Review

Target Sequence Polymorphism of Human Manganese Superoxide Dismutase Gene and Its Association with Cancer Risk: A Review

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Abstract

In normal state of a cell, endogenous antioxidant enzyme system maintains the level of reactive oxygen species generated by mitochondrial respiratory chain. Mitochondrial superoxide dismutase [SOD; manganese SOD (MnSOD) or SOD2] neutralizes highly reactive superoxide radical ($O^{\bullet}_2^-$), the first member in the plethora of mitochondrial reactive oxygen species. A polymorphism in the target sequence of MnSOD enzyme, Val[16]Ala, is known to disrupt proper targeting of the enzyme from cytosol to mitochondrial matrix where it acts on $O^{\bullet}_2^-$ to dismutate it to hydrogen peroxide (H_2O_2). A change in the level of $O^{\bullet}_2^-$ and of H_2O_2 in mitochondria modulates the molecular mechanisms of apoptosis, cellular adhesion, and cell proliferation and thus play key role in cancer development. Previous studies investigating the association between *MnSOD* Val[16]Ala polymorphism and cancer risk have revealed inconsistent results. We conducted a meta-analysis on these studies. Our meta-analysis on total of 7,366 cancer cases and 9,102 controls from 13 published case-control studies showed no overall association of this polymorphism either with breast cancer risk or for cancer risk as such (for Ala homozygous odds ratio, 0.98; 95% confidence interval, 0.90-1.07 and odds ratio, 1.02; 95% confidence interval, 0.91-1.14, respectively). Also, there was no major effect in either recessive or dominant model for the *MnSOD* Val[16]Ala. However, a proper evaluation of this polymorphism with cancer link demands experiments involving large sample size, cross-tabulation of gene-gene, gene-environment interactions, and linkage studies, as cell biological experiments clearly correlate critical levels of mitochondrial $O^{\bullet}_2^-$ and H_2O_2 to carcinogenesis. (Cancer Epidemiol Biomarkers Prev 2008;17(12):3298–305)

Reactive oxygen species (ROS) are constantly generated in aerobic organisms as a consequence of normal metabolism. ROS include free oxygen radicals [e.g., superoxide ($O^{\bullet}_2^-$), hydroxyl radical (•OH), nitric oxide (•NO), alkoxyl- (RO•), and peroxyl- (ROO•) radicals] as well as nonradical ROS [e.g., hydrogen peroxide (H_2O_2), organic hydroperoxides, and hypochloride (1)]. A low level of ROS is indispensable in several physiologic processes of cell including cell proliferation, apoptosis, cell cycle arrest, cell senescence, etc. (1). However, an increased level of ROS causes oxidative stress and creates a potentially toxic environment to the cells. In normal physiologic condition, a balance between ROS generation and oxidative defenses exists in cell. A significant role is played by endogenous antioxidant enzymes such as manganese superoxide dismutase (MnSOD), catalase, glutathione peroxidase (Gpx1), and peroxiredoxins (Prx) in the cellular defense against oxidative stress. This stressful condition may appear either due to antioxidant depletion or due to an exposure to toxic agents and/or pathologic processes or both. This stressful condition is known to play a major role in cancer development mainly by enhancing DNA damage and by modifying some key cellular processes. For example, although DNA damage is caused primarily by hydroxyl radicals, which possesses the capacity to act in a nonspecific manner (1), other species such as superoxide radicals and hydrogen peroxides are known to play role in cancer development in more specific manner largely by regulating signaling cascades involved in cell proliferation, apoptosis, and cell motility.

MnSOD, one of the major antioxidant enzymes, catalyzes the dismutation of superoxide radicals to H_2O_2 and oxygen in mitochondria and thus constitutes first-line defense against ROS in mitochondria. Hence, it is conceivable that structural and/or functional polymorphisms of *MnSOD* gene are of immense importance in the maintenance of ROS level in cell. Low expression of *MnSOD* has often been accounted for different types of cancer formation, whereas overexpression of this enzyme has been linked with inhibition of cancerous growth in humans, implicating it as a tumor suppressor gene (2).

MnSOD is a homotetramer enzyme that binds one manganese ion per subunit. Human *MnSOD* gene is a nuclear gene encoding this mitochondrial protein. The

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MnSOD gene is located on chromosome 6 (6q25.3) and encodes human *MnSOD*, also known as *SOD2*. SOD has other two isoforms in humans, a homodimeric cytosolic CuZnSOD or SOD1 (21q22.1) and an extracellular homotetrameric copper and zinc containing SOD3 (4p15.3-p15.1). Among these three isoforms, *SOD2* polymorphisms have largely been implicated with cancer risk, whereas polymorphs of *SOD1* and *SOD3* have been rarely studied for cancer link and found to have no significant association with cancer risk (3).

