ROS

REVIEW ARTICLE

Superoxide Dismutases in Biology and Medicine: Essentials and Recent Advances

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ABSTRACT | Superoxide dismutases (SODs) are a family of enzymes catalyzing the dismutation of superoxide to hydrogen peroxide and molecular oxygen. SODs are among the most extensively studied antioxidant enzymes in mammalian systems. This article provides an overview on the basic biochemistry and molecular regulation of SODs and summarizes recent cutting-edge research findings on their biological functions as well as implications in health and disease.

KEYWORDS | Epigenetics; Gene regulation; Peroxidase; Redox signaling; Superoxide dismutase; Superoxide toxicity; Tumorigenesis

ABBREVIATIONS | ALS, amyotrophic lateral sclerosis; Cu,ZnSOD, copper, zinc superoxide dismutase; ECSOD, extracellular superoxide dismutase; MnSOD, manganese superoxide dismutase; mTORC1, mechanistic target of rapamycin complex 1; ROS, reactive oxygen species

CONTENTS

1. INTRODUCTION

The term superoxide dismutase (SOD) (EC 1.15.1.1) refers to a family of enzymes that catalyze the dismutation of superoxide to hydrogen peroxide and molecular oxygen. There are three isozymes of SODs in mammalian systems: (1) copper, zinc superoxide dismutase (Cu,ZnSOD or SOD1); (2) manganese superoxide dismutase (MnSOD or SOD2); and (3) extracellular superoxide dismutase (ECSOD or SOD3). Prokaryotic cells (e.g., bacteria) or plants also contain iron SOD (FeSOD) [1], nickel SOD (NiSOD) [2], or copper only SOD (CuSOD) [3]. This article focuses on the three mammalian SOD isozymes.

Cu,ZnSOD is a homodimer with a molecular mass of 32 kDa. Both MnSOD and ECSOD are homotetramers with a molecular mass of 86–88 and 135 kDa, respectively. ECSOD also contains copper and zinc. Cu,ZnSOD is present primarily in the cytosol. It is also found in the nucleus and mitochondrial intermembrane space [4]. MnSOD exists in the mitochondrial matrix. ECSOD is associated with the plasma membrane or present in the extracellular space. The human genes for CuZnSOD, MnSOD, and ECSOD are localized on chromosomes 21q22, 6q25, and 4q21, respectively. The basic characteristics of the above three SOD isozymes are summarized in **Table 1**.

2. HISTORY

The enzymatic activity of Cu,ZnSOD was first reported by J.M. McCord and I. Fridovich in 1969 [5]. But the protein was discovered before. T. Mann and D. Keilin had purified the protein 30 years earlier from bovine blood and liver as a copper-binding protein of unknown function. The protein was then known as erythrocuprein, hepatocuprein, or cytocuprein. Superoxide, the substrate of SODs was discovered by L. Pauling in the 1930s, and it was not known then if this free radical could be produced biologically. In 1969, P.F. Knowles and coworkers showed that the enzyme xanthine oxidase could produce superoxide. J.M. McCord and I. Fridovich subsequently demonstrated that the copper protein purified by T. Mann and D. Keilin could catalytically eliminate superoxide. This copper-containing protein is now known as Cu,ZnSOD. In 1970 and 1973, I. Fridovich and coworkers further discovered MnSOD and FeSOD (FeSOD is not present in mammals), respectively. The third SOD in mammals, namely ECSOD, was discovered by S.L. Marlund and coworkers in 1982 [6].

3. BIOCHEMISTRY

The three mammalian SOD isozymes catalyze dismutation of superoxide ($O_2^{\bullet -}$) to form hydrogen peroxide (H_2O_2) and molecular oxygen with a similar reaction rate constant of ~1.6×10^9 $M^{-1}s^{-1}$. The overall mechanism by which SODs function has been called the “ping-pong” mechanism as it involves the sequential reduction and oxidation of the redox metal center of the enzymes, with the concomitant oxidation and reduction of superoxide (**Figure 1**).

