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Manganese superoxide dismutase: beyond life and death

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Abstract

Manganese superoxide dismutase (MnSOD) is a nuclear-encoded antioxidant enzyme that localizes to the mitochondria. Expression of MnSOD is essential for the survival of aerobic life. Transgenic mice expressing a luciferase reporter gene under the control of the human MnSOD promoter demonstrate that the level of MnSOD is reduced prior to the formation of cancer. Overexpression of MnSOD in transgenic mice reduces the incidences and multiplicity of papillomas in a DMBA/TPA skin carcinogenesis model. However, MnSOD deficiency does not lead to enhanced tumorigenicity of skin tissue similarly treated because MnSOD can modulate both the p53-mediated apoptosis and AP-1-mediated cell proliferation pathways. Apoptosis is associated with an increase in mitochondrial levels of p53 suggesting a link between MnSOD deficiency and mitochondrial-mediated apoptosis. Activation of p53 is preventable by application of a SOD mimetic (MnTE-2-PyP⁵⁺). Thus, p53 translocation to mitochondria and subsequent inactivation of MnSOD explain the observed mitochondrial dysfunction that leads to transcription-dependent mechanisms of p53-induced apoptosis. Administration of MnTE-2-PyP⁵⁺ following apoptosis but prior to proliferation leads to suppression of protein carbonyls and reduces the activity of AP-1 and the level of the proliferating cellular nuclear antigen, without reducing the activity of p53 or DNA fragmentation following TPA treatment. Remarkably, the incidence and multiplicity of skin tumors are drastically reduced in mice that receive MnTE-2-PyP⁵⁺ prior to cell proliferation. The results demonstrate the role of MnSOD beyond its essential role for survival and suggest a novel strategy for an antioxidant approach to cancer intervention.

Keywords

MnSOD; p53; Cancer; Chemotherapy

Introduction

The mitochondrion is a double-membraned organelle (Ernster and Schatz 1981; Frey and Mannella 2000) that is the major site of ATP synthesis in the cell because the five enzymes necessary for ATP production are embedded in the inner mitochondrial membrane (Hatefi 1985). The mitochondrion is also the site of other distinct cellular functions (Logan 2006; McBride et al. 2006), such as metabolism (Bowsher and Tobin 2001; Chen et al. 2006; Eaton et al. 1996; Leverve 2007; Manoli et al. 2007; Newsholme et al. 2007), cell cycle control and stress response (Manoli et al. 2007), intracellular signaling (Goldenthal and Marin-Garcia 2004; Owusu-Ansah et al. 2008; Ryan and Hoogenraad 2007), and apoptosis (Gulbins et al. 2003; Spierings et al. 2005).

Reactive oxygen species (ROS) are a consequence of the oxygen-rich atmosphere we live in and are by-products of oxygen metabolism (Fridovich 1978). ROS are important mediators for a variety of cellular processes, including cell adhesion, immune response, apoptosis (Droge 2002), cell growth and differentiation (Boonstra and Post 2004), and they act as second messengers in intracellular signaling (Forman et al. 2004; Rhee et al. 2003). Low levels of ROS are important for carrying out these cellular processes, but aberrant production of ROS occurs in the mechanism of numerous diseases, including various neurological disorders (Waris and Ahsan 2006) and cancer (Behrend et al. 2003; Gius and Spitz 2006; Storz 2005).

Mitochondria are a major source of ROS, and superoxide is the initial ROS produced by this organelle. Superoxide is produced primarily by complex I (Dlaskova et al. 2008; Genova et al. 2001; Verkaar et al. 2007) and complex III (Han et al. 2001, 2003) of the electron transport chain. Superoxide radicals participate in the formation of the reactive nitrogen species (RNS) peroxynitrite (Huie and Padmaja 1993), as well as attack iron-sulfur centers of key mitochondrial enzymes, releasing free iron cations that participate in the production of hydroxyl radicals through the Fenton reaction (Haber and Weiss 1932).

The cell is equipped with a variety of enzyme systems to detoxify ROS (Andreyev et al. 2005; Koehler et al. 2006). Hydrogen peroxide produced in mitochondria is decomposed to water by mitochondria-specific glutathione peroxidase (GPX) (Esworthy et al. 1997; Maiorino et al. 2003), as well as by peroxyredoxins (PRX) (Oberley et al. 2001) and catalase (Nohl and Jordan 1980). Superoxide dismutases (SODs) are the primary ROS detoxifying enzymes of the cell (reviewed in Fridovich 1989) that catalyze the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen (Klug and Rabini 1972). MnSOD is a member of a family of structurally unrelated SODs encoded by different genes (reviewed in Zelko et al. 2002). This family includes copper- and zinc-containing SOD (CuZnSOD), a homodimeric enzyme found primarily in the cytoplasm (Weisiger and Fridovich 1973; Zelko et al. 2002) and the nucleus (Crapo et al. 1992; Slot et al. 1986) with small amounts within mitochondria (Okado-Matsumoto and Fridovich 2001) and a glycosylated form of CuZnSOD called extracellular SOD (ECSOD) that resides in the extracellular region of the cell (Hjalmarsson et al. 1987). MnSOD is found exclusively in the mitochondrial matrix (Okado-Matsumoto and Fridovich 2001; Weisiger and Fridovich 1973) and exists as a homotetramer (Borgstahl et al. 1992; Ravindranath and Fridovich 1975; Wispe et al. 1989). MnSOD is an evolutionarily conserved enzyme found in a variety of organisms, including *Escherichia coli* B (Keele et al. 1970), the yeast strain *Saccharomyces cerevisiae* (Ravindranath and Fridovich 1975), the red alga *Porphyridium cruentum* (Misra and Fridovich 1977), and chicken liver mitochondria (Weisiger and Fridovich 1973).

MnSOD expression is altered in myriad diseases (reviewed by Miao and St. Clair 2009), especially cancer (Oberley and Buettner 1979). MnSOD acts as a tumor suppressor and, in many cancers, MnSOD expression is reduced. A better understanding of the mechanisms by which MnSOD suppresses cancer, and why MnSOD expression is decreased in myriad cancers, is important for the development of novel and improved anticancer therapies that maximize killing cancer and simultaneously decrease the detrimental side effects of cancer treatments on normal tissues. This review focuses on the role of MnSOD in protecting normal tissues from the toxicity associated with chemotherapeutic drugs, as well as the importance of MnSOD in tumor suppression and the potential for MnSOD to enhance cancer treatment.

MnSOD is essential for aerobic life

MnSOD is vital to protect aerobic life from the toxic effects of oxygen. Many studies of various model systems demonstrate the importance of MnSOD to aerobic organisms. *E. coli* B cells grown under 100% oxygen are much more resistant to hyperbaric concentrations of oxygen (20 atm) compared to *Bacillus subtilis* (*B. subtilis*). *E. coli* B cells are also more resistant to the antibiotic streptonigrin (a superoxide-generating antibiotic) than are cells grown under normal atmospheric conditions. The difference in oxygen toxicity between *E. coli* grown under 100% oxygen and other cells tested is due to the increased expression of MnSOD induced by growth under 100% oxygen (Gregory and Fridovich 1973). In the yeast strain *Saccharomyces cerevisiae* var. *ellipsoideus*, cells grown under 1 atm oxygen are more resistant to high oxygen concentrations than cells grown under anaerobic conditions due to oxygen-stimulated expression of MnSOD (Gregory et al. 1974).

Several mouse and *Drosophila* models also demonstrate the importance of MnSOD in aerobic life. Knock-out of MnSOD enzyme activity by the creation of inactive mutants or the complete elimination of MnSOD expression leads to early death in both mouse (Li et al. 1995) and *Drosophila* (Duttaroy et al. 2003). One mechanism of early death is reduced mitochondrial activity. While there are no gross changes in mitochondrial structure in homozygous MnSOD knock-out mice, activities are significantly reduced in both succinate dehydrogenase (complex II of the electron transport chain) and aconitase (citric acid cycle enzyme) compared to wild-type mice (Li et al. 1995). Similar reduction in aconitase enzyme activity is also seen in MnSOD knock-down *Drosophila* compared to revertant controls (Paul et al. 2007).

Van Remmen et al. (2003) used heterozygous MnSOD knock-out mice to study the effects of life-long reduction in MnSOD enzyme activity. The MnSOD knock-out mice have a ~50% reduction in MnSOD enzyme activity in all tissues, resulting in an age-dependent increase in oxidative DNA damage (8-oxodeoxyguanine, 8-oxodG) in both nuclear and mitochondrial DNA compared to wild-type mice. While life-span and various markers of aging such as cataract formation and immune response are not affected by knock-down of MnSOD, there is a 100% increase in cancer incidence in the MnSOD knock-down mice compared to wild-type mice (van Remmen et al. 2003).

Reduced or complete knock-out of MnSOD causes significant cardiovascular abnormalities that contribute to diminished life-span in these animals. Li et al. created a strain of mice that expresses an enzymatically inactive mutant of MnSOD. Homozygous MnSOD knock-outs exhibit severe cardiac defects, including dilated left ventricular cavity and reduced left ventricular wall thickness, as well as myocardial hypertrophy leading to dilated cardiomyopathy (Li et al. 1995). Lebovitz et al. (1996) generated a line of MnSOD knockout mice ($SOD2^{m1BCM}/SOD2^{m1BCM}$) that live up to 3 weeks after birth. The $SOD2^{m1BCM}/SOD2^{m1BCM}$ mice show hypocellular bone marrow with diminished levels of all hematopoietic lineages, resulting in severe anemia compared to control animals. Approximately 10% of the $SOD2^{m1BCM}/SOD2^{m1BCM}$ animals have balloon-like cardiac dilation and thinning of the ventricular wall associated with substantial myocardial cell injury and death (Lebovitz et al. 1996).

Like the effects of MnSOD expression on the heart, changes in MnSOD expression or activity can have dramatic effects on the brain. Transfection of MnSOD into cultured neuronal cells prevents cell death caused by treatment with β -amyloid and iron (Keller et al. 1998). Reduced expression of MnSOD by selective inactivation of the MnSOD gene sensitizes cultured mouse cortical neurons to glutamate-induced neurotoxicity (Li et al. 1998d). Overexpression of MnSOD provides dramatic protection against *N*-methyl-D-

aspartate (NMDA) and NO toxicity in a cortical culture (Gonzalez-Zulueta et al. 1998). Animals genetically altered to overexpress MnSOD show a significant decrease in lesion volume after traumatic brain injury compared with that of control littermates (Sullivan et al. 1999). Thus, MnSOD expression is important for neurotoxic conditions where overproduction of reactive oxygen or nitrogen species is involved. A greater understanding of these effects may shed light on the mechanisms of brain injury induced by various cancer chemotherapeutic agents or result from different neurological disorders, including ischemia/reperfusion of the brain caused by stroke (Kim et al. 2002), chronic intermittent hypoxia caused by sleep apnea (Shan et al. 2007), neurological dysfunction associated with traumatic brain injury (Bayir et al. 2007; Xiong et al. 2005), and HIV-associated dementia (Saha and Pahan 2007). Homozygous MnSOD knock-out mice have noticeable neurological damage, including death of brain stem and basal ganglia neurons marked by severe mitochondrial damage (Lebovitz et al. 1996). Lynn et al. (2005) generated MnSOD wild-type (*Sod2*^{+/+}) and knock-out (*Sod2*^{-/-}) B6D2F1 mice to better understand the effects of diminished MnSOD levels on neurological function. *Sod2*^{-/-} mice develop ataxia by postnatal day 11 (P11), which quickly progresses to frequent seizures by P14. The development of these neurological abnormalities correlates with neuronal damage to the motor cortex, the thalamus, and perceptible damage to different regions of the brain stem (motor trigeminal nucleus, mesencephalic trigeminal nucleus, the olivary nucleus, and the vestibular nucleus) at P11–13, and this damage is more extensive at P15–16. The formation of vacuoles occurs in the pyramidal layer of the hippocampus, deeper layers of the motor cortex, as well as different regions of the thalamus and brain stem in *Sod2*^{-/-} mice compared to *Sod2*^{+/+} mice (Lynn et al. 2005). *Drosophila* mutants with reduced MnSOD expression show vacuole formation in the brain and an increase in apoptotic neurons, leading to a decrease in normal olfactory behavior compared to wild-type flies (Paul et al. 2007).

The effects of MnSOD on specific regions of the brain have important implications in various neurological disorders. Marcus et al. (2006) studied the expression of MnSOD in the hippocampus of both Alzheimer's disease (AD) and non-AD patients and found a statistically significant increase in MnSOD expression in the C1, CA2/3 and C4 regions of the hippocampus of AD patients compared to non-AD patients, suggesting that neurons of the hippocampus attempt to compensate for the increase in ROS associated with AD progression with an increase in MnSOD expression. However, it is unclear whether the observed immunoreactive proteins are functionally active. Overexpression of MnSOD in the Tg19959 mouse transgenic model of AD leads to a significant decrease in amyloid plaque burden in the cortex, a non-significant reduction of plaques in the hippocampus and a decrease in cortical microglia, as well as improves spatial memory retention in T19959 mice. These effects occur in the absence of any effect of MnSOD on amyloid precursor protein (APP) expression or processing (Dumont et al. 2009). Heterozygous knock-out of MnSOD in T19959 mice causes a significant increase in oxidative damage to brain proteins and plaque burden in the cortex, hippocampus, and retrosplenial/motor cortex compared to controls (Li et al. 2004).

Work by this laboratory has focused on better understanding the mechanisms by which MnSOD affects the progression of AD. In SH-SY5Y human neuroblastoma cells, Sompol et al. (2006) found that treatment with TNF- α stimulates MnSOD expression through a NF- κ B-dependent mechanism, increasing the resistance of these cells to apoptosis induced by either β -amyloid protein or FeSO₄. In a study using primary neurons isolated from wild-type mice or mice that are APP and presenilin (PS1) knock-ins (APP/PS1), Sompol et al. (2008) found that developing APP/PS1 neurons are more resistant to β -amyloid 1–42 treatment than mature APP/PS1 neurons compared to wild-type neurons of the same age. Mature APP/PS1 neurons have diminished MnSOD expression and an increase in colocalization of MnSOD with nitrotyrosine, implying an inhibition of MnSOD enzyme activity by nitrotyrosine

modification (Sompol et al. 2008). Tyrosine nitration of MnSOD results in decreased enzyme activity (Demicheli et al. 2007; MacMillan-Crow et al. 1998; MacMillan-Crow and Thompson 1999), and an increase in nitrotyrosine-modified MnSOD is observed in APP/PS1 mice compared to wild-type controls. This correlates with a decrease in MnSOD enzyme activity and diminished mitochondrial activity (Anantharaman et al. 2006), suggesting that inactivation of MnSOD by tyrosine nitration is part of the mechanism of neuronal injury in AD. Overexpression of MnSOD protects the APP/PS1 neurons against β -amyloid-induced cell death (Sompol et al. 2008).

SOD mimetics have proven valuable in protecting various tissues from the deleterious effects of diminished MnSOD expression or enzyme activity in both cardiac and neuronal tissues. Pretreatment of rats with the SOD mimetic M40403 protects from ischemia/reperfusion-induced cardiac injury (Masini et al. 2002). The SOD mimetic MnTE-2-PyP⁵⁺ accumulates in a variety of tissues with different affinities, including the heart and brain (Spasojevic et al. 2008). The mitochondrial enzymes aconitase and isocitrate dehydrogenase are partially inactivated in the hearts of diabetic rats, and treatment with MnTE-2-PyP⁵⁺ rescues the activity of these enzymes to control levels (Batinic-Haberle and Benov 2008). MnTE-2-PyP⁵⁺ protects primary cultures of neurons isolated from the APP/PS1 model of AD from β -amyloid-induced cell death (Sompol et al. 2008). Intracerebroventricular administration of the SOD mimetic Mn^{III}TDE-2-ImP⁵⁺ protects rats from ischemia/reperfusion injury-associated neurological dysfunction caused by middle cerebral artery occlusion (Sheng et al. 2009). Treatment of MnSOD-null mice with the SOD mimetic manganese 5, 10, 15, 20-tetrakis (4-benzoic acid) porphyrin (MnTBAP) increases the life-span of these animals compared to untreated controls and abrogates the dilated cardiomyopathy associated with MnSOD knock-out. Interestingly, MnTBAP-treated MnSOD-null mice show significant neurological damage characterized by vacuolization of the cortex, brain stem, and cerebellum due to a lack of MnTBAP accumulation in the brain mediated by the blood-brain barrier (BBB) (Melov et al. 1998). Improvements in SOD mimetic design that results in more specific tissue accrual, especially compounds that can pass the BBB and accumulate in the brain, may prove valuable for the treatment of various disorders associated with reduced MnSOD expression or enzyme activity or may protect normal tissues from cancer chemotherapy-induced injury.