The most commonly studied polymorphism of *MnSOD* is Val^{16}Ala on mitochondrial target sequence. It is a single nucleotide polymorphism (SNP) in codon 16. A substitution of T to C at nucleotide 47 changes the encoded amino acid from Val (GTT) to Ala (GCT) on the 16th residue of 24-amino acid signal sequence that helps in targeting the nascent protein to mitochondria. This residue is 9-amino acid upstream of the cleavage site, hence, has often been designated as Ala^{9}Val polymorphism (4). A newly synthesized MnSOD is transported to mitochondria when it is assembled into a homotetramer. Shimoda-Matsubayashi et al. (5) predicted that Val allele would encode a β-sheet conformation rather than a preferred α-helical structure of MnSOD precursor protein that leads to an impaired transport of MnSOD to mitochondria. Sutton et al. (6) found that the Ala form of MnSOD is targeted into the mitochondria, whereas the Val form is partially arrested in the inner mitochondrial membrane. Their study revealed that Ala form of MnSOD was 30% to 40% more efficiently localized to the mitochondria than the Val form. In view of these findings, it is expected that the Val form is likely to be associated with higher levels of ROS and thus predisposes to a greater risk of cancer. However, various experiments aiming to study association between this polymorphism and different carcinoma reveal a controversial picture. Although few reports find associations between Val form and higher cancer risk, major studies have shown the Ala form to be associated with the risk of different types of cancer (7). At the same time, some other studies find no association of this polymorphism with cancer risk at all.

Association with Breast Cancer. Breast cancer is the most common form of cancer among women (8). Germline mutations in genes such as *BRCA1*, *BRCA2*, and *ATM* are reportedly responsible for substantial increase in risk of breast cancer. However, these mutations are rare in the general population and account for little of the incidence of sporadic breast cancer (9). Therefore, research has been focused on associations between common polymorphisms and breast cancer risk. As oxidative stress has often been linked with breast cancer risk, polymorphisms of antioxidant enzyme MnSOD have widely been discussed for their association with this carcinoma.

The Val^{16}Ala polymorphism has been most widely studied in relation to breast cancer. Bergman et al. (10) found significantly increased risk of breast cancer for individuals with Val/Val and Val/Ala genotypes [odds ratio (OR), 2.7; 95% confidence interval (95% CI), 2.2-5.5 and OR, 3.0; 95% CI, 1.4-6.5, respectively]. Although Silva et al. (8) did not observe any significant role of this polymorphism in breast cancer susceptibility in their case-control study, when they stratified the population based on breast-feeding, they found that women who never breast-fed had a marginally decreased risk for the disease when they were having Ala allele (OR, 0.56; 95% CI, 0.32-0.99). Although only one study evidenced significant association of Val morph with higher breast cancer risk (10) and one study revealed Ala morph with a protective role (8), majority of the experiments linked Ala allele with elevated risk of breast cancer, if not in isolation, in combination with the effects of other risk factors. Joint effects of genotype and smoking were revealed in the experiment of Mitrunen et al. (11) where they found that postmenopausal women with past smoking history and an Ala allele had higher risk of cancer development (OR, 3.7; 95% CI, 1.4-9.9) than their Val allele containing counterparts. Millikan et al. (12) observed moderate joint effects between smoking and Ala-containing genotypes (OR, 1.5; 95% CI, 1.0-2.2). Ambrosone et al. (13) and Cai et al. (14) evidenced higher-risk Ala women, particularly for those who were in premenopausal phase (OR, 4.3; 95% CI, 1.7-10.8 and OR, 1.8; 95% CI, 0.9-3.7, respectively). Mitrunen et al. (11) observed significant interactions between *MnSOD* genotypes and postmenopausal use of estrogen. Postmenopausal women who had undergone estrogen replacement therapy and had Ala allele containing genotype had a 2.5-fold higher risk of breast cancer. However, some findings may bring a ray of hope to women having higher antioxidant intake habit, as Ala women taking appropriate amount of dietary antioxidants, fruits, vegetables, etc., were found to be at a decreased risk for breast cancer (14). Contradictorily, in clinically aggressive prostate cancer, Choi et al. (15) found Val/Val men with higher iron intake level had 2-fold higher risk for prostate cancer (OR, 2.3; 95% CI, 1.0-4.9). Interestingly, Cox et al. (9) showed a higher risk for Ala homozygous women (OR, 1.87; 95% CI, 1.09-3.19), although not in isolation, rather in association with another polymorphism, Leu^{198}Leu, in another oxidative stress gene, Gpx1. These two genes are active on the pathway of detoxification of ROS from superoxide ($O_2^{\bullet-}$) to H_2O_2 (by MnSOD) and further to H_2O (by Gpx1). These two polymorphisms, Val^{16}Ala of MnSOD and Pro^{198}Leu, modify the efficiency of this detoxification pathway. Hence, it is conceivable that a combination of these two polymorphisms could be associated with elevated level of breast cancer risk. Another gene-gene interaction study (16) in combination with cytochrome *P*4501B1 (*CYP1B1*) and catechol-*O*-methyltransferase (*COMT-L*) genes documented higher risk of Ala^{16} allele of MnSOD for breast cancer in the women with a body mass index greater than 24 kg/m^2 (OR, 1.42; 95% CI, 1.04-1.93).