4. MOLECULAR REGULATION

4.1. Transcription Factors

A number of transcription factors have been implicated in regulating the constitutive or inducible expression of SOD genes. These include NF-κB, AP-1, AP-2, Sp1, and CCAAT-enhancer-binding protein (C/EBP) [7]. Notably, NF-κB response elements are present in the promoter region of both Cu,ZnSOD and MnSOD [8, 9], and NF-κB may play a critical role in regulating the gene expression of these two SOD isozymes, especially MnSOD [10]. Consensus p53 binding sequences are found in the promoter regions of human MnSOD gene, and binding of p53 causes increased transcription of the MnSOD gene [11]. Recently, it was shown that the nuclear factor E2-related factor 2 (Nrf2) could also regulate Cu,ZnSOD expression via an antioxidant response element (ARE)-driven mechanism [12].

TABLE 1. Basic characteristics of mammalian SOD isozymes

Isozyme	Molecular mass (kDa)	Assembly of subunits	Metal ions	Cellular location	Chromosomal location
Cu,ZnSOD (SOD1)	32	Homodimer	Cu and Zn	Cytosol, nucleus, mitochondrial intermembrane space	21q22
MnSOD (SOD2)	86–88	Homotetramer	Mn	Mitochondrial matrix	6q25
ECSOD (SOD3)	135	Homotetramer	Cu and Zn	Plasma membrane, extracellular space	4q21

4.2. Epigenetic Regulation

In addition to the regulation by transcription factors, SOD genes are also subjected to epigenetic regulation, such as hypermethylation, leading to epigenetic silencing [13]. The term epigenetic regulation refers to heritable changes at the level of gene expression not related to the underlying DNA sequence. Epigenetic attenuation of MnSOD may initiate and sustain an inheritable form of pulmonary arterial hypertension by impairing redox homeostasis [13] as well as contribute to the development of diabetic retinopathy [14].

4.3. Other Regulatory Mechanisms

SOD gene expression is also regulated post-transcriptionally via changes in mRNA stability and mRNA translation, as well as post-translational modifications [15]. Notably, MnSOD mRNA is a direct target of the microRNA miR-212, and overexpression of miR-212 decreases the levels of MnSOD [16].

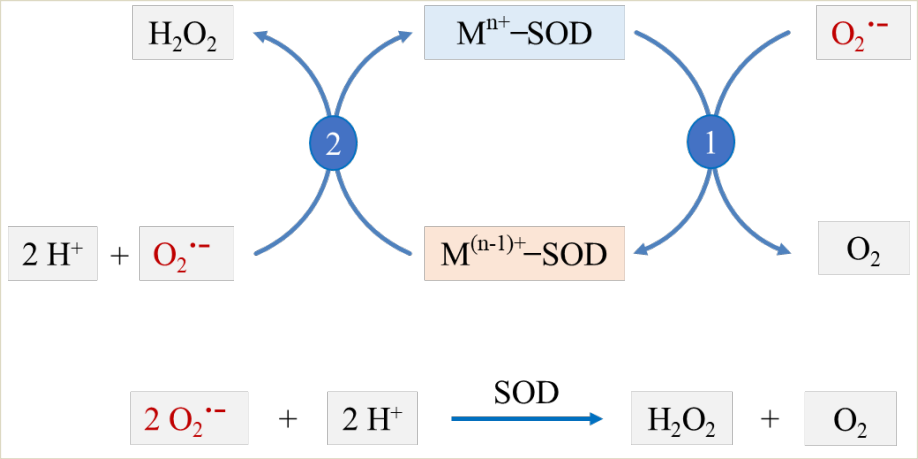

FIGURE 1. Mechanism of SOD-catalyzed dismutation of superoxide ($O_2^{\bullet-}$) to form hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). As illustrated, the overall mechanism involves two sequential reactions. In reaction (1), one superoxide reduces the SOD redox metal ion M^{n+} to $M^{(n-1)+}$ (e.g., reduction of Cu^{2+} in Cu,ZnSOD to Cu^{1+}) and the superoxide is converted to O_2. In the subsequent reaction (2), another superoxide oxidizes the $M^{(n-1)+}$ back to the original M^{n+} (e.g., oxidation of Cu^{1+} in Cu,ZnSOD to Cu^{2+}) and the superoxide, in the presence of 2 H^+, is converted to H_2O_2 and the SOD is back to the initial state. The overall mechanism is like a "ping-pong" ball in action and is hence known also as the "ping-pong" mechanism. M denotes the redox metal ion in the SOD isozymes, i.e., Cu^{2+} in CuZnSOD and ECSOD, and Mn^{3+} in MnSOD.