MnSOD in the protection of normal tissues from chemotherapy

Various chemotherapeutic drugs have been developed to treat cancer. While these anti-cancer drugs are effective in killing cancer cells, they also damage normal tissue through induction of oxidative stress (reviewed by Chen et al. 2007). Anthracyclines are among the most effective classes of anti-cancer drugs due to their ability to kill a broad spectrum of cancers (reviewed by Weiss 1992). Adriamycin (also known as doxorubicin) is a member of the anthracycline family of chemotherapeutic drugs currently used in cancer clinics. The major mechanisms by which adriamycin kills cancer cells are intercalation of DNA and inhibition of topoisomerase II activity (reviewed in Minotti et al. 2004). Adriamycin generates ROS (Doroshov 1983) through redox cycling of the quinone moiety on ring C of adriamycin (Marcillat et al. 1989), leading to increased iron uptake (Kotamraju et al. 2002; Minotti et al. 1998, 2001) and release of iron from internal stores (Minotti et al. 2004), causing further formation of ROS through iron-catalyzed reactions (Halliwell 1978). ROS production by adriamycin not only damages cancerous tissue but also causes significant toxicity in normal tissues (Brown et al. 1998; Giri et al. 2004; Singal et al. 1997). Work by this and other laboratories has focused on determining the biochemical mechanisms by which adriamycin induces both cardiotoxicity and neurotoxicity and on developing strategies to ameliorate the deleterious effects of adriamycin on normal tissue.

MnSOD and cardiotoxicity

An important side effect of chemotherapeutic drugs in cancer patients, especially adriamycin and other anthracyclines, is dose-dependent cardiotoxicity (reviewed in Simbre et al. 2005), leading to dilated cardiomyopathy and congestive heart failure (reviewed by Minotti et al. 2004). Acute effects of anthracycline treatment include transient arrhythmias and acute left ventricular failure. Risk factors for anthracycline-induced cardiotoxicity include total cumulative dose, rate of administration, and age (with younger patients being more susceptible to cardiotoxicity) (reviewed in Simbre et al. 2005).

Because the cardiotoxicity of adriamycin is mediated by production of excessive ROS, modulation of the ROS-scavenging capacity of cells can affect adriamycin-induced cardiac injury (Kang et al. 2002; Shioji et al. 2002; Sun et al. 2001). This laboratory created a series of transgenic mice expressing low, medium, or high levels of MnSOD protein. Ultrastructural analysis of heart tissue of non-transgenic mice revealed extensive, dose-dependent damage in adriamycin-treated animals, including mitochondrial damage (loss of cristae, mitochondrial swelling, and abnormal shape), as well as myofilament disarray and the presence of intracytoplasmic vacuoles. Serum markers of cardiac injury (creatinine kinase and lactate dehydrogenase) are elevated in adriamycin-treated mice. Adriamycin-induced heart damage is not present in the hearts of mice expressing low, medium, or high levels of MnSOD, suggesting MnSOD protects these mice from the detrimental effects of adriamycin (Yen et al. 1996).

The mitochondrion is a major target of adriamycin in cardiac tissue (Sarvazyan 1996). Adriamycin treatment results in statistically significant decreases in respiratory control ratio and state 3 mitochondrial respiration at both complexes I and II in non-transgenic mice. In MnSOD-overexpressing mice, only complex II activity is affected by adriamycin treatment, suggesting MnSOD protects complex I from adriamycin-induced, superoxide-mediated deactivation (Yen et al. 1999). In B6C3 mice, adriamycin causes mitochondrial injury within 3 h, with further increases at 6 and 24 h, compared to control animals. Adriamycin-induced oxidative damage occurs earlier (within 3 h) than nitrative damage (within 6 h), is more extensive in mouse heart mitochondria than in either cytoplasmic or nuclear compartments, and corresponds with ultrastructural mitochondrial damage (Chaiswing et al. 2004). Oxidative damage of mitochondrial DNA induced by adriamycin initially increases by 3 h after treatment, but diminishes by 6 h post-treatment, indicating activation of a DNA repair pathway in mitochondria. Adriamycin stimulates p53 translocation to both mitochondria and the nucleus in cardiomyocytes. p53 localization to mitochondria correlates with mitochondrial DNA damage, and p53 knock-out mice are more susceptible to adriamycin-induced mitochondrial DNA damage than controls, suggesting p53 translocation to mitochondria is a survival mechanism cardiomyocytes activate in an attempt to protect cells from adriamycin-mediated injury (Nithipongvanitch et al. 2007).

To further elucidate the role of ROS and RNS in the mechanisms of adriamycin-mediated cardiotoxicity, B6C3 transgenic mice overexpressing MnSOD (TgM^{+/+}), inducible nitric oxide synthase knock-outs (iNOSKO), or crosses (TgM + iNOSKO) were used. Compared to adriamycin-treated non-transgenic mice, TgM^{+/+} mice have decreased adriamycin-induced mitochondrial injury, while iNOSKO mice have increased mitochondrial injury, and TgM + iNOSKO mice have a statistically significant decrease in mitochondrial injury. The results suggest that formation of both ROS and RNS is involved in the early events of adriamycin-induced mitochondrial injury in mouse heart. MnSOD overexpression protects mitochondria by scavenging superoxide radicals and iNOS protects mitochondria by generating nitric oxide, which may also contribute to scavenging of superoxide radicals. The balance between ROS and RNS also contributes to adriamycin-mediated mitochondrial

injury, where the ratio of 4HNE to 3NT adduct formation (markers of ROS and RNS, respectively) inversely correlates with mitochondrial damage (Chaiswing et al. 2005).

The cardioprotective roles of nitric oxide and MnSOD are further illuminated in C57BL/6 iNOS knock-out mice (iNOS^{-/-}). Cardiac tissue from adriamycin-treated iNOS^{-/-} mice has much more mitochondrial damage and reduced cardiac function than non-transgenic controls, resulting in decreased left ventricular developed pressure (LVDP), diminished effects of isoproterenol on cardiac function and increased levels of serum creatine phosphokinase (CPK), lactate dehydrogenase (LDH), and cardiac troponin (cTnI) (markers of cardiac damage). Overexpression of MnSOD in iNOS-deficient mice blocks the effects of adriamycin, restoring cardiac function and attenuating the levels of LDH and cTnI in these animals (Cole et al. 2006).

MnSOD also enhances the cardioprotective effects of other drugs. Tamoxifen (TAM) is an artificial non-steroidal anti-estrogen used in the treatment of breast cancer (Ward 1973) that has been shown to have cardioprotective effects (Costantino et al. 1997; Love et al. 1991; Nordenskjold et al. 2005). In the VA-13 human fibroblast cell line, a combination of TAM and TNF- α enhances TNF- α -induced expression of MnSOD by modulating NF- κ B dimer binding to the MnSOD promoter, enhancing p50/p65 heterodimer binding over p50/p50 homodimer binding (Daosukho et al. 2002). Treatment with TAM increases MnSOD expression and enzyme activity in isolated cardiomyocytes and cardiac tissue of non-transgenic C57BL/6 mice, and TAM pretreatment results in reduced apoptosis in cardiomyocytes after adriamycin treatment compared to animals not receiving pretreatment (Daosukho et al. 2005). Sodium 4-phenylbutyrate (PBA), a potential anticancer drug (Carducci et al. 2001; Wang et al. 2008) with histone deacetylase activity (Lea et al. 1999), protects tissue from oxidative stress (Qi et al. 2004; Ryu et al. 2005; Vilatoba et al. 2005). A study by this laboratory has demonstrated the protective effects of PBA against adriamycin-induced cardiac injury. PBA completely abrogates the adriamycin-mediated reduction in ejection fraction and fraction shortening in mouse hearts, reduces mitochondrial and total ultrastructural cardiomyocyte damage caused by adriamycin, and diminishes adriamycin-mediated increases in serum markers of cardiac injury. PBA exerts its protective effects, in part, through induction of MnSOD protein and enzyme activity (Daosukho et al. 2007).

MnSOD mimetics have also been shown to affect adriamycin-induced cardiotoxicity. Work by this laboratory has focused on the Mn(III) *meso*-tetrakis (*N*-ethylpyridinium-2-yl) porphyrin (MnTE-2-PyP⁵⁺). In C57BL/6 mice, a single 10 mg/kg intraperitoneal dose of MnTE-2-PyP⁵⁺ results in accumulation of the porphyrin to mouse heart mitochondria at a concentration of 5.1 μ M, a concentration sufficient to protect mitochondria from oxidative damage (Spasojevic et al. 2007). Jaramillo et al. (2009) found that MnTE-2-PyP⁵⁺ protects H9c2 rat embryonic cardiomyocytes from adriamycin-induced toxicity. MnTBAP abrogates adriamycin-induced structural damage to cardiomyocytes and lactate dehydrogenase release (Konorev et al. 1999).

MnSOD and neurotoxicity

The cognitive decline in some cancer patients receiving chemotherapy, sometimes referred as chemobrain, is a potential side effect of chemotherapeutic drugs (Nelson et al. 2007; Wefel et al. 2004). Chemobrain is characterized by memory loss, as well as by decreased reaction time, attention, and concentration (reviewed in Ahles and Saykin 2007; Tannock et al. 2004). Adriamycin causes gross changes to brain structure (Brown et al. 1998; Inagaki et al. 2007) and in the activity of various brain regions (Silverman et al. 2007). Potential mechanisms of chemotherapy-induced cognitive decline include DNA damage, cytokine dysregulation, and changes in blood-brain barrier integrity (reviewed in Ahles and Saykin

2007). Cognitive dysfunction is associated with various types of chemotherapy, including adriamycin (Ahles et al. 2002), and induction of oxidative stress may be an important mechanism (Joshi et al. 2005; Tangpong et al. 2007).

The mechanisms by which this neurotoxicity occurs are only now beginning to be understood. Oxidative modification of proteins in the brains of adriamycin-treated mice has been observed, including increased protein carbonyls and 4HNE and 3NT protein adduct formation, as well as increased expression of protein-1 (MRP-1), which is associated with multidrug resistance (Joshi et al. 2005). Adriamycin treatment also decreases mitochondrial function in the brain tissue of these animals (Tangpong et al. 2006, 2007).

This laboratory was the first to report a direct biochemical mechanism by which adriamycin treatment mediates neurotoxicity. Adriamycin does not cross the blood–brain barrier due to the presence of ATP-dependent transporters (Ohnishi et al. 1995). Adriamycin was found in areas of the mouse brain outside the blood–brain barrier. Adriamycin treatment in mice results in increased serum levels of TNF- α , increased TNF- α levels in whole brain tissue homogenates, and increased TNF- α staining in the hippocampus and cortex of mouse brain. Adriamycin treatment results in significant reduction of state III respiration through decreased activity of complex I in the electron transport chain. Adriamycin also increases mitochondria-mediated apoptosis by stimulating mitochondrial localization of p53 and formation of p53/Bcl-xL complexes, as well as increased expression of the proapoptotic protein Bax, a p53 target gene. All the effects of adriamycin on mouse brain neurons are completely abrogated by co-treatment with an anti-TNF- α antibody, suggesting that the neurotoxic effects of adriamycin are mediated by increased circulating levels of TNF- α (Tangpong et al. 2006).

Modulation of the ROS detoxifying enzymes may be another mechanism of adriamycin-induced neurotoxicity. MnSOD is nitrated in mouse brain after adriamycin treatment, which correlates with decreased MnSOD enzyme activity. The effects of adriamycin on mitochondrial function and MnSOD nitration and enzyme activity were not observed in iNOS knock-out mice, suggesting a role for iNOS in neurotoxicity caused by adriamycin and the importance of MnSOD enzyme activity in preventing adriamycin-induced brain injury (Tangpong et al. 2007).

The role of MnSOD in tumor suppression

Some studies have shown that MnSOD expression is elevated in cancer cells compared to normal tissue, including gastric and esophageal (Izutani et al. 1998; Janssen et al. 2000; Malafa et al. 2000), lung (Ho et al. 2001), and colorectal cancer (Toh et al. 2000). For example, Hu et al. (2007) found increased MnSOD expression in 12 of 18 tumor samples compared to non-tumor tissue in patients with esophageal squamous cell carcinoma (ESCC). In a study of tissue sections of breast carcinomas, 78% of samples show elevated expression of MnSOD which correlates with the grade of the tumor (Tsanou et al. 2004). Higher expression of MnSOD in cancer correlates with aggressiveness (Toh et al. 2000), metastatic potential (Malafa et al. 2000), and poor prognosis (Janssen et al. 2000).

While MnSOD expression is elevated in some cancer types, a preponderance of evidence suggests a tumor suppressor role for MnSOD. One line of evidence suggesting the tumor suppressive properties of MnSOD is decreased expression of this important antioxidant enzyme in many cancers (Oberley and Buettner 1979). In a study of various invasive, in situ, and benign hyperplastic breast lesions, Soini et al. (2001) found that in situ carcinoma and benign hyperplasias, as well as non-neoplastic ductal and acinar epithelial cells, show greater MnSOD staining than invasive carcinoma. Chuang et al. (2007) found an inverse correlation between MnSOD expression and expression of the HER2/neu oncogene in

various human breast cancer cell lines. Cullen et al. (2003) found a decrease in MnSOD protein expression and enzyme activity in various pancreatic cancer cell lines (BxPC-3, AsPC-1, and MIA PaCa-2) compared to normal pancreas tissue and an inverse correlation between MnSOD expression and cell doubling time. Hu et al. found that malignant ovarian tissues adaptively respond to increased ROS by increasing expression of MnSOD, but that benign tumors and normal ovarian epithelial cells do not. Knock-down of MnSOD expression in SKOV3 human ovarian cancer cells using MnSOD-specific siRNA results in a 70% increase in superoxide production, leading to increased colony formation compared to cells transfected with empty vector in vitro and increased tumor growth in vivo (Hu et al. 2005).

Overexpression studies have further refined the role of MnSOD as a tumor suppressor. MnSOD overexpression alters many properties typical of cancer cells (growth rate, invasiveness, anchorage-independent growth, etc.) both in vitro and in vivo. UACC-903 melanoma cells overexpressing sense MnSOD cDNA have a more differentiated morphology compared to parental cells, while cells expressing the anti-sense MnSOD cDNA have a morphology similar to parental cells and grow more quickly, suggesting MnSOD overexpression suppresses tumorigenicity of these cells. Eighteen of 18 injection sites show tumor formation in mice injected with the parental cell line, while 0 of 16 sites have tumors in animals injected with MnSOD-overexpressing cells (Church et al. 1993). Overexpression of MnSOD in the MDA-MB-435 breast cancer cell line (which expresses the HER2/neu oncogene) reduces anchorage-independent cell growth and decreases chemotaxis toward conditioned medium (Chuang et al. 2007). Simultaneous expression of MnSOD and TRAIL in SW620 human colorectal tumor xenografts in BALB/c nude mice leads to complete elimination of the xenografts through induction of the mitochondrial pathway of apoptosis (Zhang et al. 2006a). Stable overexpression of MnSOD in HCT116 human colorectal carcinoma cells leads to a dose-dependent decrease in cell growth by inducing senescence in a p53-dependent manner through depolarization of the mitochondrial membrane (Behrend et al. 2005). Adenoviral vector transfection of MnSOD (*AdMnSOD*) into MIA PaCa-2 pancreatic cells results in an expression level-dependent increase in doubling time, decreased plating efficiency, and decreased colony formation in soft agar compared to cells transfected with *AdLacZ* virus or untransfected controls. Injection of *AdMnSOD* into established MIA PaCa-2 tumors in nude mice results in decreased tumor growth compared to tumors injected with serum-free medium or control virus *AdbgIII*, and expression of MnSOD in these tumors increases survival time of the animals (Weydert et al. 2003a). Similar effects of MnSOD overexpression on growth rate, plating efficiency, and anchorage-independent cell growth have been observed in SCC-25 human oral squamous carcinoma cells (Liu et al. 1997), HCPC-1 hamster cheek pouch carcinoma cells (Lam et al. 1997), and PC-3 human prostate carcinoma cells (Venkataraman et al. 2005).

