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Modulation of MnSOD in Cancer:
Epidemiological and Experimental Evidences

Aekyong Kim

School of Pharmacy, Catholic University of Daegu, Gyeongbuk 712-702, Korea

Since it was first observed in late 1970s that human cancers often had decreased manganese superoxide dismutase (MnSOD) protein expression and activity, extensive studies have been conducted to verify the association between MnSOD and cancer. Significance of MnSOD as a primary mitochondrial antioxidant enzyme is unquestionable; results from *in vitro*, *in vivo* and epidemiological studies are in harmony. On the contrary, studies regarding roles of MnSOD in cancer often report conflicting results. Although putative mechanisms have been proposed to explain how MnSOD regulates cellular proliferation, these mechanisms are not capitulated in epidemiological studies. This review discusses most recent epidemiological and experimental studies that examined the association between MnSOD and cancer, and describes emerging hypotheses of MnSOD as a mitochondrial redox regulatory enzyme and of how altered mitochondrial redox may affect physiology of normal as well as cancer cells.

Key words: MnSOD, Mitochondria, Superoxide anion, Hydrogen peroxide, Oxidative stress, Redox, Cancer, Epidemiology, Carcinogenesis

SOD AND CARCINOGENESIS

Free radicals are continuously generated as part of normal cellular events as well as due to exogenous stimuli. An example of the former is mitochondrial generation of superoxide anion ($O_2^{\cdot-}$) during oxidative phosphorylation, whereas ionizing radiation is an example for the latter. Imbalance between free radical generation and cellular antioxidant capacity causes oxidative stress during which cellular macromolecules can be transiently or permanently damaged, therein originates the terminology 'oxidative damage'.

The significance of free radicals, oxidative stress and oxidative damage to macromolecules in various diseases is well established including carcinogenesis (Klaunig *et al.*, 2010). In addition to the historical view of carcinogenesis by free radicals through mutation (tumor initiation step) and genomic instability (tumor progression step), many signaling pathways are activated by free radicals leading to increased cellular proliferation, survival and angiogenesis, therefore promoting tumorigenesis (Weinberg and Chandel, 2009).

Since the first observations were reported that cancer cells had decreased manganese superoxide dismutase (MnSOD) activities (Yamanaka and Deamer, 1974; Dionisi *et al.*,

Correspondence to: Aekyong Kim, School of Pharmacy, Catholic University of Daegu, Gyeongbuk 712-702, Korea
E-mail: aekyongkim@cu.ac.kr

1975; Sahu *et al.*, 1977), it has seemed paradoxical that decreased mitochondrial antioxidant capacity gives cancer cells proliferative advantages during carcinogenesis. This review summarizes recent epidemiological studies and experimental data regarding MnSOD and human cancer (for summary, see Table 1), and discusses emerging hypotheses that explain how MnSOD influences cellular proliferation of normal as well as cancer cells.

MnSOD, A MITOCHONDRIAL ANTIOXIDANT ENZYME

The movement of electrons liberated from highly reduced organic molecules to molecular oxygen provides cellular energy to maintain a low entropy state necessary for initiating biochemical reactions. Most biochemical reactions during which electrons move have been demonstrated to be two-electron processes to avoid the formation of products with unpaired electrons (Schafer and Buettner, 2001). Therefore, there seems to be at least two choices to detoxify $O_2^{\cdot-}$: 1) the unpaired electron of $O_2^{\cdot-}$ can be removed to generate ground state oxygen ($^3O_2$), and 2) one additional electron can be added to pair up with the unpaired electron on $O_2^{\cdot-}$, thereby generating peroxide ion (O_2^{2-}). The former strategy has been demonstrated to be utilized in the removal of an electron from $O_2^{\cdot-}$ by vitamin C or E (Gotoh and Niki, 1992; Buettner and Jurkiewicz, 1993), whereas superoxide

Table 1. Summary of epidemiological and experimental studies cited in this review

