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Major vault protein regulates cell growth/survival signaling through oxidative modifications

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ABSTRACT

Major vault protein forms a hollow, barrel-like structure in the cell called the vault, whose functions and regulation are not well understood. The present study reports that major vault protein regulates growth/survival signaling in human airway smooth muscle cells through oxidative modifications. The promotion of protein *S*-glutathionylation by asthma mediators such as interleukin-22 and platelet-derived growth factor or by knocking down glutaredoxin-1 or thioredoxin activated cell growth signaling. Mass spectrometry identified that major vault protein is glutathionylated. Major vault protein knockdown enhanced cell death and inhibited STAT3 and Akt signaling. We identified a protein partner of major vault protein that is regulated by glutaredoxin-1, namely myosin-9, which was found to serve as a cell death factor. Knocking down myosin-9 or promoting protein *S*-glutathionylation by knocking down glutaredoxin-1 inhibited the death of airway smooth muscle cells by heating to simulate bronchial thermoplasty, a clinically successful procedure for the treatment of severe asthma. These results establish a novel signaling pathway in which ligand/receptor-mediated oxidation promotes the *S*-glutathionylation of major vault protein, which in turn binds to myosin-9 to suppress the heating-induced death of airway smooth muscle cells.

1. Introduction

Major vault protein (MVP) forms a hollow, barrel-like structure in the cytosol called the vault, which seems to occur ubiquitously. While the detailed x-ray structure of the vault has been reported [1], the functions and regulation of MVP remain unclear. MVP is known to be identical to lung resistance-related protein [2], a major multidrug resistance protein that was originally found in lung cancer cells [3]. MVP is overexpressed in drug-resistant human cancer cells [3,4], and vaults are thought to export drugs from the nucleus for sequestration in cytosolic vesicles [5–7]. In drug-resistant and MVP-overexpressing non-small cell lung cancer cells, MVP is co-localized with anti-tumor drugs [8], and the siRNA knockdown of MVP promotes drug accumulation and cell death [9].

In addition to the deleterious actions of reactive oxygen species (ROS), it is now well accepted that another functional role of ROS is to mediate cell signaling [10]. In airway smooth muscle cells (SMCs), growth and survival signaling induced by ligand/receptor interactions is mediated by ROS [11–14]; however, the mechanism of ROS signaling in airway SMCs has not been well defined. Oxidation can result in protein *S*-glutathionylation, in which glutathione (GSH) is added to cysteine residues. *S*-glutathionylation reactions are regulated by deglutathionylating proteins including glutaredoxin-1 (Grx1) and thioredoxin (Trx) [15]. Understanding the process of the ROS-mediated growth and survival of airway SMCs should thus be important for the regulation of airway remodeling in asthma.

In an effort to understand the mechanism of ROS signaling in airway SMCs, the present study investigated the role of protein *S*-glutathionylation and Grx1. The experiments resulted in the discovery that MVP is expressed in human airway SMCs and that MVP is oxidatively modified via protein *S*-glutathionylation in response to ligand/receptor-mediated cell signaling. We provide evidence that this mechanism serves to inhibit the heating-induced death of airway SMCs, a process that occurs in bronchial thermoplasty, a clinically successful procedure for the treatment of asthma.

2. Materials and methods

2.1. Cell culture

Human bronchial SMCs from ScienCell Research Laboratories (Carlsbad, CA, USA) were cultured in accordance with the manufacturer's

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instructions in 5% CO_2 at 37 °C. Cells in passages 3–7 were used. Cells were growth-arrested for 2 days in media containing 0.01% fetal bovine serum for proliferation assays or serum-starved overnight for signaling studies. Cells were treated with recombinant human IL-22 (PeproTech, Inc., Rocky Hill, NJ, USA) or human recombinant PDGF-BB (Invitrogen, Carlsbad, CA, USA). For siRNA knockdown, cells were transfected with an siRNA Transfection Reagent and gene silencing siRNAs along with the controls by using a scrambled sequence from Santa Cruz Biotechnology (Dallas, TX, USA). Cells were used for experiments 2 days after transfection.

2.2. Immunoblotting and immunoprecipitation

Cell lysates were prepared and immunoblotting was performed as previously described [16]. The antibodies used were phospho-STAT3 (Tyr705), phospho-Akt (Ser473), cleaved caspase-3, LC3B (Cell Signaling Technology, Danvers, MA, USA), GAPDH, MVP, myosin-9, GSH (Santa Cruz Biotechnology and EMD Millipore Corporation, Billerica, MA, USA) and p62/SQSTM1 (Syd Labs Inc., Natick, MA). Electrophoresis through an SDS-polyacrylamide gel was followed by electroblotting onto a nitrocellulose membrane. The Enhanced Chemiluminescence System (Amersham Biosciences, Piscataway, NJ, USA) was used for detection.