Studies in this laboratory have refined the role of MnSOD in oxidative stress-dependent tumor initiation and promotion. Overexpression of MnSOD in the Fsa-II mouse fibrosarcoma cell line causes decreased cell proliferation and colony formation and induces a more differentiated cell type compared to control cells (Kiningham and St. Clair 1997). MnSOD overexpression also protects fibrosarcoma cells from 5-azacytidine (5-AzaC)-induced apoptosis while simultaneously enhancing the effects of 5-AzaC on differentiation (Zhao et al. 2001a). Using a DMBA [7,12-dimethylbenz(a)-anthracene]/TPA (12-*O*-tetradecanoylphorbol-13-acetate) model of tumor initiation/promotion, a single treatment of C57BL/6 mice with DMBA, followed by multiple applications of TPA, results in the formation of papillomas in both non-transgenic and MnSOD-overexpressing mice. The incidence and number of papillomas per animal are lower in the transgenic animals compared to non-transgenic animals, indicating MnSOD reduces the tumorigenicity of DMBA/TPA in these animals. TPA treatment causes increased 4-hydroxynonenal-adducted

proteins (4HNE, a marker of oxidative stress) in both the nucleus and mitochondria of non-transgenic and transgenic animals, but the level of 4HNE-modified proteins is higher in non-transgenic animals compared to animals overexpressing MnSOD, suggesting MnSOD protects these animals from TPA-induced oxidative stress (Zhao et al. 2001b).

Because overexpression of MnSOD results in decreased tumor formation in mice, knock-down of MnSOD was predicted to increase tumor formation. Surprisingly, MnSOD heterozygous knock-out C57BL/6 mice treated with DMBA/TPA have a similar number of papillomas as wild-type controls. The similarity in papilloma formation in the MnSOD knock-out mice and wild-type controls is due to increased proliferation and apoptosis of cells in the basal layer of the epidermis after TPA treatment. Oxidative stress is also increased in the MnSOD knock-out animals, indicated by the increase in oxidatively modified proteins (Zhao et al. 2002). Further studies to refine the mechanisms of TPA-induced papilloma formation in MnSOD knock-down mice reveal that apoptosis precedes proliferation in the basal layer of the epidermis, peaking at 6 h posttreatment, with mitosis peaking 24 h after treatment. Proliferation is greater in the knock-out animals compared to wild-type controls. Application of a MnSOD mimetic (MnTE-2-PyP⁵⁺) 12 h after each TPA treatment does not affect TPA-induced apoptosis, but significantly reduces proliferation stimulated by TPA and reduced formation of protein carbonyls. This reduction in proliferation by the MnSOD mimetic results in a 50% reduction in tumor incidence compared to animals receiving the DMBA/TPA regimen alone, suggesting an important role for oxidative stress in tumorigenesis and a potential mechanism by which MnSOD suppresses cancer development (Zhao et al. 2005a).

Mechanisms of MnSOD-mediated tumor suppression

MnSOD influences transcription factor activity

One mechanism by which MnSOD may suppress cancer growth is by altering the expression or activity of tumor suppressors and/or promoters, thereby affecting cancer cell growth. Overexpression of MnSOD in MCF-7 breast cancer cells results in increased expression of maspin (mammary serine protease inhibitor) mRNA, an important tumor suppressor, and causes a decrease in the invasiveness of the cells compared to untransfected and vector control cells (Li et al. 1998a). Increased maspin expression is due, in part, to stabilization of maspin mRNA induced by increased MnSOD expression levels (Duan et al. 2003). Overexpression of MnSOD in MDA-MB-435 human breast cancer cells, which express high levels of the HER2/neu oncogene, results in decreased expression of the HER2/neu oncogene (Chuang et al. 2007).

Redox modification of amino acids is an important mechanism for regulating the activity of a variety of transcription factors (Gabbita et al. 2000; Oktyabrsky and Smirnova 2007), including NF- κ B (Pineda-Molina et al. 2001), AP-1 (Klatt et al. 1999), HIF-1 α (Chandel et al. 1998), and p53 (Buzek et al. 2002; Cobbs et al. 2001; Furukawa et al. 2007; Rainwater et al. 1995). Changes in gene expression induced by MnSOD are due, in part, to modulation of transcription factor activity. MnSOD overexpression in MCF-7 human breast cancer cells causes biphasic suppression of HIF-1 α . At low levels of MnSOD expression, HIF-1 α protein levels are elevated in response to hypoxia, while moderate levels of MnSOD attenuate hypoxia-induced accumulation of HIF-1 α , and high levels of MnSOD again increase protein levels of HIF-1 α . Overexpression of MnSOD abrogates HIF-1 α -dependent expression and secretion of vascular endothelial growth factor (VEGF) in response to hypoxia in a biphasic manner that parallels MnSOD-dependent regulation of HIF-1 α (Wang et al. 2005).

Changes in MnSOD levels in the cell also affect the transcriptional activity of AP-1, dramatically affecting cell proliferation. In MCF-7 breast cancer cells, MnSOD

overexpression causes reduced transcriptional activity of AP-1, resulting in decreased expression of AP-1-responsive collagenase I and stromelysin (Li et al. 1998b). Similar results have been observed in this laboratory. Overexpression of MnSOD in the Fsa-II mouse fibrosarcoma cell line attenuates AP-1 DNA-binding and transcriptional activity (Kiningham and St. Clair 1997). The effects of MnSOD on AP-1 activity are mediated through altered expression of members of the Jun family of AP-1 subunits, especially JunD (Kiningham and St. Clair 1997; Zhao et al. 2001a, b, 2002). Treatment of C57BL/6 mice with a single dose of DMBA, followed by multiple treatments with TPA, results in activation of both AP-1 and p53, leading to increased proliferation and apoptosis in the basal layer of the epidermis. AP-1 activation is much greater in MnSOD knock-out animals compared to wild-type animals. AP-1 DNA-binding activity is much greater in knock-out mice treated with TPA compared to controls due to increased expression of the JunD AP-1 subunit (Zhao et al. 2002). Treatment with the MnSOD mimetic MnTE-2-PyP⁵⁺ following TPA treatment abrogates TPA-induced activation of AP-1, demonstrating the importance of MnSOD in modulating the transcriptional activity of AP-1 and suggesting a mechanism of MnSOD-mediated reduction in cell proliferation (Zhao et al. 2005a).

Another transcription factor affected by MnSOD is NF- κ B. Overexpression of MnSOD in MCF-7 breast cancer cells results in diminished transcriptional activity of NF- κ B and decreased expression of interleukin IL-1 and IL-6 (two NF- κ B responsive genes) (Li et al. 1998b). MnSOD overexpression in Fsa-II mouse fibrosarcoma cells enhances the effects of 5-AzaC in differentiation of these cells through increased NF- κ B DNA-binding activity (Zhao et al. 2001a).

Additionally, MnSOD modulates the activity of the transcription factor p53. MnSOD overexpression in the HCT116 human colorectal cancer cell line induces cellular senescence mediated by MnSOD-induced up-regulation of p53 through a p21-independent mechanism (Behrend et al. 2005). Studies in this laboratory have revealed that treatment with DMBA/TPA activates both AP-1 and p53 in a C57BL/6 mouse model of skin cancer promotion, consistent with both increased proliferation and apoptosis in this model. DMBA/TPA treatment also leads to increased mitochondrial localization of p53. Interestingly, activation of p53 by DMBA/TPA is greater in MnSOD heterozygous knock-out mice, suggesting a role for MnSOD in the regulation of p53 transcriptional activity and subcellular localization (Zhao et al. 2002). Later studies have revealed that apoptosis precedes proliferation when mice are treated with TPA. Treatment of mice with the MnSOD mimetic MnTE-2-PyP⁵⁺ 12 h after TPA treatment causes a decrease in AP-1 activity without affecting p53-mediated apoptosis, resulting in reduced tumorigenesis (Zhao et al. 2005a).

In JB6 mouse epithelial cells, treatment with TPA results in rapid (within 10 min) translocation of p53 to mitochondria and, by 1 h, p53 is found in both the nucleus and mitochondria. p53 interacts directly with MnSOD and decreases MnSOD enzyme activity, and p53 mitochondrial translocation correlates with reduced mitochondrial membrane potential. Interestingly, p53 subcellular localization is affected by the MnSOD mimetic MnTE-2-PyP⁵⁺. Treatment of JB6 cells with TPA, followed by MnTE-2-PyP⁵⁺, has no effect on p53 mitochondrial localization, but completely blocks p53 nuclear translocation and TPA-induced expression of the p53 pro-apoptotic gene Bax, suggesting that MnSOD can modulate p53 activity and that p53-induced inactivation of MnSOD enzyme activity mediates p53 transcription-dependent induction of apoptosis (Zhao et al. 2005b). Treatment of JB6 cells with mitochondrial permeability transition pore blockers cyclosporine A or bongkrekic acid prevents TPA-induced p53 mitochondrial translocation, suggesting p53 translocation to mitochondria may involve TPA-induced activation of mitochondrial permeability transition pore (Liu et al. 2008).

MnSOD affects DNA stability

Chromosomal instability (CIN) is an important hallmark of cancer (Chandhok and Pellman 2009; Ganem et al. 2007), and MnSOD has been shown to affect CIN in vivo. Increased expression of MnSOD in Lck-Bax38/1 mice has no effect on cell proliferation or apoptosis of thymocytes either in the presence or in the absence of Bax. However, MnSOD expression impedes aneuploidy in these mice. Higher expression levels of MnSOD in the MnSOD-overexpressing mice also lead to a significant decrease in the formation of spontaneous lymphomas (van de Wetering et al. 2008).

MnSOD alters ROS levels in cancer cells

ROS are an important mediator of cancer development (Behrend et al. 2003; Gius and Spitz 2006; Storz 2005), and modulation of ROS levels by MnSOD is another important mechanism of MnSOD-mediated tumor suppression (Oberley 2005; Ridnour et al. 2004; Zhang et al. 2006b). MnSOD-overexpressing PC-3 prostate carcinoma cells have much higher levels of H₂O₂ and mitochondrial membrane potential than parental cells, resulting in reduced growth rate both in vitro and in vivo (Venkataraman et al. 2005). Transfection of DU145 human prostate carcinoma cells with MnSOD cDNA results in increased ROS production as determined by increased dichlorofluorescein fluorescence compared to control cells. MnSOD overexpression results in greater sensitivity to ROS-generating agents and causes decreased growth in both in vitro in cell culture and in vivo in tumors established in nude mice (Li et al. 1998c). Overexpression of an active site mutant of MnSOD (H30N) lacking product inhibition in HEK293 human embryonic kidney cells results in ROS-dependent retardation of cell growth. Co-expression of these cells with catalase abrogates the effects of the H30N mutant on cell growth, indicating H₂O₂ is the mediator of the growth suppressive effects of the H30N mutant MnSOD (Davis et al. 2004). Ridnour et al. found that overexpression of MnSOD in the XR23M transformed X-ray immortalized rat embryo fibroblast cell line results in decreased colony formation in vitro and decreased tumor growth and lung metastases in vivo. This decrease in metastatic potential correlates with the ability of the cells to remove hydrogen peroxide. Metastatic potential decreases linearly with increased MnSOD activity, increased MnSOD:total glutathione peroxidase and increased MnSOD:catalase ratios, demonstrating that hydrogen peroxide production by MnSOD is important for its tumor suppressing properties (Ridnour et al. 2004). Stable transfection of catalase or glutathione peroxidase in MCF-7 human breast cancer cells impedes MnSOD-dependent accumulation of HIF-1 α protein in response to hypoxia at high levels of MnSOD enzyme activity (Wang et al. 2005).

Several studies have demonstrated that the enzymatic activity of MnSOD is important to its anti-tumor effects. Using site-directed mutagenesis, Zhang et al. created a mutant MnSOD that lacks enzymatic activity by mutating histidine 26 to leucine within the active site of MnSOD. Overexpression of wild-type MnSOD results in decreased cell growth and plating efficiency and increased doubling time, while overexpression of mutant MnSOD leads to cell growth, plating efficiency, and doubling time similar to control, confirming the importance of the effects of MnSOD enzyme activity on tumor suppression (Zhang et al. 2006b). MnSOD enzyme activity is a necessary condition for MnSOD-dependent abrogation of HER2/neu expression in MDA-MB-435 human breast cancer cells stably overexpressing various levels of MnSOD protein and enzyme activity. The characteristics of cell lines expressing high levels of MnSOD protein and low MnSOD enzyme activity are close to those of the parental cells, while cell lines with high MnSOD enzyme activity have greater reduction in HER2/neu and decreased anchorage-independent cell growth than parental cells (Chuang et al. 2007). Davis and collaborators generated an active site mutant of MnSOD by replacing histidine-30 with asparagine, resulting in reduced product inhibition and increased enzymatic efficiency. Overexpression of the H30N mutant in the human embryonic kidney

cell line HEK293 results in significant reduction in cell growth compared to cells overexpressing a Q143A mutant which has greatly reduced enzyme activity (Davis et al. 2004). The anti-tumor effect of MnSOD is due, in part, to increased production of hydrogen peroxide, and overexpression of glutathione peroxidase (GPX) in P U118-9 human glioma cells already overexpressing MnSOD blocks the growth suppression effects of MnSOD both in vitro and in vivo (Li et al. 2000).

Mechanisms of altered MnSOD expression in cancer

Mutations in the MnSOD promoter

Much work has been performed in this laboratory to elucidate the mechanisms by which MnSOD gene expression is regulated. The MnSOD promoter lacks both TATA and CAAT boxes, but it does contain a GC-rich region containing clusters of Sp1 and AP-2 binding sites (Wan et al. 1994). Sp1 has been identified as an important transcriptional activator of both basal and TPA-induced expression of MnSOD (Porntadavity et al. 2001; Xu et al. 2002) while AP-2 is a potent repressor of expression (Xu et al. 2002). The tumor suppressor p53 interacts with Sp1 to repress MnSOD expression (Dhar et al. 2006). An NF- κ B response element was later identified within an enhancer in the second intron of the MnSOD gene (Xu et al. 1999a), conferring NF- κ B responsiveness to MnSOD gene expression to cytokines and the phorbol ester PMA (phorbol 12-myristate 13-acetate) (Kiningham et al. 2001, 2004; Xu et al. 1999a). Nucleophosmin (NPM) is an important cofactor for NF- κ B responsiveness of MnSOD expression (Dhar et al. 2004).

This laboratory has identified three mutations in the proximal (GC-rich) region of the *MnSOD* promoter: a C \rightarrow T transition at -102 (M1), an insertion of A in the 11-straight G (11G) region at -93(M2), and a C \rightarrow G transversion at -38 (M3) in several human colorectal carcinoma (HT29, T84, and LS174T), human glioblastoma (U87), and human fibrosarcoma (HT1080) cell lines. The mutated promoters have at least a 50% reduction in transcriptional activity compared to the normal *MnSOD* promoter using a luciferase reporter assay (Xu et al. 1999b). The M3 site creates a binding site for both Sp1 and AP-2; the presence of all three mutations augments the effects of both Sp1 and AP-2; and the expression of MnSOD is dependent on the relative levels of Sp1 and AP-2 in cells. AP-2 interacts with nucleophosmin to disrupt the nucleophosmin-mediated interaction between the promoter and transcriptional enhancer in the second intron, resulting in reduced MnSOD expression (Xu et al. 2008).

The 11G region in the MnSOD promoter forms a single-stranded loop that is necessary for promoter function. Nucleophosmin binds to this loop structure and acts as a bridge between Sp1 at the promoter and NF- κ B at the second intronic enhancer to facilitate NF- κ B-dependent, cytokine-induced expression of MnSOD (Xu et al. 2007). The mutation within this 11G loop region found in cancer (Xu et al. 1999a, b) disrupts the interaction of nucleophosmin with the 11G loop, resulting in decreased cytokine responsiveness of MnSOD expression (Xu et al. 2007).

