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Cortactin mediates elevated shear stress-induced mucin hypersecretion via actin polymerization in human airway epithelial cells

Chunyi Liu [a], Qi Li [a], Xiangdong Zhou [a,*], Victor P. Kolosov [b], Juliy M. Perelman [b]

[a] Division of Respiratory Medicine, Second Affiliated Hospital, Chongqing Medical University, No. 74, Linjiang Road, Yuzhong District, Chongqing 400010, China

[b] Far Eastern Scientific Center of Physiology and Pathology of Respiration, Siberian Branch, Russian Academy of Medical Sciences, Blagoveshchensk, Russia

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ABSTRACT

Mucus hypersecretion is a remarkable pathophysiological manifestation in airway obstructive diseases. These diseases are usually accompanied with elevated shear stress due to bronchoconstriction. Previous studies have reported that shear stress induces mucin5AC (MUC5AC) secretion via actin polymerization in cultured nasal epithelial cells. Furthermore, it is well known that cortactin, an actin binding protein, is a central mediator of actin polymerization. Therefore, we hypothesized that cortactin participates in MUC5AC hypersecretion induced by elevated shear stress via actin polymerization in cultured human airway epithelial cells. Compared with the relevant control groups, Src phosphorylation, cortactin phosphorylation, actin polymerization and MUC5AC secretion were significantly increased after exposure to elevated shear stress. Similar effects were found when pretreating the cells with jasplakinolide, and transfecting with wild-type cortactin. However, these effects were significantly attenuated by pretreating with Src inhibitor, cytochalasin D or transfecting cells with the specific small interfering RNA of cortactin. Collectively, these results suggest that elevated shear stress induces MUC5AC hypersecretion via tyrosine-phosphorylated cortactin-associated actin polymerization in cultured human airway epithelial cells.

1. Introduction

Mucus hypersecretion is a remarkable pathophysiological manifestation in airway obstructive diseases, such as chronic obstructive pulmonary disease and asthma. Excessive secreted mucus accumulates in airway can aggravate airway narrowing and serve as a hotbed for bacterial growth, contributing to disturbing symptoms, and even clinical deterioration and death (Evans et al., 2009; Morcillo and Cortijo, 2006). Therefore, it is urged to clarify the disease-specific mechanism leading to mucus hypersecretion. Airway obstructive diseases are physiologically characterized as bronchoconstriction, leading to increase in the airflow-induced shear stress (frictional force per surface area) in airway (Garcia et al., 2006; Wirtz and Dobbs, 2000). It has been reported that mechanical stress of airway constriction increases mucin5AC (MUC5AC) production (Park and Tschumperlin, 2009), thus we hypothesized that the mechanical environment present in narrow airway, characterized by elevated shear stress, would cause the excessive secretion of MUC5AC, which is the prominent mucin expressed in human airway epithelial cells and is the most representative mucin in airway diseases with mucus hypersecretion.

Actin filamentous, a ubiquitous structural protein in eukaryotic cells, is responsible for a variety of cellular processes based on membrane dynamics, such as endocytosis, exocytosis, cell motility, cell morphology, locomotion, polarity and adhesion (Borisy and Svitkina, 2000; Lenne et al., 2006). Numerous studies have shown that actin polymerization plays a positive role in regulating exocytosis by transporting and guiding secretory granules to the plasma membrane docking sites (Lanzetti, 2007; Noda and Sasaki, 2008). For example, in the exocytotic process of mast cells, PC12 cells and nasal epithelial cells, secretagogue stimuli enhance secretion by mediating actin polymerization, and agents that completely depolymerize actin can significantly inhibit secretagogue stimuli-induced exocytosis (Davidovich et al., 2011; Gasman et al., 2004). However, the role of actin polymerization on mucin exocytosis in human airway epithelial cells is unknown. In the present study, we investigated the role of actin polymerization on mucin exocytosis after exposing cells to elevated shear stress.

* Corresponding author. Tel.: +86 023 6369 3207; fax: +86 023 6371 1527.
E-mail addresses: hczx-lcy@163.com (C. Liu), lqlq198210@sina.com (Q. Li), zxd999@263.net (X. Zhou), dncfpd@ramn.ru (V.P. Kolosov), jperelman@mail.ru (J.M. Perelman).

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The filamentous actin-binding protein cortactin is a well-known component of the actin polymerization machinery in various cell types. In addition, as a prominent substrate of $pp60^{Src}$, it is phosphorylated by various members of tyrosine kinases. This protein is expressed in a wide range of tissues, such as the smooth muscle of blood vessels, polarized epithelium and the hematopoietic lineage (Pollard et al., 2000; Wu and Parsons, 1993), and is overexpressed in a variety of human cancers, including the carcinomas of the head and neck, lung, liver, bladder and breast (Buday and Downward, 2007; Kirkbride et al., 2011). Multiple studies have extensively demonstrated that cortactin participates in fluid shear stress-mediated actin polymerization in endothelial cells (Birukov et al., 2002) and podocytes (Friedrich et al., 2006). During these processes, tyrosine phosphorylation of cortactin acts as a switch for cortactin activation and actin cytoskeletal polymerization (Birukov et al., 2002; Friedrich et al., 2006; Lua and Low, 2005). Considering all of the data, cortactin, as a central regulator of the actin cytoskeleton, must regulate actin polymerization under airflow shear stress stimulation in airway epithelial cells as well. Unfortunately, the role of cortactin in airflow shear stress-induced actin polymerization and the subsequent MUC5AC exocystosis is still not clearly defined, and the molecular and biochemical pathways for the cortactin-mediated shear stress-induced responses are largely unknown.

