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Cigarette Smoke Causes Small Airway Remodeling by Direct Growth Factor Induction and Release

Andrew Churg, Rong D. Wang, and Joanne L. Wright

Department of Pathology, University of British Columbia, Vancouver, British Columbia, Canada

Small airway remodeling (SAR) is one of the anatomic causes of airflow obstruction in patients with chronic obstructive pulmonary disease. The pathogenesis of SAR is poorly understood, but there are two general theories: (1) SAR is caused by smoke-induced inflammatory cells that damage the airway and evoke a repair process and (2) SAR is caused by direct induction of growth factors by cigarette smoke. To investigate this process, we briefly exposed rat tracheal explants, a model system free of exogenous inflammatory cells, to whole cigarette smoke, and then maintained the explants in organ culture for varying periods. With explants cultured in air, smoke induced up-regulation of both transforming growth factor (TGF)- β_1 and procollagen gene expression at 24 h after initial exposure. Increased procollagen gene expression was prevented in a dose-response fashion by the TGF- β antagonist fetuin, indicating that TGF- β was driving fibrosis. Collected supernatant from explants exposed to smoke and cultured in fluid medium showed increased release of TGF- β_1 , and this was abolished by the oxidant scavenger tetramethylthiourea. Gene expression of connective tissue growth factor (CTGF) was sharply increased 2 h after smoke exposure. Using pure recombinant proteins, smoke-conditioned medium oxidized TGF- β latency-associated peptide and also caused release of active TGF- β_1 from latent TGF- β_1 . We conclude that cigarette smoke directly induces release of preformed TGF- β_1 by oxidizing the latency-associated peptide in rat tracheal explants, and that release must be extremely rapid, since gene expression of CTGF, the downstream fibrogenic driver of TGF- β effects on collagen synthesis, is elevated very shortly after smoke exposure. Although tracheal explants are only an approximate model of small airways, these findings suggest that smoke-induced SAR may reflect direct induction of growth factor release and signaling and does not require smoke-evoked inflammatory cells.

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Correspondence and requests for reprints should be addressed to Andrew Churg, M.D., Department of Pathology, University of British Columbia, 2211 Wesbrook Mall, Vancouver, BC Canada V6T 2B5. E-mail: achurg@interchange.ubc.ca

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Regulation of Airway Mucin Secretion by MARCKS Protein Involves the Chaperones Heat Shock Protein 70 and Cysteine String Protein

Joungjoa Park, Shijing Fang, and Kenneth B. Adler

Department of Molecular and Biomedical Science, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina

We have reported previously that MARCKS protein is a key regulatory molecule controlling mucin secretion by airway epithelial cells *in vitro* and *in vivo*, and presented evidence supporting a mechanism whereby MARCKS regulates secretion by translocating from plasma membrane to cytoplasm, where it binds to membranes of intracellular mucin granules (1, 2). The actual mechanism whereby MARCKS is targeted to and attaches onto membranes of mucin granules has not been elucidated. We hypothesized that two specific chaperone proteins, cysteine string protein (CSP) and heat shock protein 70 (Hsp/Hsc70), play important roles in this process, and do so by complexing with MARCKS on the mucin granule membrane. Both Hsp/Hsc70 and CSP were found to be present in normal human bronchial epithelial (NHBE) cells. CSP was localized to membranes of intracellular mucin granules, as determined by ultrastructural immunohistochemistry and Western blotting of isolated mucin granule membranes. Coimmunoprecipitation and Western blotting showed that CSP within these cells complexed with both MARCKS and Hsp/Hsc70. A peptide against the C-terminus of CSP added to NHBE cells attenuated mucin secretion in a concentration-dependent manner, whereas a control missense peptide had no effect. This C-terminal peptide also blocked binding of MARCKS to mucin granule membranes. Transfection of HBE1 cells, a virally transformed human airway epithelial cell line, with siRNA constructs against MARCKS, CSP, or Hsp70 attenuated mucin secretion, indicating that these proteins are involved in the process. The results suggest that CSP, and specifically its C-terminus, associates with membranes of mucin granules in airway secretory cells and, apparently in conjunction with Hsp/Hsc70, plays a role in MARCKS attachment to granule membranes during the secretory process.

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Correspondence and requests for reprints should be addressed to Kenneth B. Adler, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street at Wm. Moore Drive, Raleigh, NC 27606. E-mail: kenneth_adler@ncsu.edu

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