5. BIOLOGY AND MEDICINE

The conventional function of all three mammalian SOD isozymes is dismutation of superoxide, thereby protecting against superoxide-mediated biological damage. In addition to this conventional function, SODs also possess atypical activities contributing to both health and disease.

5.1. Conventional Functions

5.1.1. Protection against Superoxide Toxicity

Superoxide plays a causal role in diverse disease processes, including neurodegeneration, hyperoxia-mediated pulmonary injury, and tissue ischemia-reperfusion injury, among many others [17]. The primary biological function of SODs is to dismutase superoxide and thereby protect against the above superoxide-associated disease processes, as demonstrated primarily with SOD-transgenic animal models [17].

5.1.2. Role in Embryonic Development

While homozygous deletion of Cu,ZnSOD does not affect mouse survival under normal conditions, homozygous knockout of MnSOD causes embryonic or early neonatal death in mice [18]. This suggests that MnSOD is essential for development and survival. Although Cu,ZnSOD deletion does not affect survival, its deficiency impairs olfactory sexual signaling and alters bioenergetic function in mice [19]. On the other hand, mice with ECSOD deficiency develop normally with no significant phenotypic alterations under physiological conditions [20].

5.2. Atypical Activities

5.2.1. SODs as Peroxidases

In addition to its function in catalyzing the dismutation of superoxide to hydrogen peroxide, Cu,ZnSOD may also possess other enzymatic activities. For example, Cu,ZnSOD is shown to exert a peroxidase activity. In this regard, interaction of hydrogen peroxide with Cu,ZnSOD in the presence of bicarbonate yields carbonate radical ($CO_3^{\bullet-}$), a highly reactive species [21]. In addition, Cu,ZnSOD catalyzes tyrosine nitration by peroxynitrite [22]. As described below, in cancer cells, MnSOD may become a peroxidase driving cancer progression [23].

5.2.2. Neurodegeneration Caused by Mutant Cu,ZnSOD

Mutations of Cu,ZnSOD gene, especially the alanine to valine mutation at codon 4 (A4V), are recognized as a cause of familial amyotrophic lateral sclerosis (ALS) [24, 25]. The mutant Cu,ZnSOD results in the formation of aggregates and subsequent neuron degeneration. As compared with the normal enzyme, the mutant Cu,ZnSOD generates more free radicals in the presence of hydrogen peroxide [25]. As stated earlier [21] and demonstrated elsewhere [26, 27], reaction of hydrogen peroxide with the copper redox center of Cu,ZnSOD leads to the formation of highly toxic free radicals, including hydroxyl radicals. In addition to the increased free radical production, the mutant Cu,ZnSOD aggregates may spread the disease in a prion-like fashion in the central nervous system [28] (**Figure 2**).

A recent case study reported that an infant with a homozygous truncating mutation of Cu,ZnSOD showed complete absence of erythrocyte Cu,ZnSOD activity. The complete absence of Cu,ZnSOD activity conferred the patient's cells in culture with extreme sensitivity to oxygen toxicity. The infant's phenotype was remarkable for the predominant impairment of upper motor neurons, whereas other organ systems were unaffected [29]. Although the study did not establish a causal relationship between the loss of Cu,ZnSOD activity and the impairment of motor neuron function, the finding is in line with the notion that mutant Cu,ZnSOD is a cause of familial ALS.

5.2.3. Tumor Promotion by MnSOD

Since the dismutation product of SODs is hydrogen peroxide, which is also a reactive oxygen species (ROS) of biological activity [30], SODs may exert detrimental effects under certain conditions. For example, MnSOD overexpression is found to play a role in promoting tumorigenesis [31, 32]. Several mechanisms have been suggested: (1) MnSOD maintains highly functional mitochondria by scavenging superoxide to support the high metabolic activity of the cancer cells; (2) MnSOD increases the production of H_2O_2, which in turn stimulates cancer cell