Description of study	References
Epidemiological study supporting increased risk for breast cancer by Ala16-MnSOD	Ambrosone *et al.* (1999); Mitrunen *et al.* (2001)
Epidemiological study reporting no/insufficient association between breast cancer and Ala16Val-MnSOD	Egan *et al.* (2003); Millikan *et al.* (2004); Kocabas *et al.* (2005); Cheng *et al.* (2005); Slanger *et al.* (2006); Silva *et al.* (2006); Eras-Erdogan *et al.* (2009)
Epidemiological study reporting no/insufficient association between lung cancer and Ala16Val-MnSOD	Lin *et al.* (2003); Wang *et al.* (2004); Lan *et al.* (2004); Ho *et al.* (2006)
Epidemiological study supporting increased risk for prostate cancer by Ala16-MnSOD	Woodson *et al.* (2003); Kang *et al.* (2007); Mao *et al.* (2009)
Epidemiological study reporting no/insufficient association between prostate cancer and Ala16Val-MnSOD	Li *et al.* (2005); Choi *et al.* (2007)
Altered MnSOD expression patterns in human cancers	Izutani *et al.* (1998); Janssen *et al.* (1999); Janssen *et al.* (2000); Malafa *et al.* (2000); Hermann *et al.* (2005)
In vivo multistage skin carcinogenesis study reporting decreased cancer incidence due to increased MnSOD expression	Zhao *et al.* (2001); Zhao *et al.* (2002); Oberley *et al.* (2004); Zhao *et al.* (2005)
In vitro study suggesting establishment of oxidizing redox state due to decreased MnSOD expression in cancer cells and promotion of carcinogenesis	Oberley and Buettner (1979); Oberley *et al.* (1980); Oberley *et al.* (1981); Spitz *et al.* (2000)
In vitro study suggesting increased H_2O_2 generation upon MnSOD overexpression	Wenk *et al.* (1999); Li *et al.* (2000); Rodriguez *et al.* (2000); Zhang *et al.* (2002); Droge (2002); Kinnula and Crapo (2004); Kim *et al.* (2004)
In vitro study reporting relationship between expression pattern of MnSOD and modulation of normal cellular events	Oberley *et al.* (1995); Li and Oberley (1998); Li *et al.* (1998); Kops *et al.* (2002); Kim *et al.* (2004); Kim *et al.* (2010)

dismutases (SODs) pair up the unpaired electron on $O_2^{\cdot-}$ with the unpaired electron from another $O_2^{\cdot-}$, thus utilizing both strategies.

Three isoforms of SOD present in mammalian cells: *Sod1*, *Sod2* and *Sod3*. *Sod1* gene encodes SOD containing copper and zinc in the catalytic site of enzyme, therein originates the name of enzyme, CuZnSOD. Subcellular location of CuZnSOD is nucleus, cytoplasm and mitochondrial intermembrane space. Extracellular SOD (ECSOD) also contains copper and zinc, and is encoded by *Sod3* gene. As its name implies, ECSOD presents at plasma membrane and is released into extracellular matrix. Contrary to *Sod1* and *Sod3* genes, *Sod2* gene encodes MnSOD that requires manganese in the catalytic site of enzyme and whose subcellular location is exclusively mitochondrial matrix (Miao and St Clair, 2009).

Mitochondria are a source of $O_2^{\cdot-}$ production; for a 60 kg woman, 160~320 mmol, and for a 80 kg man, 215~430 mmol of $O_2^{\cdot-}$ are produced each day (Cadenas and Davies, 2000). Mitochondria must have evolved to sufficiently protect themselves from $O_2^{\cdot-}$-mediated damage; schematic representation of mitochondrial antioxidant enzyme systems is shown in Fig. 1. Physiological significance of proper removal of mitochondrial $O_2^{\cdot-}$ has been well demonstrated by the fact that every MnSOD knockout mouse strain currently available showed either embryonic or neonatal lethality (Jang and Remmen, 2009).
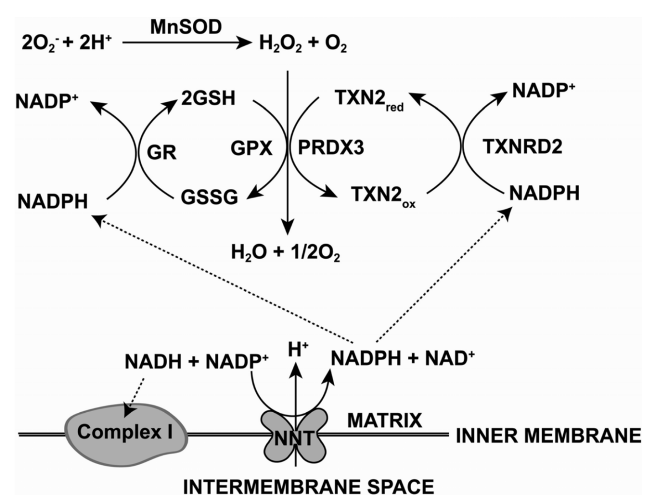

Fig. 1. Mitochondrial antioxidant enzymes involved in the removal of superoxide anion. Mitochondrial superoxide anion ($O_2^{\cdot-}$) is generated as a byproduct of oxidative phosphorylation. Enzymatic conversion of $O_2^{\cdot-}$ to H_2O_2 is catalyzed by MnSOD. H_2O_2 is further reduced to H_2O via GSH system (GPX/GR/GSH) or TXN system (PRDX3/TXNRD2/TXN2). NADPH, the reducing equivalent for these systems is regenerated by NNT at the expense of intermembrane proton gradient and NADH. Abbreviations: MnSOD, manganese superoxide dismutase; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; PRDX3, peroxiredoxin 3, TXNRD2, thioredoxin reductase 2; TXN2, thioredoxin 2; NNT, nicotinamide nucleotide transhydrogenase.