Although most of the authors find association of either Val or Ala alleles with breast cancer risk either singly or together with other polymorphisms, a population-based case-control study shows no overall association of this polymorphism with this cancer (17). Egan et al. (17) did not observe any significant risk for women either with Ala carrier or homozygous genotypes. Similar result was reported by Gaudet et al. (18), as they did not find any higher risk for these polymorphs. Also, their results did not differ by menopausal status.

Association Study with Other Types of Cancer. Val^{16}Ala polymorphism of *MnSOD* has been examined

widely for its association with risk of other types of cancers, other than breast cancer. Woodson et al. (19) examined the role of this polymorphism in the development of prostate cancer in male smokers. They found that men homozygous for Ala allele had a 70% increased risk for prostate cancer over the Val/Val men. In yet another experiment, Kang et al. (3) evaluated the association of prostate cancer with genetic polymorphisms of three main isoforms of SOD: SOD1, SOD2, and SOD3. They found that *SOD2* Ala homozygous was associated with moderately increased risk of prostate cancer (OR, 1.28; 95% CI, 1.03-1.60). However, no significant association with prostate cancer risk was observed for SOD1 and SOD3 isoforms. Ala allele was found with statistically higher risk for prostate cancer in a study on Turkish population also (20).

Ala^{16}Val polymorphism of *MnSOD* has also been identified as important risk factor for cancer of digestive system. Whereas Stoehlmacher et al. (21) linked the Ala allele of this polymorphism with an increased risk to develop colorectal cancer, Levine et al. (22) identified a protective role of Ala for distal colorectal adenomas, known precursor lesions for colorectal cancers. No association between this polymorphism and gastric cancer could be detected by Martin et al. (23). Val/Val genotype was found to be a risk factor for bladder cancer (OR, 1.91; 95% CI, 1.20-3.04) by Hung et al. (24).

Association with Diseases Other Than Cancer. Besides cancer risk, Val^{16}Ala polymorphism of *MnSOD* has often been reported to modulate the risk for some neurodegenerative diseases [Parkinson's disease (25), schizophrenia (26), etc.] Homozygosity for Ala has been found to be a major risk factor for severe alcoholic liver disease (27). Association of this polymorphism with lung cancer has not been reported much widely (28, 29). However, it has been found to be associated with chronic airway inflammation disease, asthma (30). This polymorphism has also been linked with cardiovascular risk (31, 32). Genotype for Val instead of Ala has been found to be associated with systemic lupus erythematosus (33), an autoimmune disorder, and with chronic pelvic pain syndrome in men (34).

In this article, we review association studies of Val^{16}Ala polymorphism of human *MnSOD* gene with cancer risk and analyze plausible cellular mechanisms responsible for its development and progression. Because case-control studies revealed contradictory results, we carried out a meta-analysis of the published data also to get a more precise estimation of this polymorphism as a cancer risk factor.

Materials and Methods

Identification of Relevant Studies. Studies were identified through electronic databases, MEDLINE, and BioInfoBank Library using search keywords ''MnSOD Val^{16}Ala polymorphism and cancer'' or ''MnSOD polymorphism and cancer.'' We included the articles written in English language only. Selected articles had case-control design (including nested case-control ones). Some studies could not be included as raw data were not available for calculation of risks (only abstracts of these studies could be collected). Three of them were on breast cancer (10, 17, 18) and 5 were on other cancer types (19-21, 23, 24). Case-control studies of Millikan et al. (12) and Kang et al. (3) were carried out for two ethnic groups; hence, each of these studies was considered as two reports. Study of Ambrosone et al. (13) reported genotype risk for premenopausal and postmenopausal women separately. Hence, this study was also considered as two different observations. Therefore, the present meta-analysis included 13 published case-control studies on *MnSOD* Val^{16}Ala association with cancer risk. For each study, we abstracted country name where these experiments were carried out, ethnicity of the study populations, and number of cases and controls (Table 1). We used the term ND (not defined) if ethnicity was not clearly mentioned in original article.

