It takes 2 antioxidants to tango: the interaction between manganese superoxide dismutase and glutathione peroxidase-1

Dede N. EKOUE, Alan M. DIAMOND*
Department of Pathology, University of Illinois at Chicago, Chicago, Illinois, USA

Abstract: Reactive oxygen species (ROS) are generated during mitochondrial oxidative metabolism. Accumulation of ROS without an effective antioxidant response can lead to oxidative stress, resulting in macromolecular damage that is implicated in the etiology of various diseases including cancer. ROS detoxification is regulated by various antioxidant proteins, specifically manganese superoxide dismutase (MnSOD), which catalyzes the conversion of superoxide into H₂O₂, and the subsequent conversion of H₂O₂ into water is catalyzed by glutathione peroxidase 1 (GPx-1). In vitro and in vivo evidence supports a conflicting role of MnSOD in tumor biology and indicates that an interaction between MnSOD and GPx-1 can modulate the impact of MnSOD on carcinogenesis. Additional support for this idea is provided by epidemiological data indicating that an association exists between polymorphisms in the MnSOD and GPx-1 genes and cancer risk, such that individuals who carry both at-risk polymorphisms are at a higher risk of several types of cancer. Future studies examining the impact of these 2 antioxidants on tumor biology need to consider the interplay between the 2 genes.

Key words: Oxidative stress, cancer, manganese superoxide dismutase, selenium, glutathione peroxidase

1. Oxidative Stress
The generation of ATP via oxidative phosphorylation provides the energy required by aerobic organisms to sustain themselves. This process is localized to the mitochondria, where electrons are passed among the components of the electron transport chain where there is an inevitability of leakage of the highly reactive oxygen species (ROS) superoxide, with it having been estimated that 1%–2% of the oxygen being used for mitochondrial respiration is converted to superoxide. With its short half-life and extreme reactivity, there is a strong potential for the superoxide radical to interact with biomolecules, resulting in oxidative damage that could have a profound effect on cellular function and survivability. As described below, eukaryotic cells have evolved defense mechanisms to protect themselves from the adverse effects of superoxide accumulation and the strategic location of manganese superoxide dismutase (MnSOD) in the mitochondria is a principle one of these. As a consequence of the dismutation of superoxide by MnSOD, the less reactive hydrogen peroxide (H₂O₂) is generated, which can diffuse through the mitochondrial membrane where it can have its own host of biological consequences. The management of the appropriate levels of ROS is the domain of a host of antioxidant molecules such as glutathione and enzymes that can neutralize ROS. Oxidative stress occurs when the levels of ROS exceed the cell’s abilities to maintain them at their appropriate levels. While less considered, the elimination of ROS past the ideal levels to support cellular function may also occur, and this has been referred to as reductive stress.

2. ROS as important signaling molecules
Aerobic organisms have not only evolved to take advantage of the enhanced energy production that can be achieved by the consumption of oxygen via the electron transport chain, but have also evolved to use ROS as potent signaling molecules. Fluctuations in ROS can be due to changes in environmental stimuli or intrinsic metabolic activity, and examples abound throughout phylogeny indicating the sensing of and response to one ROS, H₂O₂. As examples, plants use an oxidative burst that generates H₂O₂ that serves as a diffusible signal to induce apoptosis in pathogen-exposed cells while stimulating antioxidant enzymes in adjacent tissues (Levine et al., 1994), and a H₂O₂ gradient generated at the tissue level in zebrafish mediates wound healing and leukocyte recruitment (Niethammer et al., 2009). One of the first examples of H₂O₂ being critical for normal mammalian functions was the demonstration that the production of H₂O₂ could stimulate platelet-
derived growth factor, whose activity results in a variety of cellular responses, including DNA synthesis, replication, and chemotaxis (Sundaresan et al., 1995). A significant component of ROS signaling is the consequence of the oxidation of reactive cysteine residues in catalytic proteins and one estimate has there being over 500 individual proteins that might be regulated in this manner, with many being tyrosine and multispecific phosphatases (Weerapana et al., 2010; Finkel, 2011). Thus, there is a critical need to have an understanding of the regulation and maintenance of ROS, and H$_2$O$_2$ in particular given its relative stability compared to other ROS species, in cellular processes and homeostasis. This need extends to the roles played by the antioxidant enzymes above that are more frequently examined in the prevention of cellular damage and the response to oxidative stress.

