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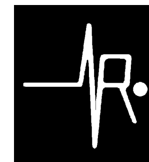

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Original Contribution

Mitochondrial redox state regulates transcription of the nuclear-encoded mitochondrial protein manganese superoxide dismutase: a proposed adaptive response to mitochondrial redox imbalance

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Abstract

Overexpression of human manganese superoxide dismutase (MnSOD) in mouse NIH/3T3 cells using an inducible retroviral system led to alterations in the mitochondrial redox state since levels of reactive oxygen species rapidly increased after induction of human MnSOD (*Antioxid. Redox Signal.* **6:**489–500; 2004). Alterations in exogenous human MnSOD led to large increases in levels of endogenous mouse MnSOD (*sod2*) and thioredoxin 2 (*txn*2) mRNAs, but smaller increases in MnSOD and thioredoxin 2 protein expression. Tight regulation of mitochondrial protein levels seems to be necessary for optimal cellular function, since mitochondrial antioxidant protein levels did not increase to the same extent as antioxidant protein mRNA levels. We hypothesize that these changes in antioxidant proteins are adaptations to the altered mitochondrial redox state elicited by MnSOD overexpression. The mitochondrial-specific antioxidant MitoQ reversed cell growth inhibition, and greatly decreased levels of endogenous *sod2* and *txn2* transcripts following induction of exogenous MnSOD. Elevated levels of mouse *sod2* transcripts resulted from transcriptional activation of the endogenous *sod2* gene since actinomycin D prevented transcription of this gene. Therefore, the mitochondrial redox state appears to modulate a nuclear-driven biochemical event, i.e., transcriptional activation of a nuclear gene encoding a protein targeted to mitochondria.
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Keywords: MnSOD; Thioredoxin 2; Transcription; mRNA; Mitochondrial redox; H_2O_2; Mitochondrial antioxidant; MitoQ; Free radicals

Introduction

In eukaryotic cells, three isoforms of superoxide dismutase (SOD) are present: extracellular copper/zinc-containing SOD (*sod3*:ECSOD), mitochondrial manganese-containing SOD (*sod2*:MnSOD), and cytoplasmic/nuclear copper/zinc-containing SOD (*sod1*:CuZnSOD), although the latter also localizes to the mitochondrial intermembrane space [1]. While the SOD isoenzymes catalyze the identical dismutation reaction involving the conversion of superoxide anion ($O_2^{\bullet-}$) to oxygen (O_2) and hydrogen peroxide (H_2O_2), the function of each SOD isoform in cellular physiology appears to be very different, and often one SOD cannot compensate for another. For example, the lethal phenotype

Abbreviations: AOE, antioxidant enzyme; DCF,2′,7′ - dichlorofluorescein diacetate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H_2O_2, hydrogen peroxide; IPTG, isopropyl β-thiogalactoside; MitoQ, a mitochondrial-specific antioxidant which is a mixture of mitoquinol and mitoquinone; MnSOD, manganese containing superoxide dismutase; $O_2^{\bullet-}$, superoxide anion; Prxns, peroxiredoxins; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; *sod2*, MnSOD gene; TNFα, tumor necrosis factor-α; *txn2*, thioredoxin 2 gene.

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exhibited by $sod2^{-/-}$ knockout mice was not corrected or delayed by *sod1* overexpression, suggesting that the subcellular location of SOD is important in physiological functions of these enzymes [2].

In a resting cell, superoxide anion is produced at 1–2% of total daily oxygen consumption during electron transfer and oxidative phosphorylation for ATP generation by mitochondria [3]. Consequently, mitochondria are the major source of reactive oxygen species (ROS) in a resting cell. Due to their ubiquitous reactivities, ROS can be converted to other radicals such as reactive nitrogen species (RNS) and reactive carbon species in subcellular location- and local redox state-dependent manners [4]. Mitochondrial ROS are now appreciated as regulators of mitochondrial functions including electron transfer chain enzymes and mitochondrial membrane potential [5,6]. In addition, they are involved in regulation of other subcellular organelles [4].

The inverse correlation between MnSOD activity and cell growth is a paradoxical phenomenon when one postulates that MnSOD functions only as an antioxidant enzyme (AOE) to protect a cell from oxidative stress caused by $O_2^{\bullet-}$ [7]. Proposed hypotheses regarding mechanisms by which MnSOD exerts growth inhibition often emphasize increased H_2O_2 production secondary to elevated MnSOD activity resulting in oxidative environments first in mitochondria and subsequently in the cytoplasm [8–10].