MnSOD REGULATION ON GENE, RNA AND PROTEIN LEVELS

The human *sod2* gene is located in chromosome region 6q25 (Church *et al.*, 1992). The promoter region of *sod2* gene contains a TATA/CAAT-less, GC-rich sequence which is often found in many housekeeping genes (Yeh *et al.*, 1998). Specificity binding protein 1 (Sp-1) and activator protein 2 (AP-2) are mainly involved in the basal expression of MnSOD through their overlapping binding sites on the proximal promoter region of the *sod2* gene (Kuo *et al.*, 1999; Xu *et al.*, 1999, 2002). Sp-1 is essential for the transcription of the *sod2* gene, whereas AP-2 is a negative regulator whose binding affinity is frequently altered in cancer cells and SV-40 transformed fibroblasts (Huang *et al.*, 1997; Xu *et al.*, 1999, 2002; Zhu *et al.*, 2001; Zhu *et al.*, 2001). Basal expression of MnSOD among various tissues seems to depend on the metabolic activity of specific organs since higher MnSOD levels are often found in heart, brain, liver and kidney (Kinnula and Crapo, , 2004).

The mRNA levels of MnSOD in human tissues increase with age (Asikainen *et al.*, 1998). Also, MnSOD expression is highly inducible, often in a stimulus-specific enhancer element and transcription factor–dependent manner (Kim *et al.*, 1999; Rogers *et al.*, 2001; Kops *et al.*, 2002; Guo *et al.*, 2003). While MnSOD can be induced under various conditions resulting in increased MnSOD activity, it has also been noted that mRNA levels are often not correlated with MnSOD activity (Asikainen *et al.*, 1998; Kinnula and Crapo, 2004). Additional regulations at post-transcriptional, translational, and post-translational levels modulate MnSOD activity (Clerch *et al.*, 1998; Kinnula and Crapo, 2004; Hopper *et al.*, 2006; Castellano *et al.*, 2009) (see Fig. 2).
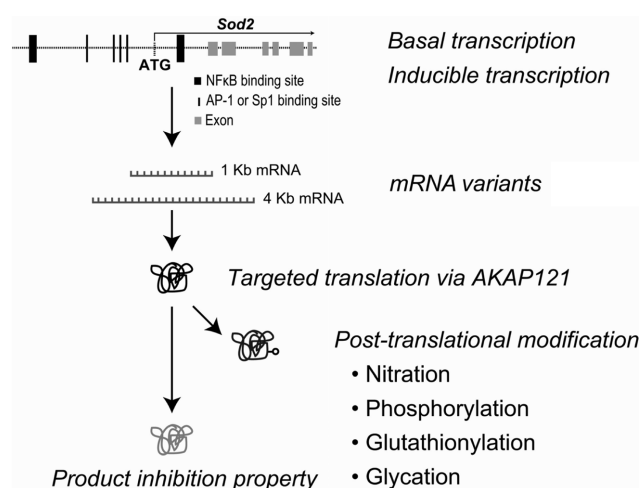
Fig. 2. Regulation of MnSOD expression at gene, RNA and protein levels. Expression and activity of MnSOD can be modulated at various levels. Detailed mechanisms are described in the text. The drawing is not to the scale.

The human *sod2* gene is transcribed into either 1 kb or 4 kb mRNA by alternative polyadenylation; the 4 kb mRNA accumulates faster but has a shorter half-life than the 1 kb mRNA (Melendez and Baglioni, 1993). The stability of MnSOD mRNA can be modulated through its coding region determinant of instability as well as the 3' UTR element/MnSOD RNA-binding protein (Chung *et al.*, 1998; Davis *et al.*, 2001; Knirsch and Clerch, 2001). Targeting of MnSOD mRNA to the outer membrane of mitochondria is mediated by a protein kinase A anchoring protein 121; this targeted translation of MnSOD has been suggested to result in increased mitochondrial MnSOD expression (Ginsberg *et al.*, 2003).

Several tyrosine residues in the MnSOD protein can be nitrated, resulting in decreased activity (Macmillan-Crow *et al.*, 1998). Other post-translational modifications on MnSOD protein such as glutathionylation, phsophorylation and glycation have been reported (Hopper *et al.*, 2006; Castellano *et al.*, 2009) but their effects on MnSOD activity are yet to be investigated. Future studies are necessary to understand how post-translational modifications on MnSOD occur under which circumstances and the pathophysiological significances of these modifications. In addition, intrinsic inhibition of MnSOD activity by H_2O_2, its own enzymatic product, has been demonstrated; a mutant form of MnSOD (His30 to Asn) devoid of characteristic product inhibition was generated with increased enzyme efficiency (Davis *et al.*, 2004).