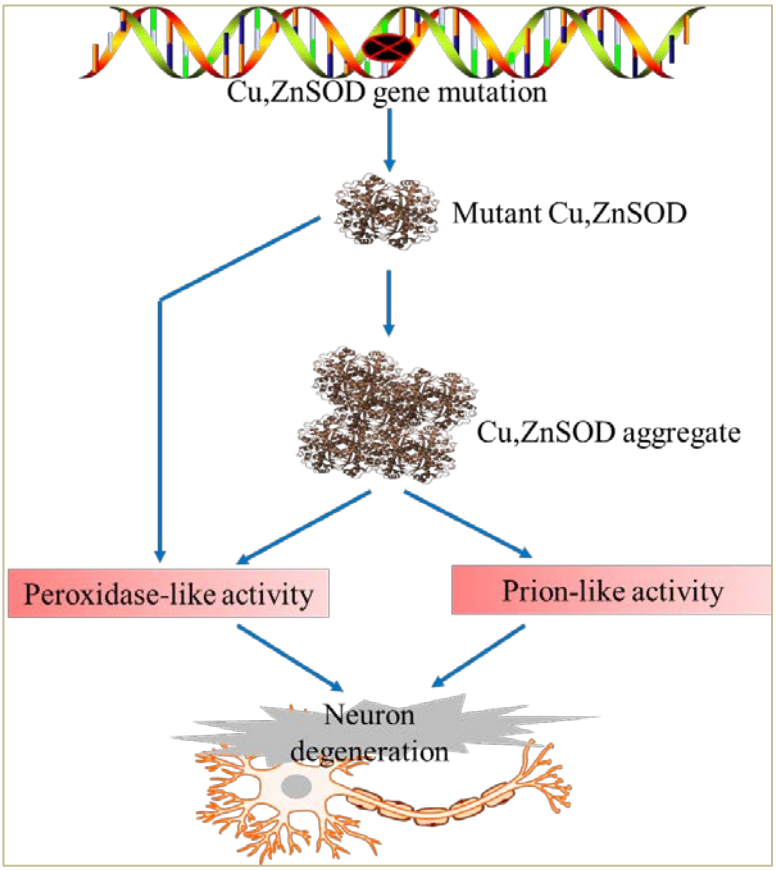

FIGURE 2. Potential mechanisms responsible for the development of familial amyotrophic lateral sclerosis (ALS) associated with Cu,ZnSOD gene mutations. As illustrated, a mutation in the Cu,ZnSOD gene (e.g., the alanine to valine mutation at codon 4) results in the production of a mutant Cu,ZnSOD, whose aggregation leads to the production of large SOD aggregates. The mutant Cu,ZnSOD and the large SOD aggregates may exhibit an augmented peroxidase activity, leading to oxidative damage to neurons. On the other hand, the SOD aggregates may also possess prion-like activity, causing neuron degeneration.

proliferation and metastasis. Indeed, MnSOD upregulation in cancer cells establishes a steady flow of H_2O_2 originating from mitochondria that sustains AMP-activated kinase (AMPK) activation and the metabolic shift to glycolysis [32]; and (3) MnSOD in tumor cells may become modified (e.g., acetylation) and behave as a tumor-promoting peroxidase, contributing to cancer progression [23].

5.2.4. Cu,ZnSOD as a Signaling Molecule

A recent study reports that Cu,ZnSOD acts as a nuclear transcription factor to regulate oxidative stress resistance [33]. In response to elevated endogenous and exogenous ROS, including H_2O_2, Cu,ZnSOD rapidly relocates into the nucleus, and in the nucleus, Cu,ZnSOD binds to promoters and regulates the expression of oxidative resistance and repair genes in yeast [33] and numerous functioning genes including oncogenes and amyotrophic lateral sclerosis-linked genes in mammalian cells [34] (**Figure 3**).