Previous studies from our laboratory demonstrated that overexpression of human MnSOD in NIH/3T3 fibroblasts utilizing an inducible retroviral system resulted in inhibition of cell growth via prolongation of G_1 and S phases of the cell cycle (see Fig. 5 for a summary of data from the previous study) [11]. We postulated that this was a physiological mechanism to regulate cell cycle progression since prolongation of G_1 and S phases was completely reversible following removal of the inducer and hence return of MnSOD expression to the original level. When cellular ROS levels were measured using DCF fluorescent dye and flow cytometry, a burst of ROS with concomitantly decreased mitochondrial membrane potential preceded transient and reversible cell cycle modulation following MnSOD induction. Sustained ROS increase was thus not necessary for MnSOD-mediated growth inhibition (see Fig. 5) [11].

As a result of these previous observations, we hypothesized that changes in the mitochondrial redox state by elevated MnSOD activity were utilized to coordinate physiological and biochemical events in two subcellular compartments. Also, MnSOD may serve as a regulatory enzyme modified by the mitochondrial redox environment [11].

In addition to a transient ROS burst after MnSOD induction, newly established steady-state levels of ROS after the initial ROS burst were approximately 50% lower than control levels (see Fig. 5) [11]. We postulated that there must be alterations in mitochondrial antioxidant proteins and/or antioxidant enzymes responsible for the newly established low steady-state levels of ROS. Guo et al. [12] have previously proposed that overexpression of MnSOD results in redox alterations with subsequent expression of stress-responsive nuclear genes. We speculated that adaptive changes in mitochondrial redox capacity following MnSOD overexpression could also occur at the transcriptional level of nuclear genes encoding mitochondrial antioxidant proteins and/or AOEs.

To test the hypothesis of mitochondrial redox state-mediated transcriptional regulation of nuclear genes encoding mitochondrial antioxidant proteins and/or AOEs, MitoQ, a mitochondrial specific antioxidant, was utilized in this study. MitoQ refers to the mixture of the mitochondrially targeted-quinol (mitoquinol) and –quinone (mitoquinone); these ubiquinone derivatives preferentially accumulate in the mitochondria matrix at levels over several hundred-fold compared to cytoplasm [13]. The antioxidant properties of MitoQ are mediated by oxidation of mitoquinol to mitoqinone, which is subsequently regenerated through reduction to mitoquinol by respiratory complexes. The efficacy of MitoQ as a mitochondrial antioxidant was previously demonstrated: (1) prevention of lipid peroxidation by H_2O_2 and ferrous iron, (2) scavenging of peroxynitrite, and (3) prevention of apoptosis by H_2O_2, but not by staurosporine or tumor necrosis factor-α (TNFα) [14].

In this paper, we utilized MnSOD as a molecular tool to manipulate the mitochondrial redox state through its antioxidant activity of removing $O_2^{\bullet-}$ and/or its pro-oxidant activity of generating H_2O_2. mRNA levels of endogenous MnSOD (*sod2*) and thioredoxin 2 (*txn2*) genes were modulated by inducible expression of exogenous MnSOD cDNA. MnSOD and thioredoxin 2 are mitochondrial proteins. In studies utilizing a mitochondrial-specific antioxidant (MitoQ), transient redox imbalance in mitochondria following MnSOD induction was shown to be responsible for transcriptional activation of the nuclear-encoded endogenous *sod2* gene. These observations provide another example, besides the previously described modulation of cell cycle kinetics [11], in which the mitochondrial redox state coordinates cellular events directed by other subcellular organelles, that is, the transcriptional regulation of a nuclear gene encoding a protein targeted to mitochondria.

Materials and methods

Chemicals and reagents

All chemicals were purchased from Sigma Chemical Co. (Saint Louis, MO), unless otherwise specified. Tissue culture supplies were from Falcon (Becton Dickinson Labware, Franklin Lakes, NJ). WI-38 VA 13 (SV-40-transformed human lung fibroblast) and NIH/3T3 (mouse embryo fibroblast) cell lines were obtained from the American Type Culture Collection (Manassas, VA). DMEM with high glucose (4.5 g/liter), and IPTG (isopropyl β-thiogalactoside) were obtained from Life Technology

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