Epigenetic regulation of MnSOD

Epigenetics, defined as heritable and transient changes in gene expression that do not involve a change in the DNA sequence of genes, has proven to be very important in the development of various diseases, including developmental disorders, autoimmune diseases, and cancer (reviewed in Hirst and Marra 2009; Sigalotti et al. 2007). Epigenetic regulation of gene expression includes methylation of cytosine residues in CpG dinucleotides within the DNA sequence and modifications of the N-terminal tails of core histones within the nucleosome (phosphorylation, methylation, acetylation, ubiquitination, etc.) (Wang et al. 2007b; Zheng et al. 2008) and remodeling of the chromatin structure (Wang et al. 2007a).

Epigenetic regulation of oncogenes and tumor suppressor genes has been shown to be important for the development and progression of cancer (Sigalotti et al. 2007; Zheng et al. 2008).

MnSOD expression is also regulated through epigenetic mechanisms, and this regulation may play an important role in cancer development (reviewed by Miao and St. Clair 2009). Cytosine hypermethylation within intron 2 is much greater in SV40 transformed human lung fibroblasts WI38 and MRC5 (WI38-VA and MRC5-VA) compared to their normal counterparts. This cytosine hypermethylation correlates with decreased MnSOD expression in these transformed cells (Huang et al. 1999). An NF- κ B enhancer has been identified within intron 2 of the MnSOD gene (Jones et al. 1997; Xu et al. 1999a), and epigenetic regulation of MnSOD at intron 2 may prove important in cancer development and progression (Huang et al. 1999). DNA hypermethylation has been identified in the promoter region of the MnSOD gene in KAS 6/1 human multiple myeloma cells (Hodge et al. 2005a) and various pancreatic cancer cell lines (Hurt et al. 2007). An inverse correlation between promoter methylation and MnSOD expression was observed in the pancreatic cancer cells studied (Hurt et al. 2007) as well as the human multiple myeloma cell line IM-9 (Hodge et al. 2005b).

Various histone modifications important for active transcription are also altered in the MnSOD gene. Treatment of C2C12 mouse myoblast cells with the histone deacetylase inhibitor trichostatin A (TSA) leads to increased expression of MnSOD through enhanced acetylation of histones 3 and 4 within the MnSOD promoter (Maehara et al. 2002). Various breast cancer cell lines (MCF-7, T47D, and MDA-MB-231) have fewer histone modifications that are key to transcription (H3K4 dimethylation and H3K9 acetylation) than the non-tumorigenic MCF-10A breast epithelial cell line. The lack of these important modifications in breast cancer cells leads to a condensed chromatin structure unfavorable for gene expression (Hitchler et al. 2008).

MnSOD genetic polymorphisms

A variety of single nucleotide polymorphisms (SNP) have been identified within the MnSOD gene. These polymorphisms include a C \rightarrow T transition resulting in the conversion of valine to alanine at amino acid 16 within the mitochondrial signaling sequence (Rosenblum et al. 1996). Other polymorphisms include a C \rightarrow T transition at amino acid 58 that converts an isoleucine to threonine (Ho and Crapo 1988) and a C \rightarrow T transition that leads to a conversion from leucine to phenylalanine at amino acid 60 (Hernandez-Saavedra and McCord 2003), as well as a G \rightarrow T transversion in the second intron of the MnSOD gene (Hernandez-Saavedra and McCord 2009). These polymorphisms affect transcription, enzyme activity, or mitochondrial localization of MnSOD.

Val16Ala SNP is one of the most extensively studied SNPs in the MnSOD gene. Val16Ala SNP has been identified in various cell lines of normal patients and patients with diseases that result in premature aging (Rosenblum et al. 1996). This particular polymorphism occurs within the mitochondrial signaling sequence, thus affecting import of MnSOD into mitochondria. The Ala16 variant is able to transverse both mitochondrial membranes quickly to enter the matrix, while most of the Val16 variant is imbedded within the inner membrane. This difference in migration capability is due, in part, to the differences in the secondary structure of the two variants. Computer models predict that the Ala16 variant has a partial α -helical structure, while the Val16 variant has a β -sheet structure (Shimoda-Matsubayashi et al. 1996; Sutton et al. 2003). More Ala16 variant MnSOD is found in the mitochondria and has higher enzyme activity than the Val16 variant. This difference in structure between the variants may affect interactions with the Tim 23 import channel within

the inner membrane, resulting in a reduced rate of import and lower activity for the Val16 variant (Sutton et al. 2003).

Different studies in diverse populations and cancer types have resulted in identifying conflicting roles for the Val16Ala MnSOD polymorphism and cancer risk (Bag and Bag 2008). Some studies have shown an association between the Val16Ala polymorphism and ovarian cancer risk (Dalan et al. 2008; Johnatty et al. 2007). Other studies suggest that the Ala/Ala genotype results in increased cancer risk while still other studies imply the Val/Val genotype increases cancer risk. A statistically significant increase in the risk of lung cancer in patients carrying the Val/Val genotype compared to the Ala/Ala genotype has been identified (Wang et al. 2001; Zejnilovic et al. 2009). Wang et al. (2001) discovered that younger patients (≤ 55 years) with the Val/Val and Val/Ala genotypes have a higher risk of lung cancer compared to older patients. A 70% increased risk of prostate cancer has been found in men homozygous for Ala compared to Val homozygous men (Woodson et al. 2003). Arsova-Sarafinovska et al. (2008) found a 5.2-fold increased risk of early-onset prostate cancer (under age 65) in men who are homozygous for the Ala allele, and a threefold increased risk for heterozygous Ala allele men, compared to Val homozygous controls. In both male and female breast cancer and prostate cancer, risk is elevated in patients carrying the MnSOD Ala/Ala genotype compared to controls (Bica et al. 2009). Individuals with the Ala/Ala genotype have a 2.89-fold increase in developing hepatocellular carcinoma, and the Ala/Ala genotype results in even greater risk for patients who are infected with hepatitis C virus (Ezzikouri et al. 2008).

While the presence of MnSOD polymorphisms alone is not always associated with increased cancer risk, their presence is often correlated with other risk factors, such as antioxidant intake. Li et al. found MnSOD Val16Ala polymorphism is not associated with overall risk of non-aggressive, aggressive, or total prostate cancer. Nevertheless, there is an association between the MnSOD genotype and prostate cancer risk when levels of antioxidants are considered. A significant and inverse relationship between plasma levels of selenium and risk of total and aggressive prostate cancer is present in men with the Ala/Ala genotype. This association is weaker in men with the Val/Val or Val/Ala genotype (Li et al. 2005). Kang et al. found a positive correlation between the Ala/Ala genotype and prostate cancer risk. This risk inversely correlates with vitamin E intake in the Ala allele patients, with patients who consume the highest levels of vitamin E having the lowest risk of prostate cancer (Kang et al. 2007). Han et al. found no overall association between Val16Ala polymorphism and risk of skin cancer (melanoma, squamous or basal cell carcinoma); however, they did find a significant interaction between this polymorphism and total vitamin C intake. The researchers also found an inverse association between intake of the dietary carotenoids α - and β -carotene and carriers of the Val/Val genotype but not the Ala/Ala genotype (Han et al. 2007). Breast cancer risk is slightly increased in women carrying the Ala/Ala genotype compared to those carrying the Val/Val genotype, especially in premenopausal women. This risk is further increased in premenopausal women with low intakes of fruits, vegetables, and various dietary supplements (antioxidant vitamins and selenium) (Cai et al. 2004; Wang et al. 2009).

The Val16Ala polymorphism also affects cancer risk in combination with polymorphisms for other genes. For non-small cell lung carcinoma, the presence of the wild-type Val/Val homozygous MnSOD allele results in increased risk in the presence of p53 or X-ray cross complementing group 1 (XRCC1) polymorphisms alone or in combination with both p53 and XRCC1 polymorphisms. No increased risk was found in patients with either the Ala/Val or Ala/Ala alleles (Liu et al. 2004). The wild-type Val/Val MnSOD genotype is also associated with increased risk of pancreatic cancer compared to the Ala/Ala or Ala/Val genotypes. The combination of the Val/Val MnSOD genotype and the G-463G

polymorphism in the promoter of the myeloperoxidase (*MPO*) gene confers greater risk of pancreatic cancer than either genotype alone (Wheatley-Price et al. 2008). Iguchi et al. (2009) found a statistically significant association between the MnSOD A/A genotype and prostate cancer in patients who also carried the rapid acetylation genotype of *N*-acetyltransferase-1 (NAT1), but no association was found between MnSOD A/A genotype and the slow acetylation genotype of NAT1.

Other polymorphisms in the *MnSOD* gene have also been identified. MnSOD exists as a homotetramer (Borgstahl et al. 1992; Ravindranath and Fridovich 1975; Wispe et al. 1989), and Ile58 is an important amino acid at the tetrameric interface between the four monomers that make up mature MnSOD protein (Borgstahl et al. 1992). The Ile58Thr polymorphism results in destabilization of this tetrameric interface, resulting in predominantly dimeric MnSOD. The Ile58Thr polymorphism also confers thermal sensitivity to mature MnSOD protein. The mutant MnSOD has about half the enzyme activity of the normal protein at all temperatures tested. Mutant MnSOD is completely inactivated even at 41.7°C, while this temperature has no appreciable effect on the activity of normal MnSOD (Borgstahl et al. 1996). Overexpression of the Thr58 variant of MnSOD in the MCF-7 human breast cancer cell line has much less tumor suppressor activity than the Ile58 variant due to reduced enzyme activity in the Thr58 variant (Zhang et al. 1999). The Leu60Phe polymorphism is present in the Jurkat human T cell leukemia cell line, leading to reduced MnSOD enzyme activity in these cells compared to human peripheral blood lymphocytes, which correlates with the malignant phenotype of Jurkat cells and confers thiol sensitivity to MnSOD in the Jurkat human T cell leukemia cell line (Hernandez-Saavedra and McCord 2003). A recently identified polymorphism (G1677T) near the enhancer element located within intron 2 of the *MnSOD* gene creates a potential glucocorticoid receptor binding site, and the T/T genotype is linked to reduced risk of lung cancer (Hernandez-Saavedra and McCord 2009).

Strategies to increase the effects of MnSOD in cancer cells

Since MnSOD expression is reduced in many cancers (Oberley and Buettner 1979) and increased expression of MnSOD inhibits cancer growth (Oberley 2001; Oberley 2005), strategies that increase expression or enzyme activity of MnSOD in patients may prove valuable in improving cancer treatments in these individuals.

Epigenetic activation of MnSOD expression

Epigenetic silencing of the MnSOD gene is a critical mechanism by which MnSOD expression is suppressed in cancer cells (Hitchler et al. 2008; Hodge et al. 2005a; Huang et al. 1999; Hurt et al. 2007). Many epigenetic drugs have been shown to increase MnSOD expression in various cancer cell types. Treatment of KAS 6/1 human multiple myeloma cells with the DNA methyltransferase inhibitor zebularine reverses methylation of the MnSOD promoter, resulting in increased MnSOD protein and enzyme activity (Hodge et al. 2005a). Zebularine treatment also increases MnSOD expression in various pancreatic carcinoma cell lines (Hurt et al. 2007) and IM-9 human multiple myeloma cells (Hodge et al. 2005b).

Treatment of breast cancer cells with histone deacetylase inhibitors trichostatin A or sodium butyrate increases MnSOD expression in these cells, suggesting epigenetic silencing of the MnSOD gene is important in the altered expression of MnSOD seen in various breast cancers (Hitchler et al. 2008). Trichostatin A also stimulates MnSOD expression in the C2C12 mouse myoblast cell line by inhibiting histone deacetylase 1 (HDAC1) activity and by stimulating early growth responsive-1 (Egr-1)-mediated dissociation of an inhibitory Sp1-HDAC1 complex from the MnSOD promoter (Maehara et al. 2002).

Enforced expression of MnSOD

Overexpression of MnSOD or CuZnSOD using adenoviral vectors (*AdMnSOD* and *AdCuZnSOD*, respectively) decreases breast cancer cell growth and reduces clonogenic survival in vitro. Direct injection of *AdMnSOD* or *AdCu-ZnSOD* into breast tumor xenografts decreases breast tumor growth in vivo, resulting in increased animal survival (Weydert et al. 2006). Similar results are observed in hamster cheek pouch carcinoma cells treated with *AdMnSOD* virus vector, both in vitro and in vivo (Lam et al. 1997).

Transfection of cancer cells with mutant MnSOD having greater enzyme activity than wild-type MnSOD may prove to be a viable strategy for cancer treatment. Davis et al. generated a mutant MnSOD in which the histidine at position 30 is replaced with arginine. This mutant has greater enzymatic efficiency than wild-type MnSOD. Overexpression of this active site mutant in A549 human lung carcinoma cells leads to significant reduction in tumor volume in NOD/SCID mice compared to vector or wild-type MnSOD-transfected cells (Davis et al. 2004).

Combining overexpression of MnSOD with other cancer therapies has proven valuable for increasing the efficacy of the therapies. Zhang et al. (2006a) found that combined intratumoral injection of SW620 human colorectal carcinoma xenografts with modified adenoviruses expressing both MnSOD and TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) results in complete elimination of the established tumor xenografts compared to either virus alone.

Combination of MnSOD and chemotherapeutic drugs is more effective in suppressing cancer growth than either treatment alone. One such therapy is adriamycin, an anthracycline commonly used in chemotherapeutic regimens. Administration of recombinant human MnSOD protein (rhMnSOD) or adriamycin alone inhibits sarcoma 180 cell tumor growth in vivo, while a combination of adriamycin and rhMnSOD inhibits tumor growth greater than either treatment alone through MnSOD-mediated stimulation of immune response and lymphocyte recruitment to the tumor (Chen et al. 2008).

Another cancer therapy is 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU), a chemotherapy drug that acts through two mechanisms to suppress tumor growth: alkylation of DNA and inhibition of glutathione reductase (GR) and thioredoxin reductase activities (Schallreuter et al. 1990). Overexpression of CuZnSOD or MnSOD using adenoviral vectors, in combination with BCNU treatment, causes increased oxidative stress and results in greater suppression of in vitro breast cancer cell growth than either BCNU or SOD overexpression alone. Directly injecting breast tumor xenografts with *AdMnSOD* and intravenous delivery of BCNU result in significantly decreased breast tumor xenograft growth and increased animal survival compared to either treatment alone or to controls (Weydert et al. 2008). In hamster cheek pouch carcinoma (HCPC-1) or human oral squamous carcinoma cells, overexpression of MnSOD using *AdMnSOD* viral vector in combination with either BCNU or 3-amino-1,2,4-triazole (AT) decreases cell viability in vitro and inhibits tumor growth in vivo greater than any of the treatments alone (Weydert et al. 2003b). Combination of MnSOD overexpression, BCNU, and either ionizing radiation or adriamycin results in much greater decreases in clonogenic survival in vitro, decreased tumor growth in vivo, and increased animal survival than any of the treatments alone or any other combination of treatments tested (Sun et al. 2009).

Application of SOD mimetics

Many studies have shown that treatment of cancer cells with SOD mimetics also reduces tumorigenicity. Treatment with MnTE-2-PyP⁵⁺ alone significantly reduced levels of HIF-1 α and decreased levels of VEGF compared to control tumors, resulting in diminished

microvessel density in 4T1 breast cancer tumors (Rabbani et al. 2009). This laboratory has shown that application of TPA induces both apoptosis and proliferation in a mouse model of skin carcinogenesis. Treatment of these mice with MnTE-2-PyP⁵⁺ 12 h after TPA treatment inhibited TPA-induced proliferation without affecting apoptosis, resulting in markedly reduced tumorigenesis compared to controls (Zhao et al. 2005a).

Co-treatment of MnSOD mimetics with other anti-cancer therapies can enhance the effects of these therapies. MnTE-2-PyP⁵⁺ porphyrin alone suppresses growth of the WEH17.2 mouse thymic lymphoma cells, and pretreatment of these cells with MnTE-2-PyP⁵⁺ augments dexamethasone-induced apoptosis. Pretreatment with MnTE-2-PyP⁵⁺ also sensitizes WEH17.2 cells to cyclophosphamide (Jaramillo et al. 2009). MnTE-2-PyP⁵⁺ also enhances the radiation responsiveness of cancer cells. MnTE-2-PyP⁵⁺ reduces the ability of 4T1 mouse mammary carcinoma tumor cells to rescue endothelial cells from irradiation-induced damage (Moeller et al. 2005), and MnTE-2-PyP⁵⁺ in combination with ionizing radiation results in a slight reduction in tumor growth compared to radiation alone in RM-9 prostate cancer tumor xenografts. MnTE-2-PyP⁵⁺ decreases angiogenesis potential by augmenting radiation-mediated 4T1 breast tumor devascularization. MnTE-2-PyP⁵⁺ is able to protect normal tissue but does not protect tumor tissue at any level that would interfere with radiotherapy (Moeller et al. 2005). Co-administration of the SOD mimetic M40403 and interleukin-2 (IL-2) not only enhances the anti-tumor activity of IL-2, but also diminishes the detrimental hypotension associated with IL-2 therapy (Samlowski et al. 2003).