3. MnSOD
MnSOD is a nuclear-encoded and mitochondrially located enzyme that removes superoxide by reducing it to the less toxic H$_2$O$_2$, and it is essential for all aerobically living organisms (Fridovich, 1975). While numerous reports have been published indicating a protective effect of increasing MnSOD in cultured cells, others have reported enhanced toxicity, particularly in a cellular environment in which other antioxidant defenses have been reduced. For example, enhanced cytotoxicity and oxidative stress from 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) are reported when MnSOD is overexpressed in different cell types (Darby-Weydert et al., 2003; Weydert et al., 2008). BCNU has been shown to inactivate important antioxidant enzymes such as glutathione reductase and thioredoxin reductase (Schallreuter et al., 1990).

There is extensive literature that addresses the role MnSOD plays in tumorigenesis, including cell culture work, animal models, and the examination of changes in MnSOD levels in tumors relative to the corresponding normal tissues, although there are data indicating that the consequences of MnSOD levels may either suppress or promote carcinogenesis. The reader is referred to 2 excellent reviews on this subject, the first from over 30 years ago for a historical perspective (Oberley and Buettner, 1979) and the second more recent (Dhar and St Clair, 2012). A critical paper indicating that elevated MnSOD levels could suppress carcinogenesis was published in 1993 by Church et al. (Church et al., 1993). Using human UACC-903 melanoma cells, they reported that the ectopic expression of MnSOD in those cells reduced their ability to form colonies in soft agar and tumors when injected in immunosuppressed mice, both hallmarks of the transformation process (Church et al., 1993). Subsequently, there have been numerous reports of the elevated expression of MnSOD attenuating the characteristics of cancer cells in culture and limiting the ability of xenografted tumor cells in mice, and these have been recently reviewed (Holley et al., 2013). Alternatively, overexpression of MnSOD in tumor cells has been shown to be protective against tumor cell death by either serum starvation or the induction of apoptosis (Palazzotti et al., 1999; Mohr et al., 2008).

4. The interaction between MnSOD and other antioxidants
An important mechanism by which MnSOD enzyme activity could impact tumor biology is the production of H$_2$O$_2$, which could stimulate the expression of genes involved in carcinogenesis and metastasis. This concept is supported by data indicating that the increased expression of MnSOD in U87 glioma cells achieved due to transfection of an MnSOD expression construct could increase cell migration, invasive properties, activation of signaling cascades associated with transformation, levels of the MMP-1 and MMP-9 matrix metalloproteinases, and that each of these could be suppressed by the addition of the antioxidant compound N-acetyl-1-cysteine that reduces hydrogen peroxide (Li et al., 2011). The effects of these treatments were due to the specific reduction in H$_2$O$_2$, which was established using a mitochondrially targeted catalase that also reversed the effects of MnSOD overexpression (Li et al., 2011). Using HT-1080 fibrosarcoma cells, it was shown that overexpression of MnSOD increased the binding of transformation-associated transcription factors as well as the expression of matrix metalloproteinase-1, a metastasis-related protein, and these effects could also be abrogated by the enhanced expression of catalase (Nelson et al., 2003). One of the earliest reports of H$_2$O$_2$ detoxifying enzymes altering the tumorigenic effects of MnSOD was published in 2000 when Li et al. investigated the effects of overexpressing another H$_2$O$_2$ detoxifying enzyme, glutathione peroxidase 1 (GPx-1), on MnSOD-induced phenotypes associated with carcinogenesis (Li et al., 2000). Here, the human glioma P U118-9 cell line was transfected with an expression construct, which resulted in the overexpression of MnSOD. Increased levels of MnSOD led to decreased doubling time, plating efficiency, and, when implanted into nude mice, reduced tumor growth. All of these changes were suppressed by the increased expression of GPx-1 achieved by the transfection of a GPx-1 expression construct into the MnSOD-overexpressing cells (Li et al., 2000).

