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SUPEROXIDE DISMUTASE: A MULTIGENE FMILY
Comparison of the SOD1 (CuZn-SOD), SOD2 (Mn-SOD) and SOD3 (EC-SOD)

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Abstract: Superoxide dismutase is a ubiquitous family of enzymes that function to efficiently catalyze the dismutation of superoxide anions. Three unique and highly compartmentalized mammalian superoxide dismutases have been biochemically and molecularly characterized to date. SOD1, or CuZn-SOD, was the first enzyme to be characterized and is a copper and zinc-containing homodimer that is found almost exclusively in intracellular cytoplasmic spaces. SOD2, or Mn-SOD, exists as a tetramer and is initially synthesized containing a leader peptide, which targets this manganese-containing enzyme exclusively to the mitochondrial spaces. SOD3, or EC-SOD, is the most recently characterized SOD, exists as a copper and zinc-containing tetramer, and is synthesized containing a signal peptide that directs this enzyme exclusively to extracellular spaces. What role(s) these SODs play in both normal and disease states is only slowly beginning to be understood. A molecular understanding of each of these genes has proven useful toward the deciphering of their biological roles. For example, a variety of single amino acid mutations in SOD1 have been linked to familial amyotrophic lateral sclerosis. Knocking out the SOD2 gene in mice results in a lethal cardiomyopathy. A single amino acid mutation in human SOD3 is associated with 10 to 30-fold increases in serum SOD3 levels. As more information is obtained, further insights will be gained.

Introduction:

The evolution of aerobic organisms that can survive in oxygen-rich environments requires an effective defense system against reactive oxygen species (ROS), which are produced following single electron reductions of molecular oxygen. While physiological concentrations of ROS in aerobic organisms are beneficial and involve cell signaling pathways and survival from invading pathogens, an unbalanced, elevated concentration of ROS may contribute to the development of various diseases, such as cancer, hypertension, diabetes, atherosclerosis, inflammation, and premature aging. The superoxide dismutases (SODs) are the first and most important line of antioxidant enzyme defense systems against ROS and particularly superoxide anion radicals. At present, three distinct isoforms of SOD have been identified in mammals, and their genomic structure, cDNA, and proteins have been described. Two isoforms of SOD have Cu and Zn in their catalytic center and are localized to either intracellular cytoplasmic compartments (CuZn-SOD or SOD1) or to extracellular elements (EC-SOD or SOD3). SOD1 has a molecular mass of about 32,000 Da and has been found in the cytoplasm, nuclear compartments, and lysosomes of mammalian cells. SOD3 is the most recently discovered and least characterized member of the SOD family.

The enzyme exists as a homotetramer of molecular weight 135,000 Da with high affinity for heparin. SOD3 was first detected in human plasma, lymph, as-cites, and cerebrospinal fluids. The expression pattern of SOD3 is highly restricted to the

specific cell type and tissues where its activity can exceed that of SOD1 and SOD2. A third isoform of SODs has manganese (Mn) as a cofactor and has been localized to mitochondria of aerobic cells (Mn-SOD or SOD2). It exists as a homotetramer with an individual subunit molecular weight of about 23,000 Da. SOD2 has been shown to play a major role in promoting cellular differentiation and tumorgenesis and in protecting against hyperoxia- induced pulmonary toxicity. The numerous studies on the physiological function of SOD1 and SOD2 and their role in protection against ROS are summarized in several excellent reviews. However, the available information related to SOD3 has not been reviewed in a comparative perspective along with the other two isoforms. This review focuses on comparative characteristics of all three SOD genes, their evolution and ontogeny, and their transcriptional regulation by various intra and extracellular stimuli.