Summary

MnSOD is a vital ROS-scavenging enzyme essential for maintaining aerobic life in our oxygen-rich atmosphere and is an important tumor suppressing protein that both inhibits proliferation and enhances apoptosis through modulation of ROS levels in cancer cells. Methods that increase the expression of MnSOD, or mimic the superoxide-scavenging capability of MnSOD, not only kill cancer cells but also enhance the effectiveness of various anti-cancer therapies. MnSOD is also vital for protecting the heart and brain from the deleterious effects of chemotherapeutic drugs, especially adriamycin, one of the most effective anti-cancer agents in the arsenal of the oncologist. Current and future studies to better understand the mechanisms by which MnSOD both protects normal tissues and kills cancer cells will lead to further advances in cancer therapy that not only improve targeting of cancer cells specifically but also enhance the quality of life for patients after treatment.

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References

- Ahles TA, Saykin AJ. Candidate mechanisms for chemotherapy-induced cognitive changes. *Nat Rev Cancer*. 2007; 7:192–201. [PubMed: 17318212]
- Ahles TA, Saykin AJ, Furstenberg CT, Cole B, Mott LA, Skalla K, Whedon MB, Bivens S, Mitchell T, Greenberg ER, Silberfarb PM. Neuropsychologic impact of standard-dose systemic chemotherapy in long-term survivors of breast cancer and lymphoma. *J Clin Oncol*. 2002; 20:485–493. [PubMed: 11786578]
- Anantharaman M, Tangpong J, Keller JN, Murphy MP, Markesbery WR, Kinningham KK, St Clair DK. β -Amyloid mediated nitration of manganese superoxide dismutase: implication for oxidative stress in a APP^{NLh/NLh} X PS-1^{P264L/P264L} double knock-in mouse model of Alzheimer's disease. *Am J Pathol*. 2006; 168:1608–1618. [PubMed: 16651627]
- Andreyev AY, Kushnareva YE, Starkov AA. Mitochondrial metabolism of reactive oxygen species. *Biochem (Moscow)*. 2005; 70:246–264.

- Arsova-Sarafinovska Z, Matevska N, Petrovski D, Banev S, Dzikova S, Georgiev V, Sikole A, Sayal A, Aydin A, Suturkova L, Dimovski AJ. Manganese superoxide dismutase (*MnSOD*) genetic polymorphism is associated with risk of early-onset prostate cancer. *Cell Biochem Funct*. 2008; 26:771–777. [PubMed: 18646267]
- Bag A, Bag N. Target sequence polymorphism of human manganese superoxide dismutase gene and its association with cancer risk: a review. *Cancer Epidemiol Biomark Prevent*. 2008; 17:3298–3305.
- Batinic-Haberle I, Benov LT. An SOD mimic protects NADP⁺-dependent isocitrate dehydrogenase against oxidative inactivation. *Free Rad Res*. 2008; 42:618–624.
- Bayir H, Kagan VE, Clark RSB, Janesko-Feldman K, Rafikov R, Huang Z, Zhang X, Vagni V, Billiar TR, Kochanek PM. Neuronal NOS-mediated nitration and inactivation of manganese superoxide dismutase in brain after experimental and human brain injury. *J Neurochem*. 2007; 101:168–181. [PubMed: 17394464]
- Behrend I, Henderson G, Zwacka RM. Reactive oxygen species in oncogenic transformation. *Biochem Soc Trans*. 2003; 31:1441–1444. [PubMed: 14641084]
- Behrend L, Mohr A, Dick T, Zwacka RM. Manganese superoxide dismutase induces p53-dependent senescence in colorectal cancer cells. *Mol Cell Biol*. 2005; 25:7758–7769. [PubMed: 16107721]
- Bica CG, da Silva LLM, Toscani NV, da Cruz IBM, Sa G, Graudenz MS, Zettler CG. *MnSOD* gene polymorphism association with steroid-dependent cancer. *Pathol Oncol Res*. 2009; 15:19–24. [PubMed: 18553161]
- Boonstra J, Post JA. Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells. *Gene*. 2004; 337:1–13. [PubMed: 15276197]
- Borgstahl GEO, Parge HE, Hickey MJ, Beyer WF Jr, Hallewell RA, Tainer JA. The structure of human mitochondrial manganese superoxide dismutase reveals a novel tetrameric interface of two 4-helix bundles. *Cell*. 1992; 71:107–118. [PubMed: 1394426]
- Borgstahl GEO, Parge HE, Hickey MJ, Johnson MJ, Boissinot M, Hallewell RA, Lepock JR, Cabelli DE, Tainer JA. Human mitochondrial manganese superoxide dismutase polymorphic variant Ile58Thr reduces activity by destabilizing the tetrameric interface. *Biochemistry*. 1996; 35:4287–4297. [PubMed: 8605177]
- Bowsher CG, Tobin AK. Compartmentation of metabolism within mitochondria and plastids. *J Exp Bot*. 2001; 53:513–527. [PubMed: 11373301]
- Brown MS, Stemmer SM, Simon JH, Stears JC, Jones RB, Cagnoni PJ, Sheeder JL. White matter disease induced by high-dose chemotherapy: longitudinal study with MR imaging and proton spectroscopy. *AJNR*. 1998; 19:217–221. [PubMed: 9504468]
- Buzek J, Latonen L, Kurki S, Peltonen K, Laiho M. Redox state of tumor suppressor p53 regulates its sequence-specific DNA binding in DNA-damaged cells by cysteine-277. *Nucleic Acids Res*. 2002; 30:2340–2348. [PubMed: 12034820]
- Cai Q, Shu X-O, Wen W, Cheng J-R, Dai Q, Gao Y-T, Zheng W. Genetic polymorphism in the manganese superoxide dismutase gene, antioxidant intake, and breast cancer risk: results from the Shanghai breast cancer study. *Breast Cancer Res*. 2004; 6:R647–R655. [PubMed: 15535847]
- Carducci MA, Gilbert J, Bowling MK, Noe D, Eisenberger MA, Sinibaldi V, Zabelina Y, Chen T-I, Grochow LB, Donehower RC. A phase I clinical and pharmacological evaluation of sodium phenylbutyrate on an 120-h infusion schedule. *Clin Cancer Res*. 2001; 7:3047–3055. [PubMed: 11595694]
- Chaiswing L, Cole MP, St Clair DK, Ittarat W, Szweda LI, Oberley TD. Oxidative damage precedes nitrate damage in adriamycin-induced cardiac mitochondria injury. *Toxicol Path*. 2004; 32:536–547. [PubMed: 15605432]
- Chaiswing L, Cole MP, Ittarat W, Szweda LI, St Clair DK, Oberley TD. Manganese superoxide dismutase and inducible nitric oxide synthase modify early oxidative events in acute adriamycin-induced mitochondrial toxicity. *Mol Cancer Therap*. 2005; 4:1056–1064. [PubMed: 16020663]
- Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc Natl Acad Sci*. 1998; 95:11715–11720. [PubMed: 9751731]
- Chandhok NS, Pellman D. A little CIN may cost a lot: revisiting aneuploidy and cancer. *Curr Opin Genet Dev*. 2009; 19:74–81. [PubMed: 19195877]

- Chen D, Yu Z, Zhu Z, Lopez CD. The p53 pathway promotes efficient mitochondrial DNA base excision repair in colorectal cancer cells. *Cancer Res.* 2006; 66:3485–3494. [PubMed: 16585172]
- Chen Y, Jungsuwadee P, Vore M, Butterfield DA, St Clair DK. Collateral damage in cancer chemotherapy: oxidative stress in nontargeted tissues. *Mol Interv.* 2007; 7:147–156. [PubMed: 17609521]
- Chen C-S, Zhao Q, Wang J, Rong J-J, Yuan Q-S, Guo Q-L, Wu W-T. Enhanced anti-tumor effects achieved in a murine tumor model using combination therapy of recombinant human manganese superoxide dismutase and adriamycin. *Biochem Biophys Res Commun.* 2008; 370:663–668. [PubMed: 18413139]
- Chuang T-C, Liu J-Y, Lin C-T, Tang Y-T, Yeh M-H, Chang S-C, Li J-W, Kao M-C. Human manganese superoxide dismutase suppresses HER2/neu-mediated breast cancer malignancy. *FEBS Lett.* 2007; 581:4443–4449. [PubMed: 17719580]
- Church SL, Grant JW, Ridnour LA, Oberley LW, Swanson PE, Meltzer PS, Trent JM. Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. *Proc Natl Acad Sci USA.* 1993; 90:3113–3117. [PubMed: 8464931]
- Cobbs CS, Samanta M, Harkins LE, Gillespie GY, Merrick BA, MacMillan-Crow LA. Evidence for peroxynitrite-mediated modifications to p53 in human gliomas: possible functional consequences. *Arch Biochem Biophys.* 2001; 394:167–172. [PubMed: 11594730]
- Cole MP, Chaiswing L, Oberley TD, Edelmann SE, Piascik MT, Lin S-M, Kinningham KK, St Clair DK. The protective roles of nitric oxide and superoxide dismutase in adriamycin-induced cardiotoxicity. *Cardiovasc Res.* 2006; 69:186–197. [PubMed: 16157314]
- Costantino JP, Kuller LH, Ives DG, Fisher B, Dignam J. Coronary heart disease mortality and adjuvant tamoxifen therapy. *J Natl Cancer Inst.* 1997; 89:776–782. [PubMed: 9182975]
- Crapo JD, Oury T, Rabouille C, Slot JW, Chang L-Y. Copper, zinc superoxide dismutase is primarily a cytosolic protein in human cells. *Proc Natl Acad Sci USA.* 1992; 89:10405–10409. [PubMed: 1332049]
- Cullen JJ, Weydert C, Hinkhouse MM, Ritchie J, Domann FE, Spitz D, Oberley LW. The role of manganese superoxide dismutase in the growth of pancreatic adenocarcinoma. *Cancer Res.* 2003; 63:1297–1303. [PubMed: 12649190]
- Dalan AB, Ergen A, Yilmaz H, Karateke A, Isbar T. Manganese superoxide dismutase gene polymorphism, MnSOD plasma levels and risk of epithelial ovarian cancer. *J Obstet Gynaecol Res.* 2008; 34:878–884. [PubMed: 18834346]
- Daosukho C, Kinningham K, Kasarskis EJ, Ittarat W, St Clair DK. Tamoxifen enhancement of TNF- α induced MnSOD expression: modulation of NF- κ B dimerization. *Oncogene.* 2002; 21:3603–3610. [PubMed: 12032862]
- Daosukho C, Ittarat W, Lin S-m, Sawyer DB, Kinningham K, Lien Y-C, St Clair DK. Induction of manganese superoxide dismutase (MnSOD) mediates cardioprotective effect of tamoxifen (TAM). *J Mol Cell Cardiol.* 2005; 39:792–803. [PubMed: 16140321]
- Daosukho C, Chen Y, Noel T, Sompol P, Nithinpongvanitch R, Velez JM, Oberley TD, St Clair DK. Phenylbutyrate, a histone deacetylase inhibitor, protects against adriamycin-induced cardiac injury. *Free Radic Biol Med.* 2007; 42:1818–1825. [PubMed: 17512461]
- Davis CA, Hearn AS, Fletcher B, Bickford J, Garcia JE, Leveque V, Melendez JA, Silverman DN, Zucali J, Agarwal A, Nick HS. Potent anti-tumor effects of an active site mutant of human manganese-superoxide dismutase. Evolutionary conservation of product inhibition. *J Biol Chem.* 2004; 279:12769–12776. [PubMed: 14688256]
- Demicheli V, Quijano C, Alvarez B, Radi R. Inactivation and nitration of human superoxide dismutase (SOD) by fluxes of nitric oxide and superoxide. *Free Radic Biol Med.* 2007; 42:1359–1368. [PubMed: 17395009]
- Dhar SK, Lynn BC, Daosukho C, St Clair DK. Identification of nucleophosmin as an NF- κ B co-activator for the induction of the human *SOD2* gene. *J Biol Chem.* 2004; 279:28209–28219. [PubMed: 15087454]
- Dhar SK, Xu Y, Chen Y, St Clair DK. Specificity protein 1-dependent p53-mediated suppression of human manganese superoxide dismutase gene expression. *J Biol Chem.* 2006; 281:21698–21709. [PubMed: 16740634]

- Dlaskova A, Hlavata L, Jezek P. Oxidative stress caused by blocking of mitochondrial complex I H⁺ pumping as a link in aging/disease vicious cycle. *Int J Biochem Cell Biol.* 2008; 40:1792–1805. [PubMed: 18291703]
- Doroshov JH. Anthracycline antibiotic-stimulated superoxide, hydrogen peroxide, and hydroxyl radical production by NADH dehydrogenase. *Cancer Res.* 1983; 43:4543–4551. [PubMed: 6309369]
- Droge W. Free radicals in the physiological control of cell function. *Physiol Rev.* 2002; 82:47–95. [PubMed: 11773609]
- Duan H, Zhang HJ, Yang J-Q, Oberley LW, Futscher BW, Domann FE. MnSOD up-regulates maspin tumor suppressor gene expression in human breast and prostate cancer cells. *Antiox Redox Signal.* 2003; 5:677–688.
- Dumont M, Wille E, Stack C, Calingasan NY, Beal MF, Lin MT. Reduction of oxidative stress, amyloid deposition, and memory deficit by manganese superoxide dismutase overexpression in a transgenic mouse model of Alzheimer's disease. *FASEB J.* 2009; 23:2459–2466. [PubMed: 19346295]
- Duttaroy A, Paul A, Kundu M, Belton A. A *Sod2* null mutation confers severely reduced adult life span in drosophila. *Genetics.* 2003; 165:2295–2299. [PubMed: 14704205]
- Eaton S, Bartlett K, Pourfarzam M. Mammalian mitochondrial beta-oxidation. *Biochem J.* 1996; 320:345–357. [PubMed: 8973539]
- Ernster L, Schatz G. Mitochondria: a historical review. *J Cell Biol.* 1981; 91:227s–255s. [PubMed: 7033239]
- Esworthy RS, Ho Y-S, Chu F-F. The *Gpx1* gene encodes mitochondrial glutathione peroxidase in the mouse liver. *Arch Biochem Biophys.* 1997; 340:59–63. [PubMed: 9126277]
- Ezzikouri S, El feydi AE, Chafik A, Afifi R, El kihal L, Benazzouz M, Hassar M, Pineau P, Benjelloun S. Genetic polymorphism in the manganese superoxide dismutase gene is associated with an increased risk for hepatocellular carcinoma in HCV-infected Moroccan patients. *Mut Res.* 2008; 649:1–6. [PubMed: 18023606]
- Forman HJ, Fukuto JM, Torres M. Redox signaling: thiol chemistry defines which reactive oxygen and nitrogen species can act as second messengers. *Am J Physiol Cell Physiol.* 2004; 287:C246–C256. [PubMed: 15238356]
- Frey TG, Mannella CA. The internal structure of mitochondria. *TIBS.* 2000; 25:319–324. [PubMed: 10871882]
- Fridovich I. The biology of oxygen radicals. *Science.* 1978; 201:875–880. [PubMed: 210504]
- Fridovich I. Superoxide dismutases. An adaptation to a paramagnetic gas. *J Biol Chem.* 1989; 264:7761–7764. [PubMed: 2542241]
- Furukawa A, Tada-Oikawa S, Kawanishi S, Oikawa S. H₂O₂ accelerates cellular senescence by accumulation of acetylated p53 via decrease in the function of SIRT1 by NAD⁺ depletion. *Cell Physiol Biochem.* 2007; 20:45–54. [PubMed: 17595514]
- Gabbita SP, Robinson KA, Steward CA, Floyd RA, Hensley K. Redox regulatory mechanisms of cellular signal transduction. *Arch Biochem Biophys.* 2000; 376:1–13. [PubMed: 10729185]
- Ganem NJ, Storchova Z, Pellman D. Tetraploidy, aneuploidy and cancer. *Curr Opin Genet Dev.* 2007; 17:157–162. [PubMed: 17324569]
- Genova ML, Ventura B, Giuliano G, Bovina C, Formiggini G, Castelli GP, Lenaz G. The site of production of superoxide radical in mitochondrial complex I is not a bound ubisemiquinone but presumably iron-sulfur cluster N2. *FEBS Lett.* 2001; 505:364–368. [PubMed: 11576529]
- Giri SN, Al-Bayati MA, Du X, Schelegle E, Mohr FC, Margolin SB. Amelioration of doxorubicin-induced cardiac and renal toxicity by pirfenidone in rats. *Cancer Chemother Pharmacol.* 2004; 53:141–150. [PubMed: 14564477]
- Gius D, Spitz DR. Redox signaling in cancer biology. *Antiox Redox Signal.* 2006; 8:1249–1252.
- Goldenthal MJ, Marin-Garcia J. Mitochondrial signaling pathways: a receiver/integrator organelle. *Mol Cell Biochem.* 2004; 262:1–16. [PubMed: 15532704]
- Gonzalez-Zulueta M, Ensz LM, Mukhina G, Lebovitz RM, Zwacka RM, Engelhardt JF, Oberley LW, Dawson VL, Dawson TM. Manganese superoxide dismutase protects nNOS neurons from NMDA and nitric oxide-mediated neurotoxicity. *J Neurosci.* 1998; 18:2040–2055. [PubMed: 9482791]

