stabilizing transition metal radicals such as copper and iron (Young and Woodside, 2001).

Nrf2 and Ref1-Mediated Redox Cellular Signaling

In order to prevent oxidative stress, the cell must respond to ROS by mounting an antioxidant defence system. Antioxidant enzymes play a major role in reducing ROS levels; therefore, redox regulation of transcription factors is significant in determining gene expression profile and cellular response to oxidative stress. Ref-1 is a multi-functional protein that not only regulates transcription factor activity but also mediates base excision repair. The transcriptional regulatory function of Ref-1 is mediated through its redox activity on several transcription factors such as activator protein 1 (AP-1), p53, nuclear factor kappa B (NFkB), and hypoxia inducible factor 1 (HIF-1α) (Tell et al., 2009). The N-terminus region of Ref-1 is responsible for redox activity; while the AP-endonuclease activity domain is located at the C-terminal region (Xanthoudakis et al., 1994). Ref-1 activates the AP-1 transcription factor, Fos-Jun, through redox regulation of cysteine residues in the Fos-Jun DNA binding domains (Xanthoudakis and Curran, 1992).

Phytochemical Antioxidants: Oxidative Stress, Disease, and Epigenome

Cellular antioxidant defence machinery has been unequivocally established as an oxidative stress-counteracting entity. Antioxidant supplementation/treatment has been adopted for either prevention of or protection against several disorders and pathophysiological
states; wherein, oxidative stress has been established as a causative mechanism (Arts and Hollman, 2005). Naturally occurring phytochemical antioxidants have occupied a prominent position as effective antioxidants for the prevention and/or treatment of several disorders and diseases among humans (Hollman et al., 2011; Spatafora and Tringali, 2011). The premise for this has been the antioxidant actions of the phytochemicals as free-radical scavengers, oxidative stress relievers, and lipoperoxidation inhibitors (Scalbert et al., 2005). Phytochemical antioxidants include simple molecule antioxidants such as vitamins C, E, and K; plant pigments such as carotenoids (β-carotene), xanthophylls, lycopene, anthocyanins, and phaeophytins; and secondary plant metabolites, including simple phenolics to more complex polyphenols (Bors and Michel, 2002). Some of these phytochemical polyphenols, in addition to acting as antioxidants, will also function as pro-oxidants that cause oxidative stress (Lambert and Elias, 2010). The pro-oxidant action of tea polyphenols has been linked to their anticancer actions (Forester and Lambert, 2011). Also, Morinda citrifolia (Noni) has been found to alter oxidative stress marker, Malondialdehyde (MDA) and Antioxidant Activity (Superoxide Dismutase and Catalase activity) in Cervical Cancer Cell Lines (Gupta and Singh, 2013). Polyphenols are known for their complexing abilities (chelation) with trace metals. Polyphenols have also been shown to attenuate the iron-induced DNA damage by complexation with iron and keeping the Fe in the +3 state after oxidation of Fe in the +2 state in presence of molecular oxygen (Perron et al., 2010). Animals and humans obtain these phytochemical polyphenols from diet or nutritional supplementation. In recent times, plant polyphenols have been the attraction as effective antioxidants (from diet or supplementation) in prevention and treatment of several diseases, including CVDs, cerebrovascular diseases, Alzheimer’s disease, airway disease, and cancer, with a focus to alleviate the oxidative stress as the causative mechanism in those diseases (Wood et al., 2010; Choi et al., 2012).

In spite of the beneficial effects of the phytochemical polyphenolic antioxidants in humans, their mechanisms of action (physiologically and pharmacologically) in the mammalian systems (including humans) are just emerging. The regulation of gene expression by dietary polyphenols in cellular models such as the vascular endothelial cells is evident (Nicholson et al., 2010). One of the noteworthy current discoveries that have emerged from the phytochemical-antioxidant interactions in mammalian model systems is their nature of modulating the mammalian epigenome (Duthie, 2011). Totally, novel disciplines such as nutrigenomics and nutrigenetics have emerged with a focus on the phytochemical nutrient-gene interactions toward cancer prevention in which signaling pathways, networks, and epigenetic phenomena are investigated (Ferguson and Schlotheau, 2012).

Summary

This Forum illustrates the progress and critical issues in the understanding of ROS in disease and cancer. Current insight, although far from being comprehensive and complete, justifies a closer look at the molecular mechanisms through which ROS and antioxidants directly interact with critical signaling molecules to initiate signaling in a broad variety of cellular processes.

Development and treatment of various diseases involves ROS and antioxidants. On the other hand, increase or decrease level of ROS can affect disease properties and treatment effect. It is hoped that this Forum can stimulate substantial interest in more mechanistic studies on the biology of ROS and antioxidants in disease and in the development of effective ROS-targeted disease treatment modalities by antioxidants.

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