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Dossier: Superoxide dismutases: recent advances and clinical applications

Mechanism of the tumor suppressive effect of MnSOD overexpression

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Abstract

The mitochondrial antioxidant protein manganese-containing superoxide dismutase (MnSOD) has been shown to be a new type of tumor suppressor protein. Overexpression of MnSOD protein inhibits growth in a wide variety of cancer types. This review examines the molecular mechanism of the tumor suppressive effect of MnSOD. Three species have been proposed to cause the tumor suppressive effect: superoxide radical, hydrogen peroxide and nitric oxide. At the present time, the evidence appears strongest that hydrogen peroxide is the effector molecule since both catalase and glutathione peroxidase has been shown to modulate the effect. Surprisingly, in different cancer cell lines, overexpression of GPx has been found to both decrease and increase the growth inhibitory effect of MnSOD overexpression. Knowledge of which molecule causes the tumor suppressive effect of MnSOD and the mechanism of action will likely lead to new therapies for the treatment of cancer.

Keywords: Tumor suppressor; Manganese-containing superoxide dismutase; Glutathione peroxidase; Catalase

1. Introduction

The superoxide dismutase (SOD) family of proteins is necessary to protect oxygen-utilizing cells from the toxicity of the reactive oxygen species (ROS) produced during normal metabolism. Besides being protective proteins, these enzymes are also key components of signaling pathways that regulate cell physiology. The SODs catalyze the reaction: $2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$. Hydrogen peroxide is then removed by catalases (CATs) and peroxidases, of which glutathione peroxidase (GPx) has been the most widely studied. There are three known forms of SOD in mammalian cells: a copper- and zinc-containing superoxide dismutase (CuZnSOD) found mainly in the cytoplasm and nucleus, a manganese-containing superoxide dismutase (MnSOD) found in the mitochondria, and an extracellular superoxide dismutase (ECSOD) found primarily in the extracellular compartments. The purpose of this review article is to discuss the role of MnSOD as a tumor suppressor protein and to suggest possible mechanisms for its tumor suppressive ability.

It has been over 30 years now since the first report was published demonstrating that the activity of MnSOD was diminished in transformed cells when compared to an appropriate normal cell control [43]. Since that time, numerous papers have been published showing altered levels of antioxidant enzymes in cancer cells; this subject matter has been reviewed many times [27–32]. Cancer cells are nearly always low in MnSOD and catalase (CAT) activity, and usually low in CuZnSOD activity [27–32]. Glutathione peroxidase (GPx) activity is variable. Recently, it has been shown that in some cancer cells, reduced expression of MnSOD is due to mutations in the promoter of the gene [42], while in other types of cancer, reduced levels of MnSOD are due to abnormal methylation [11], loss of heterozygosity [26,18], or mutation in the

Abbreviations: BCNU, 1.3 bis (2-chloroethyl)-1-nitrosourea; BSO, buthionine sulfoximine; CAT, catalase protein; *CAT*, catalase gene; CuZnSOD, copper- and zinc-containing superoxide dismutase protein; *CuZnSOD*, copper- and zinc-containing superoxide dismutase gene; ECSOD, extracellular superoxide dismutase protein; eNOS, endothelial nitric oxide synthase protein; GPx, glutathione peroxidase protein; *GPx*, glutathione peroxidase gene; GR, glutathione reductase protein; GSH, reduced glutathione; GSSG, glutathione disulfide; MnSOD, manganese-containing superoxide dismutase protein, *MnSOD*, manganese-containing superoxide dismutase gene; MOI, multiplicity of infection; mtCAT, mitochondrial form of catalase protein; NOS, nitric oxide synthase protein; *NOS*, nitric oxide synthase gene; ROS, reactive oxygen species; SOD, superoxide dismutase protein; TNF, tumor necrosis factor.

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coding sequence [10]. Thus, MnSOD loss is similar in mechanism to that reported for other tumor suppressor genes.

Even though there is a large body of literature linking free radicals and antioxidant enzymes to cancer, most of the evidence is correlative and does not demonstrate a causal relationship. There are several lines of evidence that do imply a causal relationship. Powerful evidence for a causal relationship is that in various model systems, ROS cause cancer; moreover, antioxidants in general, and SOD and SOD-mimetics in particular, inhibit malignant transformation [6,29,31]. Molecular biological techniques have been also used to demonstrate an important role for SOD in transformation; overexpression of MnSOD by cDNA transfection led to inhibition of radiation-induced transformation in a mouse fibroblast cell line [37]. Recently, it has been shown that a life-long reduction in MnSOD activity (in transgenic heterozygotic mice with a 50% reduction in MnSOD activity) results in a much higher incidence of cancer [39].