- Gregory EM, Fridovich I. Oxygen toxicity and the superoxide dismutase. *J Bacteriol.* 1973; 114:1193–1197. [PubMed: 4197269]
- Gregory EM, Goscin SA, Fridovich I. Superoxide dismutase and oxygen toxicity in a eukaryote. *J Bacteriol.* 1974; 117:456–460. [PubMed: 4590469]
- Gulbins E, Dreschers S, Bock J. Role of mitochondria in apoptosis. *Exp Physiol.* 2003; 88:85–90. [PubMed: 12525857]
- Haber F, Weiss J. On the catalysis of hydroperoxide. *Naturwissenschaften.* 1932; 20:948–950.
- Halliwell B. Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates. Is it a mechanism for hydroxyl radical production in biochemical systems? *FEBS Lett.* 1978; 92:321–326. [PubMed: 212302]
- Han D, Williams E, Cadenas E. Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space. *Biochem J.* 2001; 353:411–416. [PubMed: 11139407]
- Han D, Antunes F, Canali R, Rettori D, Cadenas E. Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol. *J Biol Chem.* 2003; 278:5557–5563. [PubMed: 12482755]
- Han J, Colditz GA, Hunter DJ. Manganese superoxide dismutase polymorphism and risk of skin cancer (United States). *Cancer Causes Control.* 2007; 18:79–89. [PubMed: 17186424]
- Hatefi Y. Themitochondrial electron transport chain and oxidative phosphorylation system. *Annu Rev Biochem.* 1985; 54:1015–1069. [PubMed: 2862839]
- Hernandez-Saavedra D, McCord JM. Paradoxical effects of thiol reagents on Jurkat cells and a new thiol-sensitive mutant form of human mitochondrial superoxide dismutase. *Cancer Res.* 2003; 63:159–163. [PubMed: 12517793]
- Hernandez-Saavedra D, McCord JM. Association of a new intronic polymorphism of the *SOD2* gene (G1677T) with cancer. *Cell Biochem Funct.* 2009; 27:223–227. [PubMed: 19405048]
- Hirst M, Marra MA. Epigenetics and human disease. *Int J Biochem Cell Biol.* 2009; 41:136–146. [PubMed: 18852064]
- Hitchler MJ, Oberley LW, Domann FE. Epigenetic silencing of *SOD2* by histone modifications in human breast cancer cells. *Free Radic Biol Med.* 2008; 45:1573–1580. [PubMed: 18845242]
- Hjalmarsson K, Marklund SL, Engstrom A, Edlund T. Isolation and sequence of complimentary DNA encoding human extracellular superoxide dismutase. *Proc Natl Acad Sci USA.* 1987; 84:6340–6344. [PubMed: 3476950]
- Ho Y-S, Crapo JD. Isolation and characterization of complementary DNAs encoding human manganese-containing superoxide dismutase. *FEBS Lett.* 1988; 229:256–260. [PubMed: 2831093]
- Ho, JC-m; Zheng, S.; Comhair, SAA.; Farver, C.; Erzurum, SC. Differential expression of manganese superoxide dismutase and catalase in lung cancer. *Cancer Res.* 2001; 61:8578–8585. [PubMed: 11731445]
- Hodge DR, Peng B, Pompeia C, Thomas S, Cho E, Clausen PA, Marquez VE, Farrar WL. Epigenetic silencing of manganese superoxide dismutase (SOD-2) in KAS 6/1 human multiple myeloma cells increases cell proliferation. *Cancer Biol Therap.* 2005a; 4:585–592. [PubMed: 15908783]
- Hodge DR, Xiao W, Peng B, Cherry JC, Munroe DJ, Farrar WL. Enforced expression of superoxide dismutase 2/manganese superoxide dismutase disrupts autocrine interleukin-6 stimulation in human multiple myeloma cells and enhances dexamethasone-induced apoptosis. *Cancer Res.* 2005b; 65:6255–6263. [PubMed: 16024627]
- Hu Y, Rosen DG, Zhou Y, Feng L, Yang G, Liu J, Huang P. Mitochondrial manganese-superoxide dismutase expression in ovarian cancer: role in cell proliferation and response to oxidative stress. *J Biol Chem.* 2005; 280:39485–39492. [PubMed: 16179351]
- Hu H, Luo M-l, Du X-l, Feng Y-b, Zhang Y, Shen X-m, Xu X, Cai Y, Han Y-l, Wang M-R. Up-regulated manganese superoxide dismutase expression increases apoptosis resistance in human esophageal squamous cell carcinomas. *Chin Med J.* 2007; 120:2092–2098. [PubMed: 18167182]
- Huang Y, He T, Domann FE. Decreased expression of manganese superoxide dismutase in transformed cells is associated with increased cytosine methylation of the *SOD2* gene. *DNA Cell Biol.* 1999; 18:643–652. [PubMed: 10463060]

- Huie RE, Padmaja S. The reaction of NO with superoxide. *Free Radic Res Commun*. 1993; 18:195–199. [PubMed: 8396550]
- Hurt EM, Thomas SB, Peng B, Farrar WL. Molecular consequences of SOD2 expression in epigenetically silenced pancreatic carcinoma cell lines. *Br J Cancer*. 2007; 97:1116–1123. [PubMed: 17895890]
- Iguchi T, Sugita S, Wang CY, Newman NB, Nakatani T, Haas GP. *MnSOD* genotype and prostate cancer risk as a function of *NAT* genotype and smoking status. *In Vivo*. 2009; 23:7–12. [PubMed: 19368118]
- Inagaki M, Yoshikawa E, Matsuoka Y, Sugawara Y, Nakano T, Akechi T, Wada N, Imoto S, Murakami K, Uchitomi Y. Group TBCSBMD. Smaller regional volumes of brain gray and white matter demonstrated in breast cancer survivors exposed to adjuvant chemotherapy. *Cancer*. 2007; 109:146–156. [PubMed: 17131349]
- Izutani R, Asano S, Imano M, Kuroda D, Kato M, Ohyanagi H. Expression of manganese superoxide dismutase in esophageal and gastric cancers. *J Gastroenterol*. 1998; 33:816–822. [PubMed: 9853553]
- Janssen AML, Bosman CB, van Duijn W, Oostendorp-van de Ruit MM, Kubben FJGM, Griffioen G, Lamers BBHW, van Krieken JHJM, van de Velde CJH, Verspaget HW. Superoxide dismutases in gastric and esophageal cancer and the prognostic impact in gastric cancer. *Clin Cancer Res*. 2000; 6:3183–3192. [PubMed: 10955802]
- Jaramillo MC, Frye JB, Crapo JD, Briehl MM, Tome ME. Increased manganese superoxide dismutase expression or treatment with manganese porphyrin potentiates dexamethasone-induced apoptosis in lymphoma cells. *Cancer Res*. 2009; 69:5450–5457. [PubMed: 19549914]
- Johnatty SE, Nagle CM, Spurdle AB, Chen X, Study ABCF, Webb PM, Chenevix-Trench G. The *MnSOD Val9Ala* polymorphism, dietary antioxidant intake, risk and survival in ovarian cancer (Australia). *Gynecol Oncol*. 2007; 107:388–391. [PubMed: 17936883]
- Jones PL, Ping D, Boss JM. Tumor necrosis factor alpha and interleukin-1 β regulate the murine manganese superoxide dismutase gene through a complex intronic enhancer involving *C/EBP- β* and *NF- κ B*. *Mol Cell Biol*. 1997; 17:6970–6981. [PubMed: 9372929]
- Joshi G, Sultana R, Tangpong J, Cole MP, St Clair DK, Vore M, Estus S, Butterfield DA. Free radical mediated oxidative stress and toxic side effects in brain induced by the anticancer drug adriamycin: insight into chemobrain. *Free Rad Res*. 2005; 29:1147–1154.
- Kang YJ, Sun X, Chen Y, Zhou Z. Inhibition of doxorubicin chronic toxicity in catalase-overexpressing transgenic mouse hearts. *Chem Res Toxicol*. 2002; 15:1–6. [PubMed: 11800590]
- Kang D, Lee K-M, Park SK, Berndt SI, Peters U, Reding D, Chatterjee N, Welch R, Chanock S, Huang W-Y, Hayes RB. Functional variant of manganese superoxide dismutase (*SOD2 V16A*) polymorphism is associated with prostate cancer risk in the prostate, lung, colorectal, and ovarian cancer study. *Cancer Epidemiol Biomark Prevent*. 2007; 16:1581–1586.
- Keele BB Jr, McCord JM, Fridovich I. Superoxide dismutase from *Escherichia coli* B. A new manganese-containing enzyme. *J Biol Chem*. 1970; 245:6176–6181. [PubMed: 4921969]
- Keller JN, Kindy MS, Holtsberg FW, St Clair DK, Yen H-C, Germeyer A, Steiner SM, Bruce-Keller AJ, Hutchins JB, Mattson MP. Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. *J Neurosci*. 1998; 18:687–697. [PubMed: 9425011]
- Kim GW, Kondo T, Noshita N, Chan PH. Manganese superoxide dismutase deficiency exacerbates cerebral infarction after focal cerebral ischemia/reperfusion in mice: implications for the production and role of superoxide radicals. *Stroke*. 2002; 33:809–815. [PubMed: 11872908]
- Kinningham KK, St Clair DK. Overexpression of manganese superoxide dismutase selectively modulates the activity of Jun-associated transcription factors in fibrosarcoma cells. *Cancer Res*. 1997; 57:5265–5271. [PubMed: 9393747]
- Kinningham KK, Xu Y, Daosukho C, Popova B, St Clair DK. Nuclear factor κ B-dependent mechanisms coordinate the synergistic effect of PMA and cytokines on the induction of superoxide dismutase 2. *Biochem J*. 2001; 353:147–156. [PubMed: 11115408]

- Kiningham KK, Daosukho C, St Clair DK. $\kappa B\alpha$ (inhibitory $\kappa B\alpha$) identified as labile repressor of MnSOD (manganese superoxide dismutase) expression. *Biochem J.* 2004; 384:543–549. [PubMed: 15330761]
- Klatt P, Molina EP, de Locaba MG, Padilla CA, Martínez-Galisteo E, Bárcena JA, Lamas S. Redox regulation of c-Jun DNA binding by reversible S-glutathionylation. *FASEB J.* 1999; 13:1481–1490. [PubMed: 10463938]
- Klug D, Rabini J. A direct demonstration of the catalytic action of superoxide dismutase through the use of pulse radiolysis. *J Biol Chem.* 1972; 247:4839–4842. [PubMed: 4626367]
- Koehler CM, Beverley KN, Leverich EP. Redox pathways of the mitochondrion. *Antiox Redox Signal.* 2006; 8:813–822.
- Konorev EA, Kennedy MC, Kalyanaraman B. Cell-permeable superoxide dismutase and glutathione peroxidase mimetics afford superior protection against doxorubicin-induced cardiotoxicity: the role of reactive oxygen and nitrogen intermediates. *Arch Biochem Biophys.* 1999; 368:421–428. [PubMed: 10441396]
- Kotamraju S, Chitambar CR, Kalivendi S, Joseph J, Kalyanaraman B. Transferrin receptor-dependent iron uptake is responsible for doxorubicin-mediated apoptosis in endothelial cells. Role of oxidant-induced iron signaling in apoptosis. *J Biol Chem.* 2002; 277:17179–17187. [PubMed: 11856741]
- Lam EWN, Zwacka RM, Engelhardt JF, Davidson BL, Domann FE Jr, Yan T, Oberley LW. Adenovirus-mediated manganese superoxide dismutase gene transfer to hamster cheek pouch carcinoma cells. *Cancer Res.* 1997; 57:5550–5556. [PubMed: 9407966]
- Lea MA, Randolph VM, Hodge SK. Induction of histone acetylation and growth regulation in erythroleukemia cells by 4-phenylbutyrate and structural analogs. *Anticancer Res.* 1999; 91:1971–1976. [PubMed: 10470142]
- Lebovitz RM, Zhang H, Vogel H, Cartwright J Jr, Dionne L, Lu N, Huang S, Matzuk MM. Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proc Natl Acad Sci USA.* 1996; 93:9782–9787. [PubMed: 8790408]
- Leverve XM. Mitochondrial function and substrate availability. *Crit Care Med.* 2007; 35:S454–S460. [PubMed: 17713393]
- Li Y, Huang T-T, Carlson EJ, Melov S, Ursell PC, Olson JL, Noble LJ, Yoshimura MP, Berger C, Chan PH, Wallace DC, Epstein CJ. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet.* 1995; 11. [PubMed: 7647778]
- Li J-J, Colburn NH, Oberley LW. Maspin gene expression in tumor suppression induced by overexpressing manganese-containing superoxide dismutase cDNA in human breast cancer cells. *Carcinogenesis.* 1998a; 19:833–839. [PubMed: 9635871]
- Li J-J, Oberley LW, Fan M, Colburn NH. Inhibition of AP-1 and NF- κB by manganese-containing superoxide dismutase in human breast cancer cells. *FASEB J.* 1998b; 12:1713–1723. [PubMed: 9837861]
- Li N, Oberley TD, Oberley LW, Zhong W. Overexpression of manganese superoxide dismutase in DU145 human prostate carcinoma cells has multiple effects on cell phenotype. *Prostate.* 1998c; 35:221–233. [PubMed: 9582091]
- Li Y, Copin JC, Reola LF, Calagui G, Gobbel GT, Chen SF, Sato S, Epstein CJ, Chan PH. Reduced mitochondrial manganese-superoxide dismutase activity exacerbates glutamate toxicity in cultured mouse cortical neurons. *Brain Res.* 1998d; 814:164–170. [PubMed: 9838093]
- Li S, Yan T, Yang J-Q, Oberley TD, Oberley LW. The role of cellular glutathione peroxidase redox regulation in the suppression of tumor cell growth by manganese superoxide dismutase. *Cancer Res.* 2000; 60:3927–3939. [PubMed: 10919671]
- Li F, Calingasan NY, Yu F, Mauck WM, Toidze M, Almeida CG, Takahashi RH, Carlson GA, Beal MF, Lin MT, Gouras GK. Increased plaque burden in brains of APP mutant MnSOD heterozygous knockout mice. *J Neurochem.* 2004; 89:1308–1312. [PubMed: 15147524]
- Li H, Kantoff PW, Giovannucci E, Leitzmann MF, Gaziano JM, Stampfer MJ, Ma J. Manganese superoxide dismutase polymorphism, prediagnostic antioxidant status, and risk of clinical significant prostate cancer. *Cancer Res.* 2005; 65:2498–2504. [PubMed: 15781667]