Meta-analysis. Allelic frequency of the variant allele (^{16}Ala) in control group was calculated for each study and was tested for Hardy-Weinberg equilibrium using the χ^2 statistic. OR of cancer association with *MnSOD* Val^{16}Ala polymorphism as well as their corresponding 95% CI was recalculated for each study. Risk of the variant allele (Ala/Ala or Val/Ala) was calculated first, comparing with the wild-type (Val/Val) genotype. Then, we evaluated the risk of Ala/Ala versus Val/Ala + Val/Val and Ala/Ala + Val/Ala versus Val/Val assuming recessive and dominant effects of ^{16}Ala allele, respectively.

Heterogeneity across the studies was determined by χ^2-based Q test using the formula: $Q = \Sigma\ \text{weight}_i \times (\ln\text{OR}_{MH} - \ln\text{OR}_i)^2$, where $\text{weight}_i = 1 / \text{variance}_i$. The heterogeneity was considered significant for $P < 0.05$. We estimated between study heterogeneity and cancer risk for all 13 studies those included different cancer types (termed in this text as ''all cancers'') and also for breast cancer separately.

Results

Present meta-analysis of 13 studies on association of cancer risk and *MnSOD* Val^{16}Ala polymorphism comprised 7,366 cancer cases and 9,102 controls. The frequency of the ^{16}Ala allele in control population varied from 14% (China) to 56% (Turkey), suggesting an ethnic distribution. All studies, except 3 of them (8, 13, 16), indicated that, in control population, distribution of genotype was consistent with Hardy-Weinberg equilibrium (Table 1). Table 2 shows recalculated OR values and their corresponding 95% CI values for individual studies. Conceivably, in some cases, OR values were not exactly same as reported due to different statistical methods followed in the original articles. There was no between-studies heterogeneity (Table 3), except in one case where we stratified the data by cancer type (for Ala/Ala + Val/Val versus Val/Val in breast cancer studies). Individuals with Ala/Ala allele did not have elevated cancer risk compared with the individuals with Val/Val genotype in both categories: ''all cancers'' and ''breast cancer'' (OR, 0.98; 95% CI, 0.90-1.07 and OR, 1.02; 95% CI, 0.91-1.14, respectively). Further, no association with breast cancer risk was found either in the recessive (Ala/Ala versus Val/Val + Val/Ala: OR, 1.03; 95% CI, 0.93-1.14) or in dominant model of ^{16}Ala allele (Ala/Ala + Val/Ala versus Val/Val: OR, 0.99; 95% CI, 0.91-1.07). Overall results did not differ for mixed cancer types (''all

Table 1. Studies included in meta-analysis

First author year (ref.)	Country	Ethnicity	Cancer type	Control			^{16}Ala	χ^2 HWE*	Case		
				Val/Val	Val/Ala	Ala/Ala			Val/Val	Val/Ala	Ala/Ala
Tamimi 2004 (2)	United States	ND	Breast	297	612	296	0.50	0.23	255	468	245
Silva 2006 (8)	Portugal	Caucasian	Breast	99	276	82	0.48	20†	59	146	36
Cai 2004 (14)	China	Asian	Breast	884	290	23	0.14	0.02	831	266	28
Millikan 2004 (12)	United States	African American	Breast	196	357	124	0.45	3.01	259	372	129
Millikan 2004 (12)	United States	White	Breast	266	586	283	0.51	1.22	273	681	311
Mitrunen 2001 (11)	Finland	Finnish Caucasian	Breast	153	231	98	0.44	0.40	124	255	100
Ambrosone 1999 (13)	United States	Caucasian	Breast (premenopausal)	25	62	23	0.49	1.8	16	53	45
Ambrosone 1999 (13)	United States	Caucasian	Breast (postmenopausal)	38	107	40	0.51	4.55†	23	84	45
Kocabas 2005 (16)	Turkey	ND	Breast	25	40	38	0.56	4.57†	23	32	29
Kang 2007 (3)	United States	Caucasian	Prostate	376	686	320	0.48	0.04	275	578	297
Kang 2007 (3)	United States	African American	Prostate	122	194	79	0.45	0.9	31	57	15
Levine 2002 (22)	United States	Mixed	Distal colorectal adenomas	140	234	121	0.48	1.4	139	209	108
Choi 2008 (15)	United States	Mixed	Prostate	327	635	317	0.50	0.06	119	245	105

*χ^2 for testing Hardy-Weinberg equilibrium.
†$P < 0.05$.

cancers'': for recessive model, OR, 1.00; 95% CI, 0.92-1.08 and for dominant model, OR, 0.97; 95% CI, 0.91-1.04).