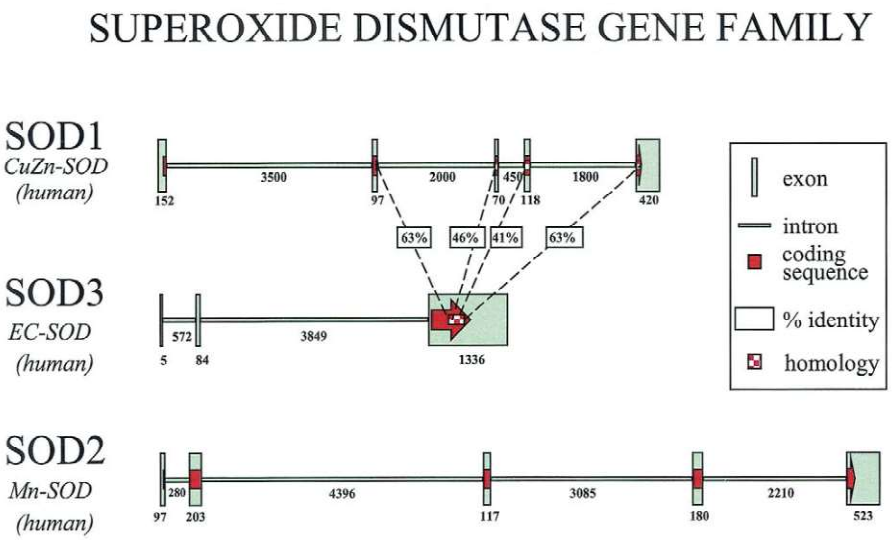

Fig. 1. Genomic organization of the three known members of the human SOD enzyme family. SOD3 was placed in the middle in order to demonstrate areas of amino acid sequence homology between SOD1 and SOD3. SOD2 has no significant amino acid sequence homology with either SOD1 or SOD3. The size of each exon and intron, in base pairs, is shown in association with that fragment.

Gene Structure

SOD 1: The genomic sequence for SOD1 has been identified in the rat, mouse, and human. The genomic organization of SOD1 gene shows striking similarity among species and has five exons and four introns (Fig.1). The TATA and CCAAT boxes, as well as several highly conserved GC-rich regions, have been localized in all three species with a similar pattern in the proximal promoter region. Such a high level of homology in the 5' flanking sequence suggests that intense evolutionary factors have preserved key regulatory regions for this gene. The 3' end of SOD1 gene possesses several poly(A) signal sequences that terminate the mRNA species with different lengths. The consensus sequences YGTGTTYY and a G/T cluster required for efficient formation of 3' -termini have also been located downstream from the

polyadenylation signal in the rat SOD1 gene. The promoter region of the human SOD1 gene has been studied and several putative binding sites for NF1, Sp1, AP1, AP2, GRE, HSF, and NF-κB transcription factors have been found. The role of Sp1 and Egr-1 transcription factors in basal and inducible expression of human SOD1 has been confirmed.

SOD 2: The complete genomic structure for SOD2 has been determined for the human, rat, and mouse. Partial identification and characterization of a bovine SOD2 gene has been described. All of these species show marked conservation of structure and sequence. The physical structure of the SOD2 gene is composed of 5 exons and 4 introns (see Fig. 1). Genomic southern blotting supports the existence of one SOD2 gene for human murine and bovine species, whereas two genes per haploid genome have been described in the rat. The promoter regions in all four species share common features. There are no upstream TATA or CAAT box elements identified. However, GC-rich regions are present in all four species. Such features can be typical of "housekeeping" genes. The human and mouse genes each contain putative NF-κB transcription regulatory element. For humans, it is located in the 3'-flanking region of the gene while the mouse contains two potential elements in the 5'-flanking region. Also present in the promoter region of all four species are multiple copies of Sp-1 and AP-2 consensus sequences.

SOD 3: The genomic structure for human SOD3 has been determined. A partial genomic clone encoding the complete open reading frame for mouse SOD3 has been reported. Currently, SOD3 cDNA clones for the human; rat, mouse, and rabbit have been isolated and sequenced. The SOD3 gene shares 40–60% similarity with the SOD1 gene at the exon level, but shows no similarity with SOD2 (Fig. 1). The mouse SOD3 gene consists of two exons separated by a 4 kb intron while in human three exons have been found. The promoter region of human and mouse SOD3 apparently lacks classical TATA or CCAAT boxes. In humans, several putative transcriptional response elements have been identified and include a metal regulatory element, an AP-1 site as well as two potential antioxidant response elements. In contrast, the mouse proximal promoter, characterized by unusually GA-rich sequence, has multiple putative binding sites for Kruppel-like and Ets-family transcription factors. The functional importance of these sites is not clear at this time.