5. Glutathione peroxidase 1 (GPx-1)
One protein involved in the regulation of H$_2$O$_2$ levels is the selenium-dependent enzyme GPx-1. GPx-1 is a member of a highly unusual family of proteins that contain the essential trace element selenium in the form of the amino
acid selenocysteine (Hatfield and Gladyshev, 2002). For these proteins, selenium is inserted cotranslationally in response to one or more in-frame UGA codons, destined to be recognized as selenocysteine due to the presence of a selenium insertion sequence element in the 3' untranslated region of the selenoprotein mRNA (Berry et al., 1991; Caban and Copeland, 2006). GPx-1 was the first characterized selenoprotein and functions to detoxify hydrogen and lipid peroxides using reducing equivalents from glutathione, although broader roles in reactive oxygen-mediated signaling pathways have been supported by experimental data in addition to the more focused-upon role as an antioxidant [see Lubos et al. (2010) for a comprehensive review].

GPx-1 is a selenium-dependent enzyme that is critical in eliminating H₂O₂. Approximately 50% of GPx-1 is located in the mitochondria, where superoxide is formed as a consequence of electron transport and where MnSOD resides (Arthur, 2000). A polymorphism in the GPx-1 gene, resulting in a leucine (leu) at codon 198 rather than a proline (pro), is associated with increased risk of cancers of the lung, breast, bladder, and liver as well as lymphoma [reviewed by Zhuo and Diamond (2009)]. In addition to cancer, allelic variants of GPx-1 have also been associated with the risk of cardiomyopathy (Lei et al., 2009), coronary heart disease (Winter et al., 2003; Tang et al., 2008), hypertension (Kato et al., 2008), autism (Ming et al., 2010), intracerebral hemorrhage (Pera et al., 2008), asthma (Solodilova et al., 2007), and metabolic syndrome (Solodilova et al., 2007). The at-risk GPx-1 leu allele encodes a protein that is less responsive to selenium as compared to the same protein with a proline at that position (Hu and Diamond, 2003; Zhuo et al., 2009).

6. Epidemiological evidence for a role of MnSOD in cancer

In addition to data obtained using cultured cells and animal models, there is also considerable human data indicating a role for MnSOD in carcinogenesis. It is difficult to arrive at a clear indication as to whether MnSOD levels are beneficial or harmful to patients with cancers because, as recently summarized, the levels of MnSOD in tumors can be higher or lower than that observed in the corresponding normal tissue (Dhar and St Clair, 2012). However, a variant MnSOD allele encoding an alanine (A) rather than a valine (V) at codon 16 has been described, and in several reports it has been associated with an elevated cancer risk, including the risk of prostate cancer, in human epidemiological studies (Li et al., 2005; Sutton et al., 2006; Kang et al., 2007; Mikhak et al., 2008). As a consequence of alanine being at this position in the mitochondrial import signal peptide, there is increased transport of MnSOD into the mitochondria as well as increased MnSOD mRNA stability (Sutton et al., 2005). While it might be counterintuitive that elevated levels of an antioxidant enzyme would increase risk, several studies have shed light on the likely explanation. Li et al. reported an impressive 10-fold swing in the risk of aggressive prostate cancer among men who expressed the AA genotype between the lowest quartile of total antioxidant consumption and the highest, with those consuming the lowest levels of dietary antioxidants being at the greatest risk (Li et al., 2005). Similarly, it was reported that there was a 3-fold increase risk of aggressive prostate cancer for AA men with low carotenoid status (P = 0.02, confidence interval: 1.37–7.02) (Mikhak et al., 2008). As originally proposed by Li et al., it is therefore possible that increased mitochondrial transport of MnSOD as a consequence of a codon 16 alanine is beneficial when antioxidant activity is high and the MnSOD dismutation product, H₂O₂, can be reduced to water (Li et al., 2005). A low antioxidant status, defined either by individual genetics and/or dietary intake, could facilitate the cycling of H₂O₂ to more ROS that are potentially mutagenic and therefore carcinogenic. Alternatively, peroxidase activity of the MnSOD protein may directly result in the oxidation of protein and DNA (Ansenger-Fricano et al., 2013). Thus, the same allelic variant may be beneficial under some circumstances and detrimental under others.