Sod2 POLYMORPHIC VARIANTS

Several polymorphic variants of the *sod2* gene have been identified. An amino acid substitution (Ala16 to Val) in the mitochondrial leading sequence (MLS) is prevalent among healthy human population. Shimoda-Matsubayashi and colleagues first reported Ala16Val dimorphism in MLS of MnSOD precursor protein among healthy Japanese and patients with Parkinson's disease (Shimoda-Matsubayashi *et al.*, 1996). Allelic frequencies of Ala16 and Val16 in the MLS of MnSOD precursor protein among Caucasian women are approximately even, whereas MnSOD precursor protein with Val16 is a dominant form among Asian women at over 85% (Silva *et al.*, 2006). Sutton's group has shown that an α-helical structure of the MLS of Ala16-MnSOD precursor protein seems to be favorable during mitochondrial translocation process compared to a β-sheet structure of the MLS of Val16-MnSOD precursor protein (Sutton *et al.*, 2003). Impaired mitochondrial transporting of Val16-MnSOD precursor protein and subsequently decreased its mRNA stability due to impaired cotranslational import resulted in 40% lower MnSOD activity than Ala16-MnSOD precursor protein in mitochondrial matrix (Sutton *et al.*, 2003; Sutton *et al.*, 2005).

Another naturally occurring polymorphic variant of MnSOD is an amino acid substitution in the mature protein (Ile58 to

Thr) (Ho and Crapo, 1988). Packing defects in the tetrameric interface of Thr58-MnSOD result in a thermodynamically unstable enzyme with only one third of Ile58-MnSOD enzymatic activity (Borgstahl et al., 1992; Borgstahl et al., 1996; Zhang et al., 1999). Studies reported the lack of threonine at amino acid position 58 of MnSOD protein among 63 German Caucasian Parkinson's disease patients (Grasbon-Frodl et al., 1999) or 1197 healthy subjects and 1125 breast cancer patients from urban Shanghai China (Cai et al., 2004). From a human T-cell leukemia cell line Jurkat, a thiol-sensitive mutant form of MnSOD (Leu60 to Phe) was identified. Zhang and colleagues speculated that Phe60-MnSOD might have similar packing defects in the tetrameric interface as Thr58-MnSOD rendering thiol-sensitivity (Hernandez-Saavedra and Mccord, 2003). However, it is yet to be shown that Thr58 or Phe60-MnSOD is also prevalent among healthy human population.

Ala16Val MnSOD DIMORPHISM AND CANCER: EPIDEMIOLOGICAL EVIDENCES

In human, almost all tumors have decreased MnSOD activities (Oberley, 2005). However current epidemiological data does not strongly suggest the role of MnSOD in the etiology of cancer. Extensive epidemiological studies have mostly been focused on the Ala16Val dimorphism of MnSOD as a risk factor for breast cancer. Ambrosone and colleagues were the first to report the Ala16-MnSOD as a breast cancer risk factor (Ambrosone, et al., 1999). This study was a case-control study with 266 breast cancer cases and 295 controls whose study subjects were Caucasian women from western New York USA and their menopausal status and dietary antioxidants intakes were considered as other risk modifiers. They reported a 4.3-fold increased breast cancer risk among premenopausal women homozygous for the A allele compared to those with homozygous V alleles. This risk was more pronounced among premenopausal women with low fruit/vegetable consumption or dietary antioxidants intakes with increased risks over 4 to 7.7-folds. Similar observations were made among women from urban Shanghai China in a case-control study consisted of 1125 breast cancer cases and 1197 controls although most odd ratios lacked statistical power due to low AA alleles frequency among study population (Cai et al., 2004).

Another study reported the Ala16-MnSOD as a risk factor for breast cancer (Mitrunen et al., 2001). The study cohort was consisted of 483 breast cancer cases and 482 controls of Finnish Caucasian women whose alcohol consumption, history of smoking, use of oral contraceptives, postmenopausal estrogen use (presumed risk factors) and antioxidants supplements intake (presumed preventive factor) were examined as other risk modifiers. Women with MnSOD gene homozygous for the A allele had a 1.4-fold increased risk for breast cancer compared to those with homozygous

for the V allele. This risk was more pronounced among subjects with any of presumed risk factors although they were mostly lacked a statistical power presumably due to small subject numbers. There were two discrepancies from this study when compared to one by Ambrosone et al. (1999): 1) menopausal status of study subjects from this study was not associated with the risk of MnSOD AA alleles, and 2) risk of MnSOD AA alleles compared to MnSOD VV alleles was more pronounced among those taking dietary antioxidant supplements.

The first study contradicting previous reports of MnSOD AA alleles as a breast cancer risk factor was also made by Ambrosone's group (Egan et al., 2003). The study cohort was consisted of 476 breast cancer cases and 502 controls of mostly Caucasian women (>98%) with European ancestry (>98%) from Massachusetts and New Hampshire USA. Presumed risk factors examined included history of smoking, alcohol consumption, oral contraceptives, hormone replacement therapy and body mass index, whereas presumed preventive factor fruit/vegetable consumption. They reported lack of association between Ala16Val MnSOD dimorphism and breast cancer risk. Through a Meta-analysis of three studies (Ambrosone et al., 1999; Mitrunen et al., 2001; Egan et al., 2003), Ambrosone's group suggested that MnSOD AA alleles was associated with breast cancer risk at a marginal range of 30~50% increase both in pre- and post menopausal women but lacking statistical power (Egan et al., 2003).