In addition, Cu,ZnSOD appears to be a key component of the mechanistic target of rapamycin complex 1 (mTORC1) nutrient signaling that modulates energy metabolism, redox homeostasis, and cellular aging [35]. Specifically, mTORC1 pathway regulates

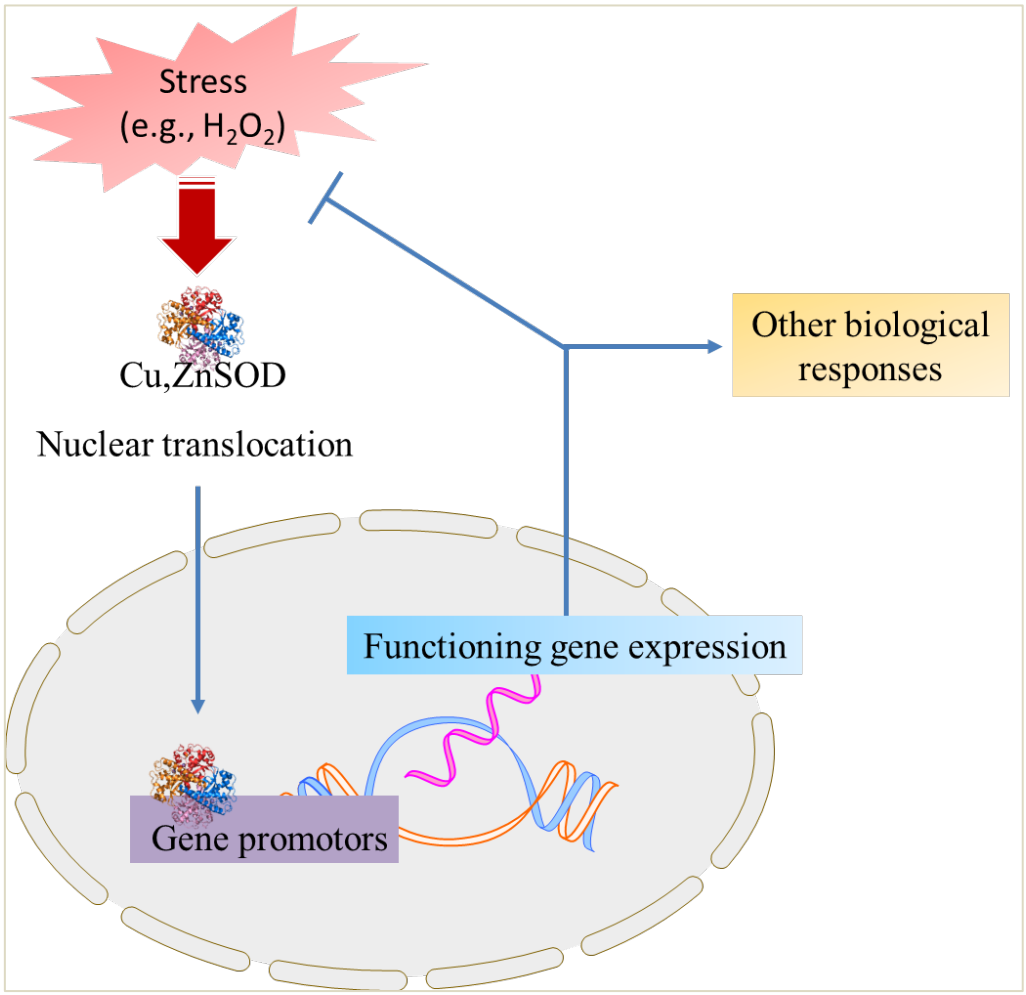

FIGURE 3. Cu,ZnSOD as a transcription factor. As illustrated, under stress conditions, such as increased intracellular H_2O_2 levels, Cu,ZnSOD undergoes nuclear translocation and binds to the promoter regions of multiple functioning genes, leading to increased transcription of genes whose products are involved in oxidative stress resistance as well as other biological processes.

Cu,ZnSOD activity through reversible phosphorylation at Ser39 in yeast and at Thr40 in humans in response to nutrients, thereby controlling cellular redox and preventing oxidative damage [35].

The implications of the above findings in health and disease remain to be elucidated. Nevertheless, they open the door for exciting investigations on Cu,ZnSOD as a signaling molecule in regulating cellular redox homeostasis and possibly other cellular processes involved in aging and cancer development [36, 37].

6. CONCLUSION

In addition to their well-established function in catalyzing the dismutase of superoxide to form H_2O_2 and molecular oxygen, native and altered SODs may possess atypical biological activities, such as causing neurodegeneration, tumor promotion, and peroxidation, as well as participating in transcriptional and redox regulation. Deciphering the complex biological activities of SODs would allow the development of effective modalities for the intervention of human

diseases associated with superoxide toxicity or SOD dysfunction.

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