Discussion

Association studies linking *MnSOD* Val^{16}Ala polymorphism and cancer risk reveal contradictory results. Present meta-analysis shows that there was no overall association between Val^{16}Ala polymorphism and cancer risk, in recessive and dominant models, or for Ala homozygotes over Val homozygotes or for Ala/Val heterozygotes. Stratification by cancer type did not bring any change in the results. Present meta-analysis, however, could be considered as preliminary due to its comparatively small sample size and limited number of studies included. Moreover, the fact that *MnSOD* constitutes a first-line defense against ROS, it would be a premature conclusion to remark that this polymorphism has no role in cancer development. Another point to be considered is that some polymorphisms might be associated with cancer risk for some ethnic communities only not for other ones (35, 36).

Meta-analysis is widely accepted and most commonly recommended method for a systematic review, but meta-analysis has its own limitations (37). In the present meta-analysis, we considered all cases for each study, which actually included a mixed group of individuals with different physiologic states (e.g., menopausal status and body mass index), different habits (smoking, alcohol consumption, and antioxidant intake), etc. We observed that, in some of these original studies, consideration of all individuals for genetic risk assessment did not establish ^{16}Ala as risk factor, whereas stratification of the data according to the habits or physiologic condition revealed an association of this genotype with cancer risk (2, 12, 14). It is known today that smoking generates polycyclic aromatic hydrocarbon that modulates cellular ROS level. Hence, it can be stated that *MnSOD* Val^{16}Ala of the smokers or of individuals exposed to other ROS resources should be more exhaustively studied to get an answer for its link to cancer. We combined the data of Tamimi et al. (2), Millikan et al. (12), and Mitrunen et al. (11) for smoking and found a nonsignificant association of Ala morph (Ala/Ala or Val/Ala) with cancer risk in smokers (OR, 1.26; 95% CI, 0.95-1.68). Cell biological studies establishing a relation between a critical level of mitochondrial $O_2^{\cdot-}$ and H_2O_2 and carcinogenesis (38, 39) insist that *MnSOD* Val^{16}Ala polymorphism may have certain link with cancer risk. According to Sutton et al. (6), Ala form, not the Val form, is targeted into mitochondria; it is expected that the Val form would be associated with higher cancer risk. However, a very few studies document Val form with higher cancer risk against a large data for Ala allele as a risk factor not only for cancer but also for other diseases. This paradox has often overwhelmed researchers. Superoxide radicals spontaneously dismutates to H_2O_2 and O_2. The rate of this reaction is speeded up by SOD2. It has been accepted that superoxide radicals are vectorially released into the mitochondrial matrix from their generation site, the inner mitochondrial membrane (40, 41), and are acted upon by MnSOD in matrix to generate H_2O_2. However, inhibition of SOD2 (as in Val form) causes accumulation of superoxide radicals. This, in turn, can lead to damage

Table 2. Genetic polymorphism of *MnSOD* Val[16]Ala and cancer risk

First author year (ref.)	Val/Val vs Ala/Ala, OR (95% CI)	Val/Ala vs Val/Val, OR (95% CI)	Ala/Ala vs Val/Val + Val/Ala, OR (95% CI)	Ala/Ala + Val/Ala vs Val/Val, OR (95% CI)
Tamimi 2004 (2)	0.96 (0.76-1.22)	0.89 (0.72-1.09)	1.04 (0.85-1.26)	0.91 (0.75-1.11)
Silva 2006 (8)	0.73 (0.44-1.22)	0.88 (0.60-1.29)	0.80 (0.52-1.22)	0.85 (0.59-1.23)
Cai 2004 (14)	1.29 (0.74-2.25)	0.97 (0.80-1.18)	1.30 (0.75-2.26)	0.99 (0.83-1.20)
Millikan 2004 (12)	0.78 (0.57-1.07)	0.78 (0.62-0.99)	0.91 (0.69-1.19)	0.78 (0.63-0.98)
Millikan 2004 (12)	1.07 (0.84-1.35)	1.13 (0.92-1.38)	0.98 (0.81-1.18)	1.11 (0.91-1.34)
Mitrunen 2001 (11)	1.25 (0.87-1.81)	1.36 (1.01-1.83)	1.03 (0.75-1.41)	1.33 (1.00-1.76)
Ambrosone 1999 (13)	3.05 (1.37-6.78)	1.33 (0.65-2.74)	2.46 (1.36-4.44)	1.80 (0.90-3.56)
Ambrosone 1999 (13)	1.85 (0.95-3.62)	1.29 (0.72-2.33)	1.52 (0.93-2.49)	1.45 (0.82-2.55)
Kocabas 2005 (16)	0.83 (0.39-1.73)	0.87 (0.42-1.80)	0.90 (0.49-1.64)	0.85 (0.44-1.63)
Kang 2007 (3)	1.26 (1.01-1.58)	1.15 (0.95-1.39)	1.15 (0.96-1.38)	1.18 (0.99-1.42)
Kang 2007 (3)	0.74 (0.38-1.46)	1.15 (0.70-1.88)	0.68 (0.37-1.23)	1.03 (0.64-1.66)
Levine 2002 (22)	0.89 (0.63-1.27)	0.90 (0.66-1.21)	0.95 (0.71-1.29)	0.89 (0.68-1.18)
Choi 2008 (15)	0.91 (0.67-1.23)	1.06 (0.82-1.37)	0.87 (0.68-1.12)	1.01 (0.79-1.28)