Supporting results by Ambrosone's group (Egan et al., 2003), a study thus far with the largest cohort size of 2025 breast cancer cases and 1812 controls composed of African American and whites from North Carolina USA concluded the lack of association between MnSOD AA alleles and breast cancer risk (Millikan et al., 2004). Similar conclusions were drawn from more studies whose study subjects were Turkish population (Kocabas et al., 2005; Eras-Erdogan et al., 2009), Taiwanese (Cheng et al., 2005), German Caucasian (Slanger et al., 2006), Portugal Caucasian (Silva et al., 2006). Therefore, current epidemiological data suggest that Ala16Val MnSOD dimorphism is not an independent risk factor for breast cancer.

In addition, four epidemiological studies have examined the Ala16Val MnSOD dimorphism as a lung cancer risk factor among Caucasian (Wang et al., 2004) or Chinese population (Lin et al., 2003; Lan et al., 2004; Ho et al., 2006), and reported lack of overall association between Ala16Val MnSOD dimorphism and lung cancer.

A few epidemiological studies have examined the Ala16Val MnSOD dimorphism as a prostate cancer risk factor and reported conflicting results (Woodson et al., 2003; Li et al., 2005; Choi et al., 2007; Kang et al., 2007; Mao et al., 2009). Woodson and colleagues were the first to report an association between Ala16Val MnSOD dimorphism and prostate cancer risk (Woodson et al., 2003). They con-

ducted a case-control study whose cohort was consisted of 197 prostate cancer cases and 190 controls of Finnish males. Man with MnSOD gene homozygous for the A allele had a 1.72-fold increased risk for prostate cancer and a 2.72-fold for high-degree of tumor compared to those with homozygous for the V allele. Alpha-tocopherol supplementation did not affect the degree of association between Ala16Val MnSOD dimorphism and prostate cancer risk.

Kang and colleagues also reported the MnSOD AA alleles as a prostate cancer risk factor (Kang et al., 2007). This study was a nested case-control study whose cohort was consisted of 1320 prostate cancer cases (1213 Caucasian and 107 African-American) and 1842 controls (1433 Caucasian and 409 African-American) from ten US cities. Men with MnSOD gene homozygous for the A allele had a 1.28-fold increased risk for prostate cancer compared to those with homozygous for the V allele. This risk was more pronounced among subjects with low dietary antioxidants intakes with increased risks over 1.34 to 1.56-folds. Contrary to reports concluding positive association (Woodson et al., 2003; Kang et al., 2007), two studies reported lack of association between Ala16Val MnSOD dimorphism and prostate cancer risk among Caucasian males (Li et al., 2005; Choi et al., 2007).

Recently, Mao and colleagues retrieved 12 eligible studies from PubMed and Embase (some of these studies are mentioned above) and performed a Meta-analysis between Ala16Val MnSOD dimorphism and the risk for prostate cancer (Mao et al., 2009). They reported that Ala16 genotype significantly increased prostate cancer incidence overall, and that ethnically, Ala16 genotype was a risk factor for prostate cancer in Caucasians, not in Africans. Therefore, current epidemiological data suggest that Ala16Val MnSOD dimorphism is an independent risk factor for prostate cancer in certain ethnic groups.

It is not intuitively clear how to explain elevated cancer risks by MnSOD AA alleles considering that MnSOD is a mitochondrial antioxidant enzyme and that Ala16-MnSOD precursor protein may result in higher MnSOD activity in the mitochondrial matrix (Sutton et al., 2003, 2005). Wang and colleagues (Wang et al., 2004) reported only piece of epidemiological data suggesting an increased cancer risk by MnSOD VV alleles, which complements the fact that in human almost all tumors have decreased MnSOD activities (Oberley, 2005). They reported that when subjects had no or low asbestos exposure, there was a 2.14-fold increased lung cancer risk among Caucasian males with MnSOD VV alleles compared to those with MnSOD AA alleles although it lacked statistical power due to small sample size. Another coinciding, albeit indirect, observation from an epidemiological study is that a breast cancer resistant population, Xavnate Indians from Sangradouro Mato Grosso Brazil, has unusually high A allelic frequency (77%) and AA genotypic frequency (59.2%) in MnSOD, suggesting a decreased cancer risk by MnSOD AA alleles (Silva et al., 2006).

Mitrunen et al. speculated the negative role of Ala16-MnSOD in cancer incidence may be due to the accumulation of H_2O_2 under poor H_2O_2-scavenging conditions with increased MnSOD activity (Mitrunen et al., 2001). This may explain why association between MnSOD AA alleles and cancer risks often become stronger when subjects have other risk modifiers such as low dietary antioxidant intakes or low serum antioxidant levels (Cai et al., 2004; Li et al., 2005; Kang et al., 2007). Therefore, future epidemiological studies to better understand the roles of MnSOD in etiology of cancer should simultaneously consider other antioxidant enzyme profiles as well as antioxidant capacity of study subjects.