2. Effect of increasing SOD on the cancer phenotype

If SODs are important in cancer, then normalization of the levels of these enzymes should result in reversal of at least part of the cancer cell phenotype. This hypothesis was first suggested by Oberley and Buettner [28] and has been tested with regards to SOD in three different ways: (1) elevation of SOD by exposure to a superoxide generator and subsequent isolation of resistant cells [9]; (2) addition of liposomal CuZn-SOD protein [3] and (3) elevation of SOD, particularly MnSOD, by sense cDNA transfection. Each of these techniques has supported the Oberley–Buettner hypothesis. For brevity, only cDNA transfection will be discussed.

3. Increasing MnSOD by cDNA transfection

The first paper using cDNA transfection of MnSOD was published in 1993 [7]. In collaboration with Drs. Sue Church and James Grant at Washington University, we demonstrated that the transfection of *MnSOD* cDNA into cultured human melanoma cells resulted in the loss of the malignant phenotype. The malignant phenotype was tested both in vitro by assays such as mitotic rate and growth in soft agar and, more importantly, in vivo by growth in nude mice. All of these tests showed a loss of the malignant phenotype in clones that overexpressed MnSOD by at least five-fold. The most important observations were that in the nude mouse assay, 18 out of 18 sites injected with the parental melanoma cell line developed tumors, while 0 out of 16 sites injected with high MnSOD overexpressing cells developed tumors.

We and others have published papers on many other cancer cell types and one virally-transformed cell line showing that overexpression of MnSOD in each of these cell lines led to suppression of cell growth both in vitro and in vivo. Growth suppression was observed in human breast carcinoma MCF-7 cells [14], virally-transformed WI-38 human lung fibroblasts [44], A172R rat glioma [48], U118 human glioma [47], human oral squamous carcinoma SCC-25 [21], mouse [35,36] and human fibrosarcoma [25], human prostatic carcinoma DU145 [15], and human pancreatic cancer cells [40]. Therefore, in all these tumor types, overexpression of MnSOD led to suppression of at least part of the tumor cell phenotype. This work has been done at five different institutions (University of Iowa, Washington University, University of Kentucky, University of Wisconsin and Albany Medical College). Thus, the evidence appears substantial that MnSOD elevation by cDNA transfection can suppress the malignant phenotype in a great variety of tumors. On the basis of this work showing growth suppression and the fact that loss of heterozygosity (LOH) for *MnSOD* has been found in human melanoma [26] and glioma [18], we and others have proposed that *MnSOD* is a new type of tumor suppressor gene [5].

4. MnSOD enzymatic activity causes the tumor suppressive effect of MnSOD protein

It has been reported that the MnSOD protein has two variants at amino acid 58; either isoleucine (Ile) or threonine (Thr) can be at this position in the protein [4]. It is still unclear whether this variation is a polymorphism or is a cancer mutation. Isolated Ile58 protein was found to possess twice the enzymatic activity of the Thr58 form and to be more stable against heat [4]. We sequenced the cDNA we had been transfecting and found it contained lesser activity Thr58 form. We used site directed mutagenesis to make the Ile58 form. We then transfected both forms into wild type MCF-7 cells and isolated overexpressing clones [45]. Four clones overexpressing Thr58 MnSOD and eight clones overexpressing Ile58 MnSOD were isolated and characterized. The Ile58 clones had three times the specific activity of the Thr58 form. Both forms of the MnSOD had tumor suppressive activity that was in general proportional to the MnSOD activity. The Ile58 clones had a higher tumor suppressive effect apparently because they had higher MnSOD activity. These results suggest that tumor suppressive effect of MnSOD is due to its enzymatic activity. In unpublished work, we have confirmed this observation with another MnSOD mutation that leads to partial inactivation.

We believe our results have far reaching implications. A paper has recently appeared demonstrating that another polymorphism for MnSOD caused increased risk for breast cancer [2]. A valine or alanine can be at the –9 position in the MnSOD mitochondrial targeting presequence. Premenopausal women who were homozygous for the alanine allele had a 4-fold increase in breast cancer risk compared to those with 1 or 2 valine alleles. This suggests that MnSOD polymorphisms may be important in cancer susceptibility. It is very logical that individuals who have an MnSOD protein that is less active should be more susceptible to oxidative