Therefore, in the present study, we first determined the optimal response conditions of shear stress in experiments by analyzing the effect of different response levels and periods of shear stress on MUC5AC secretion. We further analyzed the role of actin polymerization in MUC5AC secretion under shear stress by disrupting actin polymerization before exposing cells to shear stress. Then, we assessed cortactin expression in cells by RT-PCR and western blotting. By modulating the function of cortactin with wild-type, small interfering RNA (siRNA) or tyrosine phosphorylation, we determined the roles of cortactin and its tyrosine phosphorylation during elevated shear stress-mediated responses.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM) (high glucose), Roswell Park Memorial Institute (RPMI) 1640 and fetal bovine serum (FBS) were purchased from Hyclone (Logan, Utah, USA). Protein A beads, jasplakinolide, cytochalasin D, a selective inhibitor of Src-family tyrosine kinases PP2 and fluorescein isothiocyanate (FITC)-labeled phalloidin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Anti-phosphotyrosine antibody (4G10) was purchased from Upstate Biotechnology (Lake Placid, NY, USA). Anti-cortactin, anti-MUC5AC, anti-Src, anti-phospho-Src and anti-β-actin antibodies were purchased from Abcam (Cambridge, MA, USA). Horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Jinqiao Biotech (Beijing, China).

2.2. Cell culture

A human bronchial epithelial cell (16HBE) line was kindly provided by the experimental medical research center of Guangzhou Medical College (Guangzhou, Guangdong, China). 16HBE cells were seeded at 1×10^5 cells in 1 ml in each well of a 24-well plate or at 5×10^5 in 2 ml in each well of a 6-well plate (Costar, Cambridge, MA), and were maintained in DMEM (high glucose) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), glucose (4500 mg/l), L-glutamine (4 mM) and 10% FBS (v/v) at 37 °C in a 5% CO_2 humidified incubator and passaged when cells were 80–90% confluent. Before the experiments, the confluent cells were cultured in serum-free DMEM for additional 24 h to maintain the basal levels of MUC5AC production. A549 cells (human lung adenocarcinoma epithelial cells), purchased from American Type Culture Collection (Rockville, MD, USA), were maintained in RPMI1640 supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), 10% FBS (v/v) under the same conditions used for 16HBE cells.

2.3. Experimental device

A controllable rotative device (Kezhijie; Xi'an, Shanxi, China), as originally described by Tarran et al. (2005), was selected to shear the epithelial cells. In brief, cells were rotated in a go/stop manner for different periods inside a well-humidified incubator. Because of the inertia, this procedure caused an accelerated/decelerated period other than an instantaneous increase/decrease in velocity. To imitate the shear stress experienced under physiological or pathological conditions, the frequencies of acceleration/deceleration were set from 1 s (0.06 dyn/cm^2) to 10 ms (6 dyn/cm^2), whereas the frequencies of shear stress were set at 30 cycles/min to correspond to 15 breaths/min, as shear stress occurs during both inspiration and expiration. The static group was placed in the same rotative device without the oscillatory rotation.

2.4. Agent experiments

To shear the epithelial cells, cells were exposed to a range of shear stress levels for various periods of time. To investigate the role of cortactin, the expression of cortactin was modulated by transfection with siRNA targeted to cortactin or its wild-type form. To investigate the signaling pathway regulating MUC5AC secretion, cells were pretreated with the actin polymerization stabilizer jasplakinolide (1 μM, 1 h), actin disrupting agent cytochalasin D (2 μM, 1 h) or Src kinase inhibitor PP2 (10 μM, 2 h) before the experiments (Liedtke et al., 2003). The equivalent amount of dimethyl sulfoxide (DMSO) or medium alone was used as the vehicle control, and the final concentration of DMSO was less than 0.01% (v/v) for the above conditions. Agent controls were pretreated with these agents without mechanical stimulation.

2.5. Reverse transcriptase-polymerase reaction (RT-PCR)

To detect the cortactin gene expression in airway epithelial cells, total RNA was extracted from cells using Trizol solution (Takara; Dalian, Liaoning, China). 2 μg of total RNA was used for synthesizing cDNA using the RevertAid First Strand cDNA synthesis kit (Thermo; Waltham, MA, USA). cDNA (20 ng) was used for the real-time PCR reaction in the mixture of SYBR Green supermix and primers. The following primers were used: cortactin (forward) 5′-GCC GAC CGA GTA GAC AAG AG-3′ (reverse), 5′-TCC TCC AAA CCC TTT CAC ATA G-3′; GAPDH (forward) 5′-CAA GGT CAT CCA TGA CAA CTT TG -3′ (reverse), 5′-GTC CAC CAC CCT GTT GCT GTA G-3′. The PCR reaction mixture was denatured at 95 °C for 2 min, followed by 40 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 12 s, and 72 °C for another 5 min. All products were separated by 1.5% agarose gel electrophoresis at approximately 207 bp for cortactin and 496 bp for GAPDH. The expression level of cortactin mRNA was normalized to the relative ratio of the expression of GAPDH mRNA.

2.6. Transient transfection

The siRNAs and the cytomegalovirus-driven human cortactin expression plasmid (based on pcDNA3.0) were commercially synthesized by Sangon (Shanghai, China). The sequences of the siRNAs are as follows: the specific siRNA targeted for cortactin (cortactin-siRNA), 5′-AAG TAT GGG GTG CAG AAG GAT (dTdT)-3′,