- Liu R, Oberley TD, Oberley LW. Transfection and expression of MnSOD cDNA decreases tumor malignancy of human oral squamous carcinoma SCC-25 cells. *Human Gene Therap.* 1997; 8:585–595. [PubMed: 9095410]
- Liu G, Zhou W, Park S, Wang LI, Miller DP, Wain JC, Lynch TJ, Su L, Christiani DC. The *SOD2 Val/Val* genotype enhances the risk of nonsmall cell lung carcinoma by *p53* and *XRCC1* polymorphisms. *Cancer.* 2004; 101:2802–2808. [PubMed: 15534883]
- Liu J, St Clair DK, Gu X, Zhao Y. Blocking mitochondrial permeability transition prevents p53 mitochondrial translocation during skin tumor promotion. *FEBS Lett.* 2008; 582:1319–1324. [PubMed: 18358838]
- Logan DC. The mitochondrial compartment. *J Exp Bot.* 2006; 57:1225–1243. [PubMed: 16556626]
- Love RR, Wiebe DA, Newcomb PA, Cameron L, Leventhal H, Jordan VC, Feyzi J, Demets DL. Effects of tamoxifen on cardiovascular risk factors in postmenopausal women. *Ann Intern Med.* 1991; 115:860–864. [PubMed: 1952472]
- Lynn S, Huang EJ, Elchuri S, Naemuddin M, Nishinaka Y, Yodoi J, Ferriero DM, Epstein CJ, Huang T-T. Selective neuronal vulnerability and inadequate stress response in superoxide dismutase mutant mice. *Free Radic Biol Med.* 2005; 38:817–828. [PubMed: 15721992]
- MacMillan-Crow LA, Thompson JA. Tyrosine modifications and inactivation of active site manganese superoxide dismutase mutant (Y34F) by peroxynitrite. *Arch Biochem Biophys.* 1999; 366:82–88. [PubMed: 10334867]
- MacMillan-Crow LA, Crow JP, Thompson JA. Peroxynitrite-mediated inactivation of manganese superoxide dismutase involves nitration and oxidation of critical tyrosine residues. *Biochemistry.* 1998; 37:1613–1622. [PubMed: 9484232]
- Maehara K, Uekawa N, Isobe K-I. Effects of histone acetylation on transcriptional regulation of manganese superoxide dismutase gene. *Biochem Biophys Res Commun.* 2002; 295:187–192. [PubMed: 12083788]
- Maiorino M, Scapin M, Ursini F, Biasolo M, Bosello V, Flohe L. Distinct promoters determine alternative transcription of gpx-4 into phospholipid-hydroperoxide glutathione peroxidase variants. *J Biol Chem.* 2003; 278:34286–34290. [PubMed: 12819198]
- Malafa M, Margenthaler J, Webb B, Neitzel L, Christophersen M. MnSOD expression is increased in metastatic gastric cancer. *J Surg Res.* 2000; 88:130–134. [PubMed: 10644478]
- Manoli I, Alesci S, Blackman MR, Su YA, Rennert OM, Chrousos GP. Mitochondria as key components of the stress response. *Trends Endocrinol Metab.* 2007; 18:190–198. [PubMed: 17500006]
- Marcillat O, Zhang Y, Davies KJA. Oxidative and non-oxidative mechanisms in the inactivation of cardiac mitochondrial electron transport chain components by doxorubicin. *Biochem J.* 1989; 259:181–189. [PubMed: 2719642]
- Marcus DL, Strafaci JA, Freedman ML. Differential neuronal expression of manganese superoxide dismutase in Alzheimer's disease. *Med Sci Monit.* 2006; 12:BR8–BR14. [PubMed: 16369462]
- Masini E, Cuzzocrea S, Mazzon E, Marzocca C, Mannaioni PF, Salvemini D. Protective effects of M40403, a selective superoxide dismutase mimetic, in myocardial ischaemia and reperfusion injury in vivo. *Br J Pharmacol.* 2002; 136:905–917. [PubMed: 12110615]
- McBride HM, Neuspiel M, Wasiak S. Mitochondria: more than just a powerhouse. *Curr Biol.* 2006; 16:R551–R560. [PubMed: 16860735]
- Melov S, Schneider JA, Day BJ, Hinerfeld D, Coskun P, Mirra SS, Crapo JD, Wallace DC. A novel neurological phenotype in mice lacking mitochondrial manganese superoxide dismutase. *Nat Genet.* 1998; 18:159–163. [PubMed: 9462746]
- Miao L, St Clair DK. Regulation of superoxide dismutase genes: implications in disease. *Free Radic Biol Med.* 2009; 10.1016/j.freeradbiomed.2009.05.018
- Minotti G, Recalcatti S, Mordente A, Liberi G, Calafiore M, Mancuso C, Preziosi P, Cairo G. The secondary alcohol metabolite of doxorubicin irreversibly inactivates aconitase/iron regulatory protein-1 in cytosolic fractions from human myocardium. *FASEB J.* 1998; 12:541–552. [PubMed: 9576481]
- Minotti G, Ronchi R, Salvatorelli E, Menna P, Cairo G. Doxorubicin irreversibly inactivates iron regulatory proteins 1 and 2 in cardiomyocytes: evidence for distinct metabolic pathways and

- implications for iron-mediated cardiotoxicity of antitumor therapy. *Cancer Res.* 2001; 61:8422–8428. [PubMed: 11731422]
- Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol Rev.* 2004; 56:185–229. [PubMed: 15169927]
- Misra HP, Fridovich I. Purification and properties of superoxide dismutase from a red alga, *Porphyridium cruentum*. *J Biol Chem.* 1977; 252:6421–6423. [PubMed: 893415]
- Moeller BJ, Batinic-Haberle I, Spasojevic I, Rabbani ZN, Anscher MS, Vujaskovic Z, Dewhirst MW. A manganese porphyrin superoxide dismutase mimetic enhances tumor radioresponsiveness. *Int J Rad Oncol Biol Phys.* 2005; 63:545–552.
- Nelson CJ, Nandy N, Roth AJ. Chemotherapy and cognitive deficits: mechanisms, findings, and potential interventions. *Pall Support Care.* 2007; 5:273–280.
- Newsholme P, Bender K, Kiely A, Brennan L. Amino acid metabolism, insulin secretion and diabetes. *Biochem Soc Trans.* 2007; 35:1180–1186. [PubMed: 17956307]
- Nithipongvanitch R, Ittarat W, Velez JM, Zhao R, St Clair DK, Oberley TD. Evidence for p53 as guardian of the cardiomyocyte mitochondrial genome following acute adriamycin treatment. *J Histochem Cytochem.* 2007; 55:629–639. [PubMed: 17312011]
- Nohl H, Jordan W. The metabolic fate of mitochondrial hydrogen peroxide. *Eur J Biochem.* 1980; 111:203–210. [PubMed: 7439185]
- Nordenskjold B, Rosell J, Rutqvist L-E, Malmstrom P-O, Bergh J, Bengtsson N-O, Hatschek T, Wallgren A, Carstensen J. Coronary heart disease mortality after 5 years of adjuvant tamoxifen therapy: results from a randomized trial. *J Natl Cancer Inst.* 2005; 97:1609–1610. [PubMed: 16264181]
- Oberley LW. Anticancer therapy by overexpression of superoxide dismutase. *Antiox Redox Signal.* 2001; 3:461–472.
- Oberley LW. Mechanism of the tumor suppressive effect of MnSOD overexpression. *Biomed Pharmacother.* 2005; 59:143–148. [PubMed: 15862707]
- Oberley LW, Buettner GR. Role of superoxide dismutase in cancer: a review. *Cancer Res.* 1979; 39:1141–1149. [PubMed: 217531]
- Oberley TD, Verwiebe E, Zhong W, Kang SW, Rhee SG. Localization of the thioredoxin system in normal rat kidney. *Free Radic Biol Med.* 2001; 30:412–424. [PubMed: 11182297]
- Ohnishi T, Tamai I, Sakanaka K, Sakata A, Yamashita T, Yamashita J, Tsuji A. In vivo and in vitro evidence for ATP-dependency of *p*-glycoprotein-mediated efflux of doxorubicin at the blood–brain barrier. *Biochem Pharmacol.* 1995; 49:1541–1544. [PubMed: 7763297]
- Okado-Matsumoto A, Fridovich I. Subcellular distribution of superoxide dismutases (SOD) in rat liver. Cu, Zn-SOD in mitochondria. *J Biol Chem.* 2001; 276:38388–38393. [PubMed: 11507097]
- Oktyabrsky ON, Smirnova GV. Redox regulation of cellular functions. *Biochem (Moscow).* 2007; 72:132–145.
- Owusu-Ansah E, Yavari A, Mandal S, Banerjee U. Distinct mitochondrial retrograde signals control the G1-S cell cycle checkpoint. *Nat Genet.* 2008; 40:356–361. [PubMed: 18246068]
- Paul A, Belton A, Nag S, Martin I, Grotewiel MS, Duttaroy A. Reduced mitochondrial SOD displays mortality characteristics reminiscent of natural aging. *Mech Ageing Dev.* 2007; 128:706–716. [PubMed: 18078670]
- Pineda-Molina E, Klatt P, Vázquez J, Marina A, De Locaba MG, Pérez-Sala D, Lamas S. Glutathionylation of the p50 subunit of NF-kappaB: a mechanism for redox-induced inhibition of DNA binding. *Biochemistry.* 2001; 40:14134–14142. [PubMed: 11714266]
- Porntadavity S, Xu Y, Kiningham K, Rangnekar VM, Prachayasitikul V, St Clair DK. TPA-activated transcription of the human *MnSOD* gene: role of transcription factors SP-1 and Egr-1. *DNA Cell Biol.* 2001; 20:473–481. [PubMed: 11560779]
- Qi X, Hosoi T, Okuma Y, Kaneko M, Nomura Y. Sodium 4-phenylbutyrate protects against cerebral ischemic injury. *Mol Pharmacol.* 2004; 66:899–908. [PubMed: 15226415]
- Rabbani ZN, Spasojevic I, Zhang X, Moeller BJ, Haberle S, Vasquez-Vivar J, Dewhirst MW, Vujaskovic Z, Batinic-Haberle I. Anti-angiogenic action of redox modulating Mn(III) *ortho*

- tetrakis *N*-ethylpyridylporphyrin, MnTE-2-PyP⁵⁺ via suppression of oxidative stress in a mouse model of breast tumor. *Free Radic Biol Med*. 2009;10.1016/j.freeradbiomed.2009.07.001
- Rainwater R, Parks D, Anderson ME, Tegtmeyer P, Mann K. Role of cysteine residues in regulation of p53 function. *Mol Cell Biol*. 1995; 15:3892–3903. [PubMed: 7791795]
- Ravindranath SD, Fridovich I. Isolation and characterization of a manganese-containing superoxide dismutase from yeast. *J Biol Chem*. 1975; 250:6107–6112. [PubMed: 238997]
- Rhee SG, Chang T-S, Bae YS, Lee S-R, Kang SW. Cellular regulation by hydrogen peroxide. *J Am Soc Nephrol*. 2003; 14:S211–S215. [PubMed: 12874433]
- Ridnour LA, Oberley TD, Oberley LW. Tumor suppressive effects of MnSOD overexpression may involve imbalance in peroxide generation versus peroxide removal. *Antiox Redox Signal*. 2004; 6:501–512.
- Rosenblum JS, Gilula NB, Lerner RA. On signal sequence polymorphisms and diseases of distribution. *Proc Natl Acad Sci USA*. 1996; 93:4471–4473. [PubMed: 8633092]
- Ryan MT, Hoogenraad NJ. Mitochondrial-nuclear communications. *Ann Rev Biochem*. 2007; 76:701–722. [PubMed: 17227225]
- Ryu H, Smith K, Camelo SI, Carreras I, Lee J, Iglesias AH, Dangond F, Cormier KA, Cudkowicz ME, Brown RH Jr, Ferrante RJ. Sodium phenylbutyrate prolongs survival and regulates expression of anti-apoptotic genes in transgenic amyotrophic lateral sclerosis mice. *J Neurochem*. 2005; 93:1087–1098. [PubMed: 15934930]
- Saha R, Pahan K. Differential regulation of Mn-superoxide dismutase in neurons and astroglia by HIV-1 gp120: implications for HIV-associated dementia. *Free Radic Biol Med*. 2007; 42:1866–1878. [PubMed: 17512466]
- Samlowski WE, Petersen R, Cuzzocrea S, Macarthur H, Burton D, McGregor JR, Salvemini D. A nonpeptidyl mimic of superoxide dismutase, M40403, inhibits dose-limiting hypotension associated with interleukin-2 and increases its antitumor effects. *Nat Med*. 2003; 9:750–755. [PubMed: 12730689]
- Sarvazy N. Visualization of doxorubicin-induced oxidative stress in isolated cardiac myocytes. *Am J Physiol Heart Circ Physiol*. 1996; 271:H2079–H2085.
- Schallreuter KU, Gleason FK, Wood JM. The mechanism of action of the nitrosourea anti-tumor drugs on thioredoxin reductase, glutathione reductase and ribonucleotide reductase. *Biochim Biophys Acta*. 1990; 1054:14–20. [PubMed: 2200526]
- Shan X, Chi L, Ke Y, Luo C, Qian S, Gozal D, Liu R. Manganese superoxide dismutase protects mouse cortical neurons from chronic intermittent hypoxia-mediated oxidative damage. *Neurobiol Dis*. 2007; 28:206–215. [PubMed: 17719231]
- Sheng H, Yang W, Fukuda S, Tse HM, Paschen W, Johnson K, Batinic-Haberle I, Crapo JD, Pearlstein RD, Piganelli J, Warner DS. Long-term neuroprotection from a potent redox-modulating metalloporphyrin in the rat. *Free Radic Biol Med*. 2009; 47:917–923. [PubMed: 19631268]
- Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y, Mizuno Y. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. *Biochem Biophys Res Commun*. 1996; 226:561–565. [PubMed: 8806673]
- Shioji K, Kishimoto C, Nakamura H, Masutani H, Yuan Z, Oka S-i, Yodoi J. Overexpression of thioredoxin-1 in transgenic mice attenuates adriamycin-induced cardiotoxicity. *Circulation*. 2002; 106:1403–1409. [PubMed: 12221060]
- Sigalotti L, Fratta E, Coral S, Cortini E, Covre A, Nicolay HJM, Anzalone L, Pezzani L, di Giacomo AM, Fonsatti E, Colizzi F, Altomonte M, Calabro L, Maio M. Epigenetic drugs as pleiotropic agents in cancer treatment: biomolecular aspects and clinical applications. *J Cell Physiol*. 2007; 212:330–344. [PubMed: 17458893]
- Silverman DHS, Dy CJ, Castellon SA, Lai J, Pio BS, Abraham L, Waddell K, Petersen L, Phelps ME, Ganz PA. Altered frontocortical, cerebellar, and basal ganglia activity in adjuvant-treated breast cancer survivors 5–10 years after chemotherapy. *Breast Cancer Res Treat*. 2007; 103:303–311. [PubMed: 17009108]

