Manganese Superoxide Dismutase: Genetic Variation and Regulation¹

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EXPANDED ABSTRACT

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Manganese superoxide dismutase (MnSOD)³ is a nuclear-encoded antioxidant enzyme that localizes to the mitochondria (1). Increased expression of MnSOD suppresses cancer phenotypes in a large number of human and murine tumors (2). MnSOD can also regulate tumor formation in MnSOD-overexpressing and MnSOD-deficient mice by modulating apoptotic and proliferation pathways in situ (3,4). The presence of MnSOD in mitochondria can influence the role of mitochondria as a source of reactive oxygen species (ROS) generation as well as a source of ROS removal. Given the evidence that links mitochondria to cell death and MnSOD to playing a role in the suppression of cancer, it has been proposed that alteration of MnSOD levels might contribute to cancer susceptibility and resistance to cancer therapeutic agents.

To elucidate the mechanisms by which the expression of the human sod2 gene is regulated, we cloned, sequenced, and characterized the human sod2 gene from various normal and human cancer cell lines as well as from human tissues. We found that sod2 is a single-copy gene consisting of 5 exons interrupted by 4 introns (5). Unique characteristics of this gene include a TATA-less promoter and an intronic enhancer element responsible for the induction of the gene (Fig. 1). The constitutive expression of this gene is controlled by a GC-rich promoter containing several specificity protein 1 (SP-1) and activating protein 2 (AP-2) binding sites. Transcription factor SP-1 is essential and sufficient, whereas AP-2 is unnecessary for and antagonistic to the constitutive expression of the gene (6,7). Interestingly, a unique set of 3 mutations was found in the promoter region of sod2 genes isolated from several human cancer cells. These mutations lead to an increase in an AP-2 binding site and a reduction in transcription activity of the basal promoter. The unique mutations were found in 60% of colon cancer cell lines examined (8). Preliminary studies using peripheral blood of human samples suggest that the unique mutations are present in a high percentage of colon cancer patients. These findings may provide an explanation for the reduced MnSOD activity found in some human cancer cells. However, a larger sample size is needed to establish the link between mutation in the transcription regulatory region of the sod2 gene and colon cancer.

Although MnSOD may be present at a low level in some normal tissues, its expression is highly inducible by a wide range of agents, including cytokines and alcohol, suggesting that MnSOD is a stress-responsive gene. The induction of MnSOD by cytokine is mediated by nuclear factor κB (NF-κB). However, the presence of NF-κB is essential but not sufficient for a high-level induction of MnSOD (9). Thus far, there are no published reports of polymorphisms in the enhancer region of this gene in the human population. Therefore, it is highly likely that a deficiency of MnSOD can be alleviated by induction of sod2. A number of recent studies indicate that the level of MnSOD is inducible by nutrient constituents, including those from cruciferous vegetables (10), retinoic acid (11), and vitamin E (12). However, the mechanisms by which these agents mediate MnSOD induction remain unclear. Understanding how endogenous defense enzymes such as MnSOD can be enhanced by dietary intake of antioxidants could provide a novel strategy for designing cancer intervention.

In addition to the transcriptional regulation, accumulating data suggest that genetic polymorphisms in the human sod2 gene may be associated with a risk of developing cancer. Polymorphisms of the human sod2 gene were found in the regions that code for the mature protein and the mitochondria targeting peptide. Two polymorphic variants coding for isoleucine or threonine at amino acid 58 (I58T) in the mature protein were found in human cells. Due to its stability at the tetrameric interface, the mature protein containing isoleucine is more stable and less susceptible to inactivation by S-thiolation reactions than the threonine-containing protein (13).

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³ Abbreviations used: AP-2, activating protein 2; I58T, isoleucine-threonine polymorphism at position 58; L60F, leucine-phenylalanine polymorphism at position 60; NF-κB, nuclear factor κB; MnSOD, manganese superoxide dismutase; ROS, reactive oxygen species; SP-1, specificity protein 1.
polymorphic variants have been identified. More recently, an additional thiol-sensitive mutant form of the variants in cancer patients remains to be established. The structural dimorphism in this region is associated to the mitochondrial matrix, whereas the valine-containing MnSOD is alanine-containing MnSOD is actively targeted into the mitochondrial superoxide dismutase. Cancer Res. 63: 159–163. 

Two polymorphic variants at codon 16 that lead to incorporation of either alanine or valine in the mitochondrial targeting sequence are frequently detected in humans. The alanine-containing MnSOD is actively targeted into the mitochondria, whereas the valine-containing MnSOD is partially arrested within the inner mitochondrial membrane. The MnSOD deficiency in this region is associated with risk for development of breast cancer (16,17). Premenopausal women who are homozygous for the A/A allele have an increased risk of breast cancer compared to those with 1 or 2 V alleles. Furthermore, the risk is more pronounced among women whose consumption of fruit and vegetables, ascorbic acid, and α-tocopherol is low. These findings support the use of sod2 gene polymorphism as a biomarker and the risk-modification benefit of dietary manipulation. In contrast to breast cancer, a study of lung cancer that involved 1101 patients with lung cancer and 1239 control subjects indicated a markedly increased risk of lung cancer in individuals with A/V and V/V genotypes (18). This result may reflect the constant chronic exposure of the lung to ROS. Ongoing studies in lung cancer models using MnSOD-deficient mice should verify this possibility.

However, identification of genetic polymorphisms of these variants in cancer patients remains to be established. More recently, an additional thiol-sensitive mutant form of the sod2 gene was identified in Jurkat human T-cell leukemia (14). The leucine-to-phenylalanine mutation occurs at position 60 (L60F) in the mature protein. Cells containing the L60F heterozygous mutation have markedly less SOD activity in the presence of thiol agents. This finding explains the observed loss of MnSOD activity in some leukemia cells and suggests paradoxical effects from supplementation by the thiols class of antioxidants.


FIGURE 1 Organization of the human sod2 gene and sites where polymorphic variants have been identified.

LITERATURE CITED


