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Exposure to bile acids alters the intracellular location and function of MnSOD in Barrett's esophagus

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ABSTRACT

Background: Oxidative stress secondary to bile-acid exposure has been associated with metaplastic degeneration of normal esophageal mucosa into Barrett's esophagus (BE) cells and eventually esophageal adenocarcinoma. We previously reported that the macromolecular response of BE cells to this stress was largely regulated by the expression of manganese-dependent mitochondrial superoxide dismutase (MnSOD). As the mitochondrion plays a vital role in MnSOD activation, this study sought to determine the location and activity of MnSOD within BE cells after exposure to oxidative stress.

Methods: A human BE cell line, BAR-T cell, was exposed 0.4 mM concentrations of taurocholic acid (Tau) or a 0.4 mM 1:1 mixture of bile salts for 4 h. Cell viability was performed with 3-(4, 5-dimthyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide assays. Proteins were extracted and separated into mitochondrial, nuclear, and cytoplasmic fractions followed by analysis by a western blot and enzymatic activities.

Results: BAR-T cell showed resistance to the bile-salt insults. Expression of MnSOD was significantly increased in the cells exposed to a mixture of bile acids and Tau *versus* control. Mitochondria MnSOD is abundant and highly active. Nuclear fraction displayed presence of both MnSOD and Cu/zinc superoxide dismutase secondary to bile-acid exposure; however, the MnSOD was inactive in nuclear fraction.

Conclusions: This is the first study to specifically evaluate cellular fraction MnSOD expression, increased in BE cells in response to the oxidative stress of bile exposure. Mitochondrial MnSOD contributes to resistance of BAR-T cells to the bile-salt insults. Further investigation is required to determine the potential correlation between bile exposure and BE to adenocarcinoma progression via MnSOD-mediated cell signaling.

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Introduction

Esophageal cancer is among the deadliest of malignancies. With an increasing incidence rate, esophageal cancer accounts for approximately 16,910 new cancer diagnoses each year with nearly 15,690 cancer-related deaths annually.[1] Current survival outcomes from esophageal adenocarcinoma (EAC) are particularly dismal, with a 5-year overall survival of approximately 18%.[1] Over the past decades, epidemiologic trends have shown a massive shift in esophageal cancer histology—most tumors are now EAC compared to the previously more present squamous cell carcinomas.[2-4] The increase in EAC incidence has been linked to Barrett's esophagus (BE), a stepwise metaplastic degeneration of esophageal epithelium secondary to gastroesophageal reflux disease, which drastically increases the risk of EAC.[3,5]

Reactive oxygen species (ROS) have been implicated among the primary causes of carcinogenesis. Recent work details its link to cell signaling and homeostasis beyond the primary effects of nucleic acid damage.[6] Gastric refluxate is composed of multiple molecular compounds that have been shown to produce ROS that may play a role in the progression of BE to EAC.[7] The use of proton pump inhibitors to quell the symptoms of chronic reflux has not changed the incidence of the disease. Instead, these medicines have merely rendered reflux episodes clinically silent, allowing for continued reflux of bile containing intestinal contents into the proximal esophagus. This course of treatment allows for continued and silent exposure of the esophageal mucosa to potential endogenous sources of ROS.

The superoxide dismutase (SOD) family is a group of natural molecular defenders against ROS in nearly all living cells exposed to oxygen. This family includes: cytosolic copper/zinc-dependent SOD (Cu/ZnSOD), iron-dependent extracellular SOD, and manganese-dependent mitochondrial SOD (MnSOD).[8,9] Mitochondria possess predominantly the mitochondrial isoform-MnSOD or SOD, which locates at the matrix level, and a small fraction of the copper/zinc SOD (Cu/ZnSOD) or SOD1, which resides in the intermembrane space of mitochondria.[10,11] Generation of ROS mainly takes place at the electron transport chain located on the inner mitochondrial membrane during the process of oxidation; the mitochondrial MnSOD represents a major cellular defense against oxidative stress.[12] SOD molecules have been shown to be decreased in BE.[13] In addition, MnSOD has been implicated as a tumor suppressor through the prevention of ROS formation and maintenance of homeostasis.[8] Current thinking links MnSOD as a potential actor in the final common pathway of C-myc/nuclear factor kappa-light-chain-enhancer of activated B cells action as well as to mitochondrial p53.[14] MnSOD must exist in its membrane-bound, tetrameric form in the mitochondrion so that it can acquire the manganese needed to reduce ROS.[10]

Previously, we have found that MnSOD expression is decreased in an animal model during the progression from reflux esophagitis to EAC.[15] Also, we have shown that MnSOD is not exclusively located within the mitochondrion following ROS stress of benign esophageal mucosa cells, instead moving to the cytoplasm where it has decreased function.[11] We have also shown that this decrease in MnSOD expression can be replicated through exposure to the molecular components of bile refluxate: cholate, deoxycholate, chenodeoxycholate, and glycocholate. Furthermore, we have shown that the response in premalignant and malignant cells is altered/attenuated in response to bile-salt exposure.[16]

The purpose of this study was to investigate alteration of MnSOD in the premalignant BE cell following exposure to bile salts as a challenging of oxidative stress. Given that bile salt–caused oxidative stress has been shown to induce cytoplasmic translocation of MnSOD in benign esophageal mucosal cells, we hypothesize that BE cells may have a similar response with possible alterations of MnSOD activity and expression and the potential for aberrant translocation. It is particularly important to note the possibility of location within the cytosol where the nascent MnSOD molecule remains inactive and unable to form the functional MnSOD necessary to reduce ROS. Understanding MnSOD location, expression, and activity within premalignant BE cells is critical to elucidate the potential mechanism by which MnSOD exerts its tumor suppressor activity.

Methods

Cell line and culture

BAR-T is a human telomerase reverse transcriptase-immortalized nonneoplastic Barrett's cell line, which was generously gifted by Rhonda Souza, MD, and S.J. Spechler at the Department of Medicine, VA North Texas Health Care System and the University of Texas Southwestern Medical School (Dallas, Texas). The BAR-T cells were maintained in keratinocyte-basal medium 2 (Clonetics) at 37°C in 75 mL flasks (Greiner Bio-One, Monroe, NC). Authentication of the BAR-T cell line for experiment was performed (Bio-Synthesis, Inc.) within 6 mo.

Bile-salt exposure and cell viability

The BAR-T cells were seeded onto 96 well plates at a density of 1×10^4 cells/well and allowed to reach 90% confluence. Cells were then exposed to varying concentrations of bile salts over various time points to determine what level of mixed bile acids resulted in cell death resistance over clinically significant potential time frames. Cells were exposed to medium alone as untreated control. For the bile-salt treatment, cells were exposed respectively to individual bile salts, including deoxycholate, chenodeoxycholate, taurocholate, and glycocholate (Sigma, St. Louis, MO) or a mixture of bile acids (1:1 mixed of each bile salt). Cell survivability was assessed via a 3-(4, 5-dimthyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction assay according to the previous report.[11] In brief, cells were washed with phosphate-buffered saline (PBS) after bile-salt treatment, and then were incubated in 100 μL of 1 mg/mL MTT solution for 4 h. Following this, the supernatant was removed and 100 μL dimethyl sulfoxide (Sigma, St. Louis, MO) was added and the cells were allowed to incubate for 10 min. Absorption was then determined via an optical density plate reader using measurements at 570 nm and 620 nm. Final