For immunoprecipitation, cell lysates were incubated with 1 μl of antibodies and 10 μl GammaBind G-Sepharose (Amersham) overnight at 4 °C with gentle shaking. After washing twice, the pellet was boiled in Laemmli buffer and centrifuged, and the supernatant was subjected to reducing 12% SDS-polyacrylamide gel electrophoresis and electroblotted onto a nitrocellulose membrane. The membrane was then blocked with 5% milk for 1 h at room temperature and incubated overnight at 4 °C with antibodies, followed by incubations with HRP-conjugated secondary antibodies and ECL detection.

2.3. Mass spectrometry

Protein bands were excised from the Coomassie Blue-stained gels. Mass spectra were recorded by using a matrix-assisted laser desorption/ionization–time of flight, time of flight (MALDI-TOF-TOF) spectrometer (4800 Proteomics Analyzer, Framingham, MA, USA). Peptide masses were compared with the theoretical masses derived from the sequences contained in SWISS-PROT/NCBI databases by using MASCOT.

2.4. Statistical analysis

Means ± SEM were calculated. The comparisons between two groups were analyzed by using a two-tailed Student's *t* test, while those between three or more groups were analyzed by using two-way ANOVA with a Student–Newman–Keuls post-hoc test with the Kolmogorov–Smirnov test for normality. $P < 0.05$ was considered to be significant.

3. Results

3.1. Role of glutathionylation in growth/survival signaling in airway SMCs

Protein *S*-glutathionylation can be reversed by Grx1 or Trx. STAT3, an important growth/survival signaling molecule, was phosphorylated

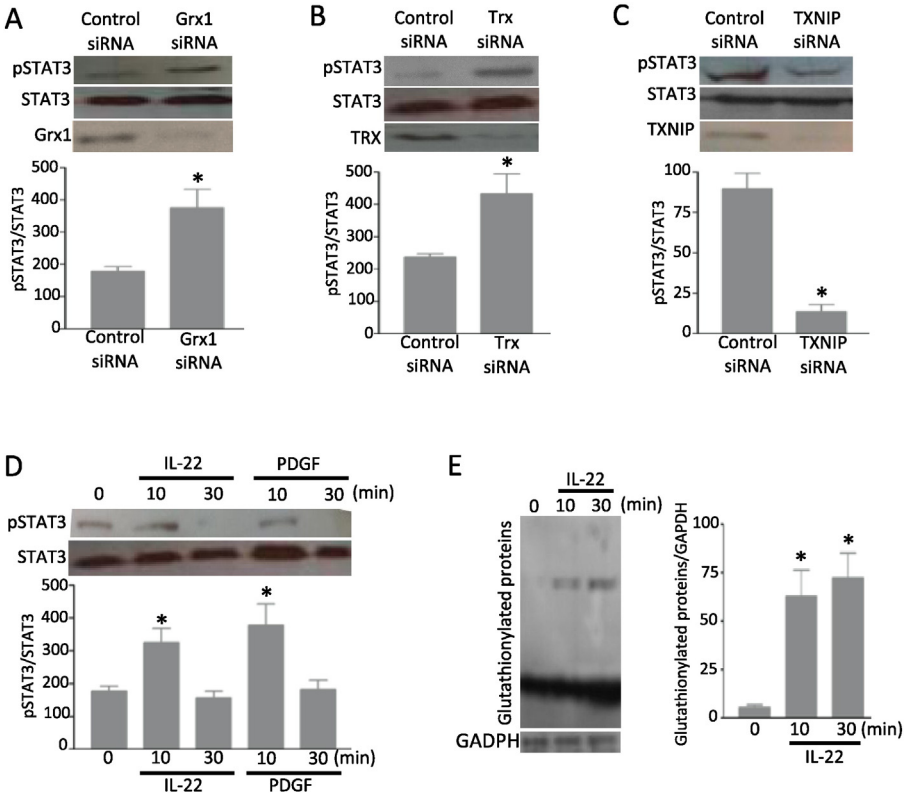

Fig. 1. Protein *S*-glutathionylation confers cell growth/survival/signaling. (A–C) Human bronchial SMCs were transfected with control siRNA and siRNAs to knockdown (A) Grx1 (n = 6), (B) Trx (n = 6), or (C) TXNIP (n = 6) to monitor phosphorylated STAT3 (pSTAT3) by immunoblotting. (D) Human bronchial SMCs were treated with IL-22 (10 ng/ml) and PDGF (10 ng/ml) for durations indicated. Cell lysates were subjected to immunoblotting to monitor phosphorylated STAT3 (n = 4). (E) Human bronchial SMCs were treated with IL-22 (10 ng/ml) for 10 and 30 min, levels of GSH protein adducts were monitored by nonreducing SDSPAGE and immunoblotting using the GSH antibody (n = 4). Bar graphs represent means ± SEM. * denotes values are significantly different from control at $p < 0.05$.