of mitochondrial membrane, as it is known that highly reactive free radicals affect *in situ* the cellular parts situated immediate to their generation site, thus causing most of the cellular parts vulnerable. This causes release of cytochrome *c* from mitochondrial membrane; consequently, apoptosis takes place. Recent experiments by Dasgupta et al. (38) reveals very interesting and important information regarding ROS-mediated apoptosis. They showed that steady-state increase in intracellular production of H_2O_2 by SOD2 can block the activation of key processes involved in induction of programmed cell death, the tumor necrosis factor-α-mediated apoptosis. According to them, an increased H_2O_2 level was associated with decreased sensitivity to tumor necrosis factor-α-mediated apoptosis. Hence, if SOD2 is inhibited to enter mitochondrial matrix, as in the case of Val form, superoxides cannot be dismutated to H_2O_2, and this may bring increased rate of programmed cell death. Hence, although there would be enormous amount of cellular damage by superoxide free radicals, at the same time death of the handicapped cells may check cancer. This may be the plausible explanation for association of the Val form with lesser risk of cancer. On the other hand, if MnSOD efficiently dismutates superoxide to H_2O_2, in the absence of oxidative stress, the later species will be neutralized with the action of Gpx1 in mitochondria and also to some extent by mitochondrial catalase (42-44) and by mitochondrial peroxiredoxin, PrxIII (45). However, if not quenched, H_2O_2 can potentially react to yield other ROS and thus brings more harm to cell. Mostly H_2O_2 is converted to more toxic hydroxyl radicals. Although H_2O_2 itself is not reactive to DNA, •OH is highly reactive to DNA. Hydroxyl radical has been reported to activate certain oncogenes also [e.g., *K-Ras* (39)]. As increased level of H_2O_2 reduces chance of apoptosis (38), these mutations will be propagated to a new generation of cells that may give rise to cancer. Moreover, it has been established that SOD2-dependent H_2O_2 production contributes to the signaling mechanism that regulates the rate of metastasis (46, 47). According to Ranganathan et al. (47), SOD2-dependent production of H_2O_2 up-regulates matrix metalloproteinase (MMP) expression (MMP1 and also potentially other MMP family members). MMPs are responsible for the degradation of extracellular matrix and hence are likely to promote metastasis. The MMP family is composed of at least 20 zinc-dependent extracellular endopeptidases that are primarily expressed as zymogens (48). Their expression is low in normal tissues and is induced when extracellular matrix remodeling is required. It is known that, as a group, MMPs can digest almost all different extracellular matrix components, although individual family member has its function limited to extracellular matrix molecule they can attack. According to Ranganathan et al. (47), SOD2-dependent increase in H_2O_2 causes extracellular signal-regulated kinase 1/2 phosphorylation and activation. It leads to subsequent activation of activator protein-1. Activator protein-1 is an essential transcription factor required for the induction of MMP1 promoter (49, 50). Further work by the same group (51) asserted that MnSOD-dependent regulation of MMP-1 signals through the Ras/MEK/extracellular signal-regulated kinase pathway. Nelson et al. (51) reestablished their previous findings (47) that elevated level of SOD2 causes a consequent rise of H_2O_2 that in turn leads to higher MMP expression. They also showed a H_2O_2-mediated

Table 3. Meta-analysis of case-control studies on *MnSOD* Val[16]Ala polymorphism and cancer risk