- Simbre VC II, Duffy Sa, Dadlani GH, Miller TL, Lipshultz SE. Cardiotoxicity of cancer chemotherapy. Implications for children. *Pediatric Drugs*. 2005; 7:187–202. [PubMed: 15977964]
- Singal PK, Iliskovic N, Li T, Kumar D. Adriamycin cardiomyopathy: pathophysiology and prevention. *FASEB J*. 1997; 11:931–936. [PubMed: 9337145]
- Slot JW, Geuze HJ, Freeman BA, Crapo JD. Intracellular localization of the copper–zinc and manganese superoxide dismutases in rat liver parenchymal cells. *Laboratory Investigations*. 1986; 55:363–371.
- Soini Y, Vakkala M, Kahlos K, Paakko P, Kinnla V. MnSOD expression is less frequent in tumour cells of invasive breast carcinomas than in in situ carcinomas or non-neoplastic breast epithelial cells. *J Pathol*. 2001; 195:156–162. [PubMed: 11592093]
- Sompol P, Xu Y, Ittarat W, Daosukho C, St Clair D. NF- κ B-associated MnSOD induction protects against β -amyloid-induced neuronal apoptosis. *J Mol Neurosci*. 2006; 29:279–288. [PubMed: 17085785]
- Sompol P, Ittarat W, Tangpong J, Chen Y, Doubinskaia I, Batinic-Haberle I, Abdul HM, Butterfield DA, St Clair DK. A neuronal model of Alzheimer's disease: an insight into the mechanisms of oxidative stress-mediated mitochondrial injury. *Neuroscience*. 2008; 153:120–130. [PubMed: 18353561]
- Spasojevic I, Chen Y, Noel TJ, Yu Y, Cole MP, Zhang L, Zhao Y, St Clair DK, Batinic-Haberle I. Mn porphyrin-based superoxide dismutase (SOD) mimic, Mn^{III}TE-2-PyP⁵⁺, targets mouse heart mitochondria. *Free Radic Biol Med*. 2007; 42:1193–1200. [PubMed: 17382200]
- Spasojevic I, Chen Y, Noel TJ, Fan P, Zhang L, Reboucas JS, St Clair DK, Batinic-Haberle I. Pharmacokinetics of the potent redox-modulating manganese porphyrin, MnTE-2-PyP⁵⁺, in plasma and major organs of B6C3F1 mice. *Free Radic Biol Med*. 2008; 45:943–949. [PubMed: 18598757]
- Spierings D, McStay G, Saleh M, Bender C, Chipuk J, Maurer U, Green DR. Connected to death: the (unexpurgated) mitochondrial pathway of apoptosis. *Science*. 2005; 310:66–67. [PubMed: 16210526]
- Storz P. Reactive oxygen species in tumor progression. *Front Biosci*. 2005; 10:1881–1896. [PubMed: 15769673]
- Sullivan PG, Bruce-Keller AJ, Rabchevsky AG, Cristakos S, Clair DK, Mattson MP, Scheff SW. Exacerbation of damage and altered NF-kappaB activation in mice lacking tumor necrosis factor receptors after traumatic brain injury. *J Neurosci*. 1999; 19:6248–6256. [PubMed: 10414954]
- Sun X, Zhou Z, Kang YJ. Attenuation of doxorubicin chronic toxicity in metallothionein-overexpressing transgenic mouse heart. *Cancer Res*. 2001; 61:3382–3387. [PubMed: 11309296]
- Sun W, Kalen AL, Smith BJ, Cullen JJ, Oberley LW. Enhancing the antitumor activity of adriamycin and ionizing radiation. *Cancer Res*. 2009; 69:4294–4300. [PubMed: 19401447]
- Sutton A, Khoury H, Prip-Buus C, Cepanec C, Pessayre D, Degoul F. The Ala¹⁶Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. *Pharmacogenetics*. 2003:13.
- Tangpong J, Cole MP, Sultana R, Joshi G, Estus S, Vore M, St Clair W, Ratanachaiyavong S, St Clair DK, Butterfield DA. Adriamycin-induced, TNF- α -mediated central nervous system toxicity. *Neurobiol Dis*. 2006; 23:127–139. [PubMed: 16697651]
- Tangpong J, Cole MP, Sultana R, Estus S, Vore M, St Clair W, Ratanachaiyavong S, St Clair DK, Butterfield DA. Adriamycin-mediated nitration of manganese superoxide dismutase in the central nervous system: insight into the mechanism of chemobrain. *J Neurochem*. 2007; 100:191–201. [PubMed: 17227439]
- Tannock IF, Ahles TA, Ganz PA, van Dam FS. Cognitive impairment associated with chemotherapy for cancer: report of a workshop. *J Clin Oncol*. 2004; 22:2233–2239. [PubMed: 15169812]
- Toh Y, Kuninaka S, Oshiro T, Ikeda Y, Nakashima H, Baba H, Kohnoe S, Okamura T, Mori M, Sugimachi K. Overexpression of manganese superoxide dismutase mRNA may correlate with aggressiveness in gastric and colorectal adenocarcinomas. *Int J Oncol*. 2000; 17:107–112. [PubMed: 10853026]

- Tsanou E, Ioachim E, Briasoulis E, Damala K, Charchanti A, Karavasilis V, Pavlidis N, Agnantis NJ. Immunohistochemical expression of superoxide dismutase (MnSOD) anti-oxidant enzyme in invasive breast carcinoma. *Histol Histopathol.* 2004; 19:807–813. [PubMed: 15168344]
- van de Wetering CI, Coleman MC, Spitz DR, Smith BJ, Knudson CM. Manganese superoxide dismutase gene dosage affects chromosomal instability and tumor onset in a mouse model of T cell lymphoma. *Free Radic Biol Med.* 2008; 44:1677–1686. [PubMed: 18291119]
- van Remmen H, Ikeno Y, Hamilton M, Pahlavani M, Wolf N, Thorpe SR, Alderson NL, Baynes JW, Epstein CJ, Huang T-T, Nelson J, Strong R, Richardson A. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol Genome.* 2003; 16:29–37.
- Venkataraman S, Jiang X, Weydert C, Zhang Y, Zhang HJ, Goswami PC, Ritchie JM, Oberley LW, Buettner GR. Manganese superoxide dismutase overexpression inhibits the growth of androgen-independent prostate cancer cells. *Oncogene.* 2005; 24:77–89. [PubMed: 15543233]
- Verkaart S, Koopman WJH, van Ernst-de Vries SE, Nijtmans LGJ, van den Heuvel LWPJ, Smeitink JAM, Willems PHGM. Superoxide production is inversely related to complex I activity in inherited complex I deficiency. *Biochim Biophys Acta.* 2007; 1772:373–381. [PubMed: 17289351]
- Vilatoba M, Eckstein C, Bilbao G, Smyth CA, Jenkins S, Thompson JA, Eckhoff DE, Contreras JL. Sodium 4-phenylbutyrate protects against liver ischemia reperfusion injury by inhibition of endoplasmic reticulum-stress mediated apoptosis. *Surgery.* 2005; 138:342–351. [PubMed: 16153446]
- Wan XS, Devalaraja MN, St Clair DK. Molecular structure and organization of the human manganese superoxide dismutase gene. *DNA Cell Biol.* 1994; 13:1127–1136. [PubMed: 7702755]
- Wang LI, Miller DP, Sai Y, Liu G, Su L, Wain JC, Lynch TJ, Christiani DC. Manganese superoxide dismutase alanine-to-valine polymorphism at codon 16 and lung cancer risk. *J Natl Cancer Inst.* 2001; 93:1818–1821. [PubMed: 11734599]
- Wang M, Kirk JS, Venkataraman S, Domann FE, Zhang HJ, Schafer FQ, Flanagan SW, Weydert CJ, Spitz DR, Buettner GR, Oberley LW. Manganese superoxide dismutase suppresses hypoxic induction of hypoxia-inducible factor-1 α and vascular endothelial growth factor. *Oncogene.* 2005; 24:8154–8166. [PubMed: 16170370]
- Wang GG, Allis D, Chi P. Chromatin remodeling and cancer, part I: covalent histone modifications. *Trends Mol Med.* 2007a; 13:363–372. [PubMed: 17822958]
- Wang GG, Allis D, Chi P. Chromatin remodeling and cancer part II: ATP-dependent chromatin remodeling. *Trends Mol Med.* 2007b; 13:373–380. [PubMed: 17822959]
- Wang, C-t; Meng, M.; Zhang, J-c; Jin, C-j; Jiang, J-j; Ren, H-s; Jiang, J-m; Qin, C-y; Yu, D-q. Growth inhibition and gene induction in human hepatocellular carcinoma cell exposed to sodium 4-phenylbutanoate. *Chin Med J.* 2008; 121:1707–1711. [PubMed: 19024104]
- Wang S, Wang F, Shi X, Dai J, Peng Y, Guo X, Wang X, Shen H, Hu Z. Association between manganese superoxide dismutase (MnSOD) Val-9Ala polymorphism and cancer risk—a meta-analysis. *Eur J Cancer.* 2009; 45:1016. [PubMed: 19101604]
- Ward HWC. Anti-oestrogen therapy for breast cancer: a trial of tamoxifen at two dose levels. *Br Med J.* 1973; 1:13–14. [PubMed: 4567104]
- Waris G, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. *J Carcinogen.* 2006; 5:14–21.
- Wefel JS, Lenzi R, Theriault R, Buzdar AU, Cruickshank S, Meyers CA. ‘Chemobrain’ in breast carcinoma? a prologue. *Cancer.* 2004; 101:466–475. [PubMed: 15274059]
- Weisiger RA, Fridovich I. Mitochondrial superoxide dismutase. Site of synthesis and intramitochondrial localization. *J Biol Chem.* 1973; 248:4793–4796. [PubMed: 4578091]
- Weiss RB. The anthracyclines: will we ever find a better doxorubicin? *Semin Oncol.* 1992; 19:670–686. [PubMed: 1462166]
- Weydert C, Roling B, Liu J, Hinkhouse MM, Ritchie JM, Oberley LW, Cullen JJ. Suppression of the malignant phenotype in human pancreatic cancer cells by the overexpression of manganese superoxide dismutase. *Mol Cancer Therap.* 2003a; 2:361–369. [PubMed: 12700280]

- Weydert CJD, Smith BB, Xu L, Kregel KC, Ritchie JM, Davis CS, Oberley LW. Inhibition of oral cancer cell growth by adenovirus *MnSOD* plus BCNU treatment. *Free Radic Biol Med*. 2003b; 34:316–329.
- Weydert CJ, Waugh TA, Ritchie JM, Iyer KS, Smith JL, Li L, Spitz DR, Oberley LW. Overexpression of manganese or copper-zinc superoxide dismutase inhibits breast cancer growth. *Free Radic Biol Med*. 2006; 41:226–237. [PubMed: 16814103]
- Weydert CJ, Zhang Y, Sun W, Waugh TA, Teoh MLT, Andringa KK, Aykin-Burns N, Spitz DR, Smith BJ, Oberley LW. Increased oxidative stress created by adenoviral *MnSOD* or *CuZnSOD* plus BCNU (1, 3-bis(2-chloroethyl)-1-nitrosourea) inhibits breast cancer cell growth. *Free Radic Biol Med*. 2008; 44:856–867. [PubMed: 18155673]
- Wheatley-Price P, Asomaning K, Reid A, Zhai R, Su L, Zhou W, Zhu A, Ryan DP, Christiani DC, Liu G. Myeloperoxidase and superoxide dismutase polymorphisms are associated with an increased risk of developing pancreatic adenocarcinoma. *Cancer*. 2008; 112:1037–1042. [PubMed: 18205184]
- Wispe JR, Clark JC, Burhans MS, Kropp KE, Korfhagen Tr, Whitsett JA. Synthesis and processing of the precursor for human manganese-superoxide dismutase. *Biochim Biophys Acta*. 1989; 994:30–36. [PubMed: 2462451]
- Woodson K, Tangrea JA, Lehman TA, Modali R, Taylor KM, Snyder K, Taylor Pr, Virtamo J, Albanes D. Manganese superoxide dismutase polymorphism, α -tocopherol supplementation and prostate cancer risk in the alpha-tocopherol, beta-carotene cancer prevention study (Finland). *Cancer Causes Control*. 2003; 14:513–518. [PubMed: 12948282]
- Xiong Y, Shie F-S, Zhang J, Lee C-P, Ho Y-S. Prevention of mitochondrial dysfunction in post-traumatic mouse brain by superoxide dismutase. *J Neurochem*. 2005; 95:732–744. [PubMed: 16248885]
- Xu Y, Kiningham KK, Devalaraja MN, Yeh C-C, Majima H, Kasarskis EJ, St Clair DK. An intronic NF- κ B element is essential for induction of the human manganese superoxide dismutase gene by tumor necrosis factor- α and interleukin- 1β . *DNA Cell Biol*. 1999a; 18:709–722. [PubMed: 10492402]
- Xu Y, Krishnan A, Wan XS, Majima H, Yeh C-C, Ludewig G, Kasarskis EJ, St Clair DK. Mutations in the promoter reveal a cause for the reduced expression of the human manganese superoxide dismutase gene in cancer cells. *Oncogene*. 1999b; 18:93–102. [PubMed: 9926924]
- Xu Y, Porntadavity S, St Clair DK. Transcriptional regulation of the human manganese superoxide dismutase gene: the role of specificity protein 1 (Sp1) and activating protein-2 (AP-2). *Biochem J*. 2002; 362:401–412. [PubMed: 11853549]
- Xu Y, Fang F, Dhar SK, St Clair WH, Kasarskis EJ, St Clair DK. The role of a single-stranded nucleotide loop in transcriptional regulation of the human *sod2* gene. *J Biol Chem*. 2007; 282:15981–15994. [PubMed: 17426024]
- Xu Y, Fang F, Dhar SK, Bosch A, St Clair WH, Kasarskis EJ, St Clair DK. Mutations in the *SOD2* promoter reveal a molecular basis for an activating protein 2-dependent dysregulation of manganese superoxide dismutase expression in cancer cells. *Mol Cancer Res*. 2008; 6:1881–1893. [PubMed: 19074833]
- Yen H-C, Oberley TD, Vichitbandha S, Ho Y-S, St Clair DK. The protective role of manganese superoxide dismutase against adriamycin-induced acute cardiac toxicity in transgenic mice. *J Clin Invest*. 1996; 98:1253–1260. [PubMed: 8787689]
- Yen H-C, Oberley TD, Gairola CG, Szveda LI, St Clair DK. Manganese superoxide dismutase protects mitochondrial complex I against adriamycin-induced cardiomyopathy in transgenic mice. *Arch Biochem Biophys*. 1999; 362:59–66. [PubMed: 9917329]
- Zejniliovic J, Akev N, Yilmaz H, Isbir T. Association between manganese superoxide dismutase polymorphism and risk of lung cancer. *Cancer Genet Cytogenet*. 2009; 189:1–4. [PubMed: 19167605]
- Zelko IN, Mariani TJ, Felz RJ. Superoxide dismutase multigene family: a comparison of *CuZn-SOD* (*SOD1*), *Mn-SOD* (*SOD2*), and *EC-SOD* (*SOD3*) gene structure, evolution, and expression. *Free Radic Biol Med*. 2002; 33:337–349. [PubMed: 12126755]

- Zhang HJ, Yan T, Oberley TD, Oberley LW. Comparison of effects of two polymorphic variants of manganese superoxide dismutase on human breast MCF-7 cancer cell phenotype. *Cancer Res.* 1999; 59:6276–6283. [PubMed: 10626823]
- Zhang Y, Gu J, Zhao L, He L, Qian W, Wang J, Wang Y, Qijun Qian, Qian C, Liu XY. Complete elimination of colorectal tumor xenograft by combined manganese superoxide dismutase with tumor necrosis factor-related apoptosis inducing ligand gene virotherapy. *Cancer Res.* 2006a; 66:4291–4298. [PubMed: 16618754]
- Zhang Y, Smith BJ, Oberley LW. Enzymatic activity is necessary for the tumor-suppressive effects of MnSOD. *Antiox Redox Signal.* 2006b; 8:1283–1293.
- Zhao Y, Kiningham KK, Lin S-M, St Clair DK. Overexpression of MnSOD protects murine fibrosarcoma cells (F5a-II) from apoptosis and promotes a differentiation program upon treatment with 5-azacytidine: involvement of MAPK and NF κ B pathways. *Antiox Redox Signal.* 2001a; 3:375–386.
- Zhao Y, Xue Y, Oberley TD, Kiningham KK, Lin S-M, Yen H-C, Majima H, Hines J, St Clair DK. Overexpression of manganese superoxide dismutase suppresses tumor formation by modulation of activator protein-1 signaling in a multistage skin carcinogenesis model. *Cancer Res.* 2001b; 61:6082–6088. [PubMed: 11507057]
- Zhao Y, Oberley TD, Chaiswing L, Lin S-m, Epstein CJ, Huang T-T, St Clair DK. Manganese superoxide dismutase deficiency enhances cell turnover via tumor promoter-induced alterations in AP-1 and p53-mediated pathways in a skin cancer model. *Oncogene.* 2002; 21:3836–3846. [PubMed: 12032821]
- Zhao Y, Chaiswing L, Oberley TD, Batinic-Haberle I, St Clair W, Epstein CJ, St Clair DK. A mechanism-based antioxidant approach for the reduction of skin carcinogenesis. *Cancer Res.* 2005a; 65:1401–1405. [PubMed: 15735027]
- Zhao Y, Chaiswing L, Velez JM, Batinic-Haberle I, Colburn NH, Oberley TD, St Clair DK. p53 translocation to mitochondria precedes its nuclear translocation and targets mitochondrial oxidative defense protein-manganese superoxide dismutase. *Cancer Res.* 2005b; 65:3745–3750. [PubMed: 15867370]
- Zheng YG, Wu J, Chen Z, Goodman M. Chemical regulation of epigenetic modifications: opportunities for new cancer therapy. *Med Res Rev.* 2008; 28:645–687. [PubMed: 18271058]