Evolution: The appearance of SOD enzymes was triggered by the proliferation of photosynthetic organisms that began to produce oxygen about 2 billion years ago. A variety of antioxidant enzymes evolved to neutralize the toxic effects of sub products of oxygen utilization. Two major kinds of superoxide dismutase appeared in prokaryotes at that time, copper/zinc-containing SODs and iron/manganese-containing SODs. Is it possible that all forms of SOD originated from a single protein whose function was to protect primitive organisms from a relatively new toxin, oxygen? Although both types of enzymes carry out the same function, their completely different crystal structures, utilization of different metal cofactors and distinctive catalytic mechanism strongly argue against a common ancestor. The evolutionary tree for CuZn containing SOD, based on multiple sequence alignments with structural superimpositions of crystal structures, shows that extracellular SOD

diverged from the cytosolic form at early stages of evolution, before the differentiation of fungi, plants, and metazoa. Our own phylogenetic analysis of all known vertebrate SOD genes show close similarities between SOD1 and SOD3 with very low homology to SOD2. The structural core of SOD1 exists as a Greek key β-barrel motif, consisting of eight β-barrels. The amino acid substitutions, as well as deletions and insertions, occur mostly outside of this structural motif. These data support the theory that CuZn-SOD evolution involved gene duplication and fusion with subsequent addition of exons I and III. Interestingly, the evolutionary rates of CuZn- and Mn-SOD differed considerably during the last billion years. While Mn-SOD proteins have evolved at a relatively constant rate, CuZn-SODs evolved unusually slowly at the beginning and erratically quickly in the most recent 100 million years. Why such an abnormal evolutionary rate took place remains unclear; one possible explanation is that CuZn containing SOD was caught in "folding block" when most changes in amino acid composition were deleterious. The accumulation of silent mutations finally led to an escape from this "evolutionary hibernation" and a return to the faster evolutionary rate. While the plausibility of this theory remains questionable, the existence of aerobic life on Earth proves that SOD successfully evolved as a potent protective enzyme against oxygen toxicity.

Ontogeny: The developmental regulation of SOD enzymes is crucial for adaptation of fetuses to the relatively high oxygen environment after parturition. The lung is one of the most important organs for protection of newborn organisms against harmful oxygen radicals, but increased SOD activity in the kidney of fetuses may have the same protective effect against extrauterine environment as in the lung. The expression of SOD enzymes in lung and kidney during development differs substantially among the species, but in most cases the level of SOD activity increases considerably just after birth as has been shown for fetal lamb or rabbit lung. It is not clear which particular SOD enzymes were attributed to this increase.

Conclusion: The past decade has brought us new evidence of SOD's involvement in a number of diseases and pathologies: ALS, Down's syndrome, and premature aging are probably just some of the pathological conditions that develop due to altered SOD activity and ROS concentration. What other discoveries await us? New, emerging questions such as what role the extracellular form of SOD plays in cardiovascular and pulmonary diseases, and how it affects our ability to learn, still need to be answered. With a wealth of information provided in this field over the last few years we are just beginning to understand the significance of SOD in biology and pathology. The further gain of knowledge about the mechanisms of cell and tissue-specific regulation of SOD gene expression and their signal transduction pathways may also lead to the design of new drugs and strategies directed at regulating levels of these enzymes in particular tissues, cell types, and compartments without affecting other cells.

Abbreviations:

C/EBP—CCAAT/enhancer binding protein
CuZn-SOD—SOD1 or copper, zinc SOD
EC-SOD—SOD3 or extracellular SOD
Egr-1— early growth response-1
GRE— glucocorticoid response element

HSF—heat shock factor
IFN-γ—interferon γ
kDa —kilodaltons
Mn-SOD—SOD2 or manganese SOD
ROS—reactive oxygen species
RT-PCR—reverse transcription-polymerase chain reaction
SOD—superoxide dismutase
NF-κB—nuclear factor kappa B
TNF-α—tumor necrosis factor α
TPA—12-O-tetradecanoylphorbol-13-acetate

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