Genotypes	All cancers		Breast cancers	
	Q*	OR (95% CI)	Q †	OR (95% CI)
Ala/Ala vs Val/Val	19.82	0.98 (0.90-1.07)	14.05	1.02 (0.91-1.14)
Val/Ala vs Val/Val	16.39	0.97 (0.90-1.04)	13.11	0.98 (0.90-1.06)
Ala/Ala vs Val/Val + Val/Ala	18.03	1.00 (0.92-1.08)	13.20	1.03 (0.93-1.14)
Ala/Ala + Val/Ala vs Val/Val	19.61	0.97 (0.91-1.04)	15.70 ‡	0.99 (0.91-1.07)

*df = 12.
† df = 8.
‡ Significant heterogeneity between studies.

degeneration of collagen by human fibrosarcoma cells. All these indicated MMP-mediated higher invasive as well as increased metastatic potential of tumor cells. It is known that, under normal physiologic conditions, steady-state increased concentrations of H_2O_2 are buffered by glutathione redox system. When SOD2 level increases, glutathione redox system may not be enough to buffer the increased level of H_2O_2. As Ala form of *MnSOD* is related to higher SOD2 activity and thus higher production of H_2O_2, it is plausible that there will be up-regulation of *MMP* expression and elevated metastatic activity in the individuals possessing Ala form of *MnSOD*. Hence, it is justified that majority of the research findings on cancer as well as on other diseases indicate involvement of the Ala form rather than Val form of *MnSOD* with elevated risk of cancer. These tight regulations of MMPs by SOD2-derived H_2O_2 advocate for the link between Ala form of *MnSOD* with higher incidence of cancers. In addition to this, as ROS can activate mitogen-activated protein kinase pathways (52), it can also be predicted that increased level of H_2O_2 can mediate higher rate of cell proliferation by activating particular members of a specific mitogen-activated protein kinase pathway, contributing further to tumor formation. These probable molecular mechanisms engaged in regulation of cancer risk involving Val[16]Ala polymorphism of *MnSOD* are summarized in Fig. 1.

However, a major aspect of concern is whether it is justified to link only a single gene as well as single polymorphism of a particular gene in isolation for this type of association studies. Although MnSOD is the first warrior in the defense against mitochondrial oxidative stress, other enzymes such as Gpx1 and mitochondrial catalase and PrxIII have major contribution in protecting cells from oxidative stress. Hence, it can be expected that only a proper balance between the activity of MnSOD (dismutates $O_2^{\bullet-}$) and Gpx1 (neutralizes H_2O_2), and to some extent mitochondrial catalase and PrxIII, can protect cells from detrimental effects of H_2O_2 and its downstream ROS. However, in a condition where there is continuous supply of MnSOD but less supply of Gpx1 or catalase or PrxIII, H_2O_2 will accumulate and promote cancer formation. Hence, it can be said that, for a better interpretation, polymorphic forms of *MnSOD* as well as *Gpx1*, *catalase*, and *PrxIII* should be considered in combination for a same individual. However, only very few studies considered cross-tabulations between genotypes of different oxidative stress genes for an association to cancer risk. Cox et al. (9) studied joint effects of two polymorphisms on two different genes involved in the detoxification pathway of ROS (e.g., *MnSOD* and *Gpx1*) with breast cancer risk. They considered Val[16]Ala of *MnSOD* and Pro[198]Leu of *Gpx1* and observed significant increase in breast cancer risk, whereas the two polymorphisms acted in combination. Individuals homozygous for the Ala[16] allele of *MnSOD* and Leu[198] of *Gpx1* were found to have a 1.87-fold higher risk for breast cancer than the individuals with Val[16] and Pro[198] carriers. This finding, no doubt, emphasizes on association studies of more than one oxidative gene with cancer risk. Further, inter-SNP studies between genes of MnSOD and its regulatory proteins (e.g., transcription factors of MnSOD such as nuclear factor-κB family and p53; ref. 53) should be carried out to get a better picture on the association between cancers and polymorphisms. In concordance with the finding of Dhar et al. (53) that overexpression of p53 down-regulates expression of MnSOD, and the known fact that p53 is proapoptotic, our present discussion on the relation between mitochondrial SOD2 level and rate of apoptosis indicates for further cross-tabulated studies involving SNP(s) of *p53* and *MnSOD* for cancer risk. The same is applicable for polymorphisms also. Only few polymorphisms on a single gene are found to be reported for statistically significant associations with cancer risk in isolation to date. It is possible that common polymorphisms that have no enough large effect on gene function alone, when combined with other polymorphisms in linkage disequilibrium up-regulate and down-regulate gene function, may be related to cancer risk. *MnSOD* Val[16]Ala polymorphism, too, could not be linked to cancer risk unequivocally. Rather, it has been proposed as low-penetrance allele (9). Very few studies, however, considered the combined effect of more than one *MnSOD* polymorphisms for cancer risk. Martin et al. (23)