MnSOD AND *IN VIVO* CANCER MODELS: EXPERIMENTAL EVIDENCES

Understanding of the roles of MnSOD in cancer etiology has been also approached by means of *in vivo* cancer progression studies in human (Izutani et al., 1998; Janssen et al., 1999, 2000; Malafa et al., 2000; Hermann et al., 2005) and multistage skin carcinogenesis models (Zhao et al., 2001, 2002; Oberley et al., 2004; Zhao et al., 2005). Although data are limited and some conflicting, these studies provide us with insights into how intimately a mitochondrial protein influences normal as well as pathological cellular events such as proliferation, differentiation, apoptosis and metastasis. It has long been predicted by Oberley and Buettner that all cancers should have decreased MnSOD activity (Oberley and Buettner, 1979), which has been proven to be mostly accurate (Oberley, 2001, 2005). However, *in vivo* progression and metastasis of cancer are orchestrated by morphologically and functionally heterogeneous cells within tumor mass (Brabletz et al., 2005), and at each stage of carcinogenesis, MnSOD expression profile seems to change (Izutani et al., 1998; Janssen et al., 1999, 2000; Malafa et al., 2000; Hermann et al., 2005).

Human esophageal/gastric and colorectal cancers are two most extensively investigated tumors through which stepwise progression of cancer, from adenoma to carcinoma and then to metastases, is well defined. When compared to normal mucosal tissue, MnSOD expression was increased in esophageal squamous cell carcinoma and gastric adenocarcinoma (Izutani et al., 1998). Jansson and colleagues reported that this was also held in colorectal carcinoma, and that liver metastases had similar MnSOD expression profile to that of carcinoma (Janssen et al., 1999). Patients with metastatic adenocarcinoma gastric cancer had increased MnSOD expression at over 90% of cases, whereas patients with non-metastatic one did so at only 44% of cases (Malafa et al., 2000). Furthermore, the higher ratio of MnSOD expression profile of tumor over normal tissue, the poorer overall survival of patient with esophageal squamous cell carcinoma

or gastric carcinoma (Janssen *et al.*, 2000). Therefore, as esophageal/gastric cancer progresses, MnSOD expression gradually increases; with increased MnSOD expression, likelihood of metastasis potentiates and prognosis of patient deteriorates (Izutani, 1998; Janssen *et al.*, 2000; Malafa *et al.*, 2000) Contrary to these studies, Hermann and colleagues reported that MnSOD expression decreased and remained lower than normal tissue during the progression of Barrett esophagus to dysplasia, then to esophageal adenocarcinoma (Hermann *et al.*, 2005). These conflicting observations warrant further studies in various *in vivo* cancer progression models to examine changes in MnSOD expression during each phase of cancer progression and underlying mechanisms and driving force of their changes.

A multistage skin carcinogenesis model is an experimental approach where initiation and promotion of cancer can be experimentally manipulated. One of the best studied is the two-stage DMBA/TPA skin carcinogenesis model where 7,12-dimethylbenz[a]anthracene (DMBA) acts as an initiator and 12-O-tetradecanoylphorbol-13-acetate (TPA) a promoter of carcinogenesis. The progression of skin cancer from papillomas to squamous carcinoma and then to invasive undifferentiated carcinoma has been well characterized morphologically as well as biochemically in this system (Zhao *et al.*, 2001; St. Clair *et al.*, 2005). Elevated MnSOD expression suppressed DMAB/TPA induced skin papilloma formation (Zhao *et al.*, 2001). There were decreased levels of oxidative stress in not only mitochondria but also nucleus and delayed mitochondrial oxidative damages in MnSOD transgenic mice when compared to wild type mice (Zhao *et al.*, 2001; Oberley *et al.*, 2004). Elevated levels of oxidative damage with decreased MnSOD expression were also demonstrated with *Sod2* heterozygous mutant mice following DMBA/TPA treatment (Zhao *et al.*, 2002). However, there was no increased incidence of papilloma formation in *Sod2* heterozygous mutant mice (Zhao *et al.*, 2002). Therefore, in the processes of carcinogenesis which are by nature complex and dynamic, levels of MnSOD expression alone may not be sufficient to predict the outcomes of progression of tumor.

Interestingly, from some of these studies, higher incidences of apoptosis as well as mitosis were reported both in MnSOD transgenic mice and *Sod2* heterozygous mutant mice (Zhao *et al.*, 2002, 2005; Oberley *et al.*, 2004). Early and sustained activation of apoptotic pathway in *Sod2* heterozygous mutant mice was demonstrated (Zhao *et al.*, 2002). Peak of apoptosis was preceded that of mitosis; preor post-treatment of a MnSOD mimetic upon TPA treatment stage dramatically decreased the number of mitotic cells without affecting apoptotic population, which resulted in decreased number and mass of papilloma (Zhao *et al.*, 2005). These observations can not sufficiently be explained by MnSOD being a mitochondrial antioxidant enzyme protecting mitochondria from oxidative stress or being a tumor suppressor inhibiting cellular proliferation. To explain these seemingly conflicting findings, one needs to understand how as well as by which molecular mechanisms, mitochondria with MnSOD expression deviated from norm influence cellular physiology. Then, one would be able to explain why it is critical to tightly control the expression levels of MnSOD to maintain normal cellular physiology.