7. The interaction between MnSOD and GPx-1 in human cancers

While the results described above indicate an effect of the intake of dietary antioxidants on the ultimate carcinogenic consequences of MnSOD genotype, there are also data on the interaction between MnSOD and GPx-1 in cancer etiology. An interaction between MnSOD and GPx-1 in contributing to cancer risk was reported by Cox et al., who initially reported that there was no association between the at-risk leu allele of GPx-1 and breast cancer risk among participants of the Nurse’s Health Study (Cox et al., 2004). However, a follow-up study by the same authors indicated that there was indeed a significant risk for breast cancer among participants of the same cohort when the MnSOD genotypes were also considered; carriers of both the AA and leu/leu genotype were at increased risk of breast cancer with an odds ratio of 1.87 (95% confidence level: 1.09–3.19) (Cox et al., 2006). These human data indicate an interaction between MnSOD and GPx-1 in influencing cancer risk. GPx-1 may be a particularly important H₂O₂-detoxifying enzyme because of its cellular location in the mitochondria and cytoplasm, as well as the nucleus (Utsunomiya et al., 1991; Asayama et al., 1994; Li et al., 2000). Further support for this interaction comes from human data indicating that polymorphisms in the gene for the selenium transport protein selenoprotein P (SEPP1),
which result in less SEPP1 in the plasma and reduced levels of GPx-1, are associated with a significant risk of aggressive prostate cancer only in men also expressing the \textit{ala16 MnSOD} allele (Cooper et al., 2008).

A dramatic 10-fold differential was found in the risk of aggressive prostate cancer among individuals homozygous for the alanine-expressing \textit{MnSOD} allele when those with low intake of antioxidants were compared to those with the greatest intake (Li et al., 2005). Additionally, it was observed that those who express this allele are also at greater risk of cancer if they express the at-risk alleles of GPx-1 or SEPP1 (Cox et al., 2006; Cooper et al., 2008). Taken together, it is apparent that the frequency of these alleles in the human population is a significant factor. The \textit{ala16 MnSOD} allele is common, with approximately 25% of healthy participants of the Physician’s Health Study having that genotype (Li et al., 2005). Genotype frequencies for the MnSOD, GPx-1 (Cox et al., 2004), and SEPP1 (Steinbrecher et al., 2010) genes are presented below in the Table. A diagrammatic representation of the dietary and genetic factors that may participate in determining the risk of cancer due to the expression of the \textit{ala16 MnSOD} allele is presented below in the Figure.

8. Conclusion
A greater understanding of the role of MnSOD in cancer etiology has been achieved using in vitro, in vivo, and epidemiological approaches. Although some results are conflicting, these studies provide insight into how GPx-1 and MnSOD function to influence the physiology of normal and cancer cells. Several mechanisms have been proposed to explain the role of MnSOD in carcinogenesis with existing evidence indicating that these effects by which MnSOD impacts carcinogenesis can be influenced by GPx-1 activity. An interaction between these 2 enzymes is apparent where the mitochondrial resident protein MnSOD detoxifies superoxide to H$_2$O$_2$, which can be further reduced to water by GPx-1, which is also located in the Table. A diagrammatic representation of the dietary and genetic factors that may participate in determining the risk of cancer due to the expression of the \textit{ala16 MnSOD} allele is presented below in the Figure.

### Table. Frequencies of anticipated genetic modifiers of MnSOD-associated disease risk.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype frequencies</th>
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<tr>
<td>MnSOD</td>
<td>Val/Val Val/Ala Ala/Ala</td>
</tr>
<tr>
<td></td>
<td>24.9% 49.6% 25.5%</td>
</tr>
<tr>
<td>GPx-1</td>
<td>Pro/Pro Pro/Leu Leu/Leu</td>
</tr>
<tr>
<td></td>
<td>47.2% 40.4% 2.4%</td>
</tr>
<tr>
<td>SEPP1</td>
<td>Ala/Ala Ala/Thr Thr/Thr</td>
</tr>
<tr>
<td></td>
<td>55.1% 39.4% 5.5%</td>
</tr>
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</table>

**Figure.** Model for the interaction among endogenous and environmental sources of oxidative stress, individual genotype, and risk of cancer and other degenerative disease. Elevated expression and/ or activity of MnSOD results in enhanced dismutation of superoxide and an additional load of H$_2$O$_2$, which, if detoxified, will be beneficial. In contrast, reduced levels of GPx-1, as a result of polymorphisms in GPx-1 or SEPP1, or reduced selenium/antioxidant levels, will increase the levels of H$_2$O$_2$ and contribute to cancer and degenerative disease risk as well.
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in the mitochondria. Epidemiological studies examining the association between MnSOD and cancer support the interaction of the 2 genes, and specifically the impact of polymorphisms in MnSOD and GPx-1. Future studies should include the examination of the levels and genotypes of both proteins in order to grasp the importance of how altered redox may affect the physiology of both normal and cancer cells.

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References