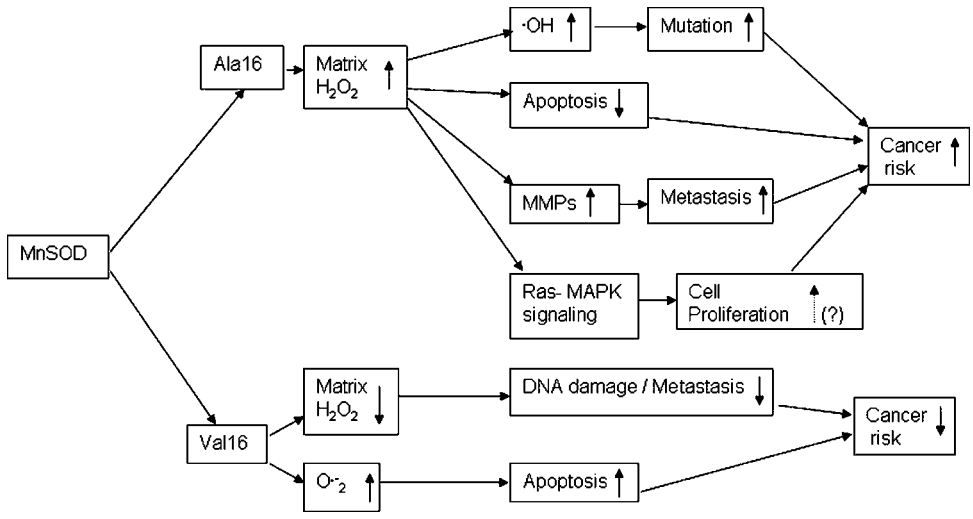

Figure 1. Probable mechanisms responsible for ROS-mediated higher and lower cancer risk in association with Val[16]Ala polymorphism of human MnSOD gene.

considered two genetic polymorphisms of *MnSOD*, -102 C/T and -9 T/C, for gastric cancer association study. *MnSOD* -102 C/T polymorphism changes the binding pattern of activator protein-2 on promoter, which leads to a reduction of transcriptional activity. They did not observe association of these two polymorphisms as gastric cancer risk factor. However, more studies involving these polymorphisms in different populations may bring different conclusion. Further, experiments recruiting other polymorphisms affecting protein function could be considered as well (e.g., $Ile^{58}Thr$ polymorphism of *MnSOD*). Although Thr^{58} is rare in population (54), because it causes two packing defects in the protein (55), a study of this polymorphism along with *MnSOD* $Val^{16}Ala$ may bring new direction to this type of experiments. According to HapMap Consortium 2003, the MnSOD lies within a single haplotype block. Wiener et al. (56) reported a strong linkage disequilibrium region in *MnSOD* spanning from a SNP at promoter region, 65 bp upstream of a nuclear factor-κB binding site (rs2758346), through well-characterized rs4880 up to a SNP on intron 3 (rs2855116). They evidenced significant association of this block with Alzheimer's disease. As dbSNP database reports >100 SNPs in this gene including coding SNP(s), and several others in its regulatory sequences, further exploration of recent genomic knowledge will help for better understanding about genetic association with cancer risk. In addition, there may be unidentified alleles in linkage disequilibrium with *MnSOD* $Val^{16}Ala$, which could affect SOD2 targeting and/or function, and also alter the risk for cancer formation. Again, it would be much more meaningful if the interaction between the genes and their environment is discussed. Lifestyle factors that elevate oxidative stress such as smoking or alcohol consumption, as well as those that decrease oxidative stress such as antioxidant supplements, are important aspects to be included in such type of association studies. Finally, a large sample size is required to get statistically sufficient power to detect such association, especially when the alleles to be studied are of low penetrance, as power (ability to detect a true association) calculations show that detecting association with alleles having frequency lower than 5% would require scanning of thousands of patients (57), except in rare cases in which the relative risk attributable to the allele is large.

In brief, variations in key antioxidant enzymes are long-speculated risk factors for the genetic susceptibility of cancer development. MnSOD, being the first component in the detoxification pathway of mitochondrial ROS, has been discussed very often for its association with cancer development. However, much more exhaustive studies involving a large sample size and considering the variables such as polymorphisms in linkage disequilibrium, gene-gene interactions, and environmental exposures are required to acquire a total knowledge on the link between *MnSOD* polymorphism and cancer risk. This understanding would certainly have huge prospect in the promising field of pharmacogenetics, which involves identification of genetically susceptible individuals for complex diseases, such as cancer, and development of genome-based drug (individualized medicine).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Target Sequence Polymorphism of Human Manganese Superoxide Dismutase Gene and Its Association with Cancer Risk: A Review

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