MnSOD AS A TUMOR SUPPRESSOR

Since the first observations (Dionisi *et al.*, 1975; Sahu *et al.*, 1977), why and how of altered MnSOD activities in malignant cells have been intensive research interests for over 30 years. Oberley and Buettner proposed that if MnSOD is a critical determinant in malignancy of tumor, increased MnSOD expression would reinstall at least some of normal cellular phenotypes into malignant cells (Oberley and Buettner, 1979). As predicted, MnSOD activity was directly correlated with cell doubling time and inversely with platting efficiency and clonogenicity of tumor cells, which resulted in decreased tumorigenicity in nude mice (Zhang *et al.*, 1999; Oberley, 2005). Hence, some consider MnSOD as a tumor suppressor (Oberley, 2005).

In light of its antioxidant enzymatic function, the observation that many types of malignant tumors often have decreased MnSOD activities seems to be quite puzzling. Oberley and colleagues hypothesized that balance between $O_2^{\cdot-}$ as a proliferation signal and H_2O_2 as a differentiation signal was disturbed in cancer cells, and that increased $O_2^{\cdot-}$ influx due to lack of MnSOD contributed to infinite growth potential and loss of differentiation potential of cancer cells (Oberley *et al.*, 1980, 1981). Secondary to altered metabolic activity, cancer cells were proposed to have more oxidizing redox environments compared to normal cells (Spitz *et al.*, 2000). Many proteins involved in signaling pathways and transcription factors are redox-sensitive (Droge, 2002; Rhee *et al.*, 2003; Kinnula and Crapo, 2004); binding of growth factors to their cell membrane receptors results in the production of ROS (Droge, 2002; Adachi *et al.*, 2004). Therefore, an oxidizing redox environment established due to loss of MnSOD would be favorable for cancer transformation (Oberley and Buettner, 1979; Oberley *et al.*, 1980, 1981; Spitz *et al.*, 2000).

The inverse correlation between cellular proliferative potential and MnSOD activity also applied to nonmalignant cells (Li and Oberley, 1998; Li *et al.*, 1998; Kim *et al.*, 2004). MnSOD activities change in different stages of the cell growth curve *in vitro* (Oberley *et al.*, 1995). In addition, MnSOD expression and its activity change during the cell cycle, that is less MnSOD activity in S phase cells compared to ones in G_0 phase (Oberley *et al.*, 1995; Li and Oberley, 1998). It has been shown that MnSOD expression in quiescent cells was mediated by FOXO3a transcription factor and its DNA binding elements on the promoter

region of the *sod2* gene (Kops *et al.*, 2002). These observations support the notion of an inverse correlation between proliferation and MnSOD activity. It was recently confirmed that MnSOD activity regulates cellular proliferation *in vivo* (Kim *et al.*, 2010).

Although it is still disputed (Liochev and Fridovich, 2007), cellular steady state levels of H_2O_2 seem to be influenced by H_2O_2, enzymatically generated by MnSOD activity (Buettner *et al.*, 2006). Independent laboratories reported increased cellular ROS levels following experimental manipulations in cell culture systems designed to increase MnSOD activities (Wenk *et al.*, 1999; Li *et al.*, 2000; Rodriguez *et al.*, 2000; Kim *et al.*, 2004). Since H_2O_2 has been demonstrated to regulate certain signaling pathways and transcription factors, H_2O_2 has been proposed to be the mediator of decreased cell growth following MnSOD overexpression (Droge, 2002; Kim *et al.*, 2004; Kinnula and Crapo, 2004). Impaired mitochondrial functions such as decreased ATP production, dysfunctional ETC due to H_2O_2 production were also implicated as possible mechanisms by which elevated MnSOD activity led to growth inhibition (Rodriguez *et al.*, 2000). In addition, enzymatically generated H_2O_2 by MnSOD enhanced matrix metalloproteinase activity (Zhang *et al.*, 2002), which potentiated migratory and invasive phenotype of cancer cells (Connor *et al.*, 2007). This may explain elevated levels of MnSOD expression often seen in metastatic and invasive tumors *in vivo* (Izutani *et al.*, 1998; Janssen *et al.*, 1999; Malafa *et al.*, 2000).

A LESSON LEARNED FROM STUDYING MnSOD AND CANCER

Current epidemiological and experimental data suggest that from the pathophysiological point of view, MnSOD itself per se may not be a determining or causative factor. However, their ability to maintain mitochondria at their optimal physiological conditions according to physiological demands seems to be critical. Under oxidative stress conditions imposed both by endogenous and exogenous initiators, MnSOD serves as the first line of mitochondrial defense mechanisms against oxidative damages by removing excessive $O_2^{\cdot-}$, thereby being a mitochondrial antioxidant enzyme. However, this capability is influenced by mitochondrial H_2O_2-removing capacity since H_2O_2 itself is another form of reactive oxygen species (see Fig. 1). The biological significance of synchronous modulation of MnSOD and mitochondrial H_2O_2-scavenging systems has been reported (Kim *et al.*, 2005a, 2010).

Furthermore, the exclusive view of MnSOD as a mitochondrial antioxidant enzyme is not sufficient to explain some of deleterious outcomes of forced MnSOD expression (Raineri *et al.*, 2001; Kim *et al.*, 2005b; Ford *et al.*, 2007). Emerging opinion regarding roles of MnSOD in cellular pathophysiology stems from the fact that the enzymatic reaction of MnSOD not only removes mitochondrial
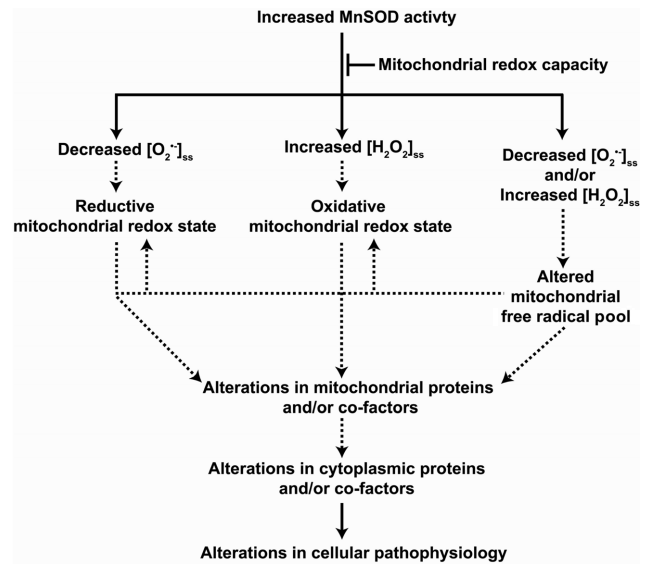
Fig. 3. Possible cellular alterations via transiently increased MnSOD activity. Putative pathways are shown in closed lines. Down-stream events shown in dashed lines may not be required for the manifestation of physiological consequences of MnSOD overexpression. See the text for detailed information.

$O_2^{\cdot-}$ but also generates H_2O_2 as a product whose characteristics differ, thereby altering mitochondrial free radical pool (Cadenas and Davies, 2000) (see Fig. 3). Non-toxic tumor suppressive effects of MnSOD have been proposed to be mediated by through enzymatically generated H_2O_2 (Oberley, 2005) and its cellular (Buettner *et al.*, 2006) and/or mitochondrial (Kim *et al.*, 2004, 2005a) redox environmental modulation. Therefore MnSOD may serve as a mitochondrial redox regulatory enzyme under physiological conditions (Kim *et al.*, 2004, 2005a).

In the light of emerging opinions for mitochondrial retrograde signaling in normal cellular physiology (Schieke and Finkel, 2006), an alternative explanation of product inhibition property of MnSOD (Davis *et al.*, 2004) could be a H_2O_2 signal-off mechanism for a postulated mitochondrial redox mediated signaling network. Altered mitochondrial redox states may result in faster kinetics of accumulation and termination of production of H_2O_2, generating more frequent bursts and higher levels of H_2O_2, which could then initiate a mitochondrial redox signaling pathway. Therefore, the degree of MnSOD activity in mitochondria could be pre-conditioned by coordination between the host cell and its mitochondria in order to optimally serve physiological needs. Through proposed function as a mitochondrial redox regulatory enzyme, alterations in mitochondrial redox state may be converted into a signal initiated by H_2O_2 enzymatically generated by MnSOD. Mitochondrial H_2O_2 generated through such a mechanism could convey 'information regarding mitochondrial redox environments' to downstream pathways in a manner analogous to cytoplasmic H_2O_2 signaling

pathway.

Although it has been proposed by Oberley and colleagues (Oberley et al., 1980, 1981), a possible role of MnSOD as a fine regulator between infinite proliferative and differentiation potentials is yet to be extensively explored, but now holds infinite possibilities with emerging theories of aging and cancer mediated by stem cells (Trosko, 2003; Brabletz et al., 2005). Only 40 years after initial recognition of significance of MnSOD in aerobic organism, we have discovered many pathophysiological roles of MnSOD. Some are supported by solid scientific evidences, but others need further studies to resolve conflicting opinions or to test emerging hypotheses. Through these efforts, we will get one step closer to the complete spectrum of roles of MnSOD in normal physiology as well as pathology of many diseases.

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