Neutrophil elastase increases MUC4 expression in normal human bronchial epithelial cells

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Fischer, Bernard M., Jacob G. Cuellar, Meredith L. Diehl, Akira M. deFreytas, Jin Zhang, Kermit L. Carraway, and Judith A. Voynow. Neutrophil elastase increases MUC4 expression in normal human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 284: L671–L679, 2003. First published December 20, 2002; 10.1152/ajplung.00220.2002.—In chronic obstructive pulmonary diseases, the airway epithelium is chronically exposed to neutrophil elastase, an inflammatory protease. The cellular response to neutrophil elastase dictates the balance between epithelial injury and repair. Key regulators of epithelial migration and proliferation are the ErbB receptor tyrosine kinases, including the epidermal growth factor receptor. In this context, we investigated whether neutrophil elastase may regulate expression of MUC4, a membrane-tethered mucin that has recently been identified as a ligand for ErbB2, the major heterodimerization partner of the epidermal growth factor receptor. In normal human bronchial epithelial cells, neutrophil elastase increased MUC4 mRNA levels in both a concentration- and time-dependent manner. RNA stability assays revealed that neutrophil elastase increased MUC4 mRNA levels by prolonging the mRNA half-life from 5 to 21 h. Neutrophil elastase also increased MUC4 glycoprotein levels as determined by Western analysis, using a monoclonal antibody specific for a nontandem repeat MUC4 sequence. Therefore, airway epithelial cells respond to neutrophil elastase exposure by increasing expression of MUC4, a potential activator of epithelial repair mechanisms.

PATIENTS WITH CHRONIC BRONCHITIS (CB) and cystic fibrosis (CF) are plagued by recurrent exacerbations of productive cough, dyspnea, and progressive deterioration of lung function. Although the etiologies of these diseases are different, they share a common pathogenic mechanism characterized by an imbalance between proteolytic injury and antiprotease defenses. Neutrophils dominate the inflammatory response during acute exacerbations of CB (45) and CF (55). Airway obstruction, poor pulmonary function, and chronic expectoration are directly associated with sputum neutrophil concentrations (48). Neutrophils release elastase (NE; EC 3.4.21.37), a serine protease found in high concentrations (μM) in the airways of patients with exacerbations of CB (49) and CF (32). NE exposure injures airway epithelium resulting in ciliary dismotility and injury (1, 34), increased mucin production (6, 10), mucin secretion (13, 24, 28), mucin gene expression (53), and epithelial loss (21). After NE exposure, there is either normal epithelial restitution or secretion and/or squamous metaplasia (21, 45, 55). The mechanisms regulating epithelial proliferation and differentiation following injury are critical for understanding the airway remodeling that occurs in CB and CF but are not yet defined.

Activation of the epidermal growth factor receptor (EGFR), a member of the ErbB receptor tyrosine kinase family, is required for epithelial proliferation and migration (3, 23) following epithelial injury (reviewed in Ref. 27). Three members of the ErbB family have been detected in human airways: EGFR (ErbB1), ErbB2, and ErbB3 (37). When activated, these receptors may homodimerize or heterodimerize (17). ErbB2 is the preferred heterodimerization partner for the other ErbB receptors (16). Importantly, the first ligand specific for ErbB2 activation has recently been identified, the membrane-tethered mucin MUC4 (8).

MUC4 is one member of a family of membrane-tethered mucins that have protein domains and expression patterns that are very different from the secreted, gel-forming mucins. MUC4 cDNA encodes a large domain of tandemly repeating amino acids rich in serine and threonine that is the site of O-linked glycosylation, characterizing the molecule as a mucin (33, 38). In addition to the tandem repeat domain, the carboxyl moiety of human MUC4, MUC4B, contains several interesting protein domains, including two epidermal growth factor (EGF)-like domains, a non-EGF cysteine-rich domain, a transmembrane domain, and a cytoplasmic domain that contains potential phosphorylation residues (31). Human MUC4 is expressed in branching airways during fetal lung development (7, 43). It is also highly expressed in superficial ciliated and secretory airway epithelial cells from large airways to bronchioles in adults (2, 7). The protein domains of MUC4 and the expression of MUC4 during...
airway development and in differentiated airway epithelium suggest that MUC4 has important functions in the airway.

With this background, we sought to determine whether NE regulated the expression of MUC4 mRNA and glycoprotein. We first established that in normal human bronchial epithelial (NHBE) cells both MUC4 mRNA and protein are expressed. We also show that MUC4 glycoprotein is expressed in superficial airway epithelial cells. We then demonstrate that NE increases expression of MUC4 mRNA and glycoprotein. We found that NE regulates MUC4 by enhancing mRNA stability. The increased expression of MUC4 in NHBE cells, following exposure to NE, suggests that MUC4 may have an important role in the epithelial response to injury.

MATERIALS AND METHODS

Reagents. Dulbecco's modified Eagle's medium, T4 kinase, and 5× SSC were from Gibco-BRL/Invitrogen (Carlsbad, CA). NHBE cells, bronchial epithelial basic medium, and SingleQuot supplements were from Clonetics/BioWhittaker (Walkersville, MD). Six-well plates were purchased from Corning Life Sciences (Cambridge, MA), and 60-mm tissue culture dishes from BD Biosciences (Bedford, MA). EGF and bovine serum albumin (BSA) were from Intergen/Serologicals (Norcross, GA). NE (875 U/mg protein) was from Elastin Products (Owensville, MO). Nylon filter (Nytrel Supercharged) was from Schleicher and Schuell (Keene, NH). Acrylamide was from National Diagnostics (Atlanta, GA). Prime-It II random primer labeling kit was purchased from Stratagene (La Jolla, CA). Enhanced Chemiluminescence Plus kit, streptavidin-horseradish peroxidase conjugate, sheep anti-mouse IgG, peroxidase-linked species-specific whole antibody, microspin G-25 columns, HiTrap Protein G HP column, [α-32P]dCTP, and [γ-32P]ATP were from Amersham Biosciences (Piscataway, NJ). Kaleidocoesst prestained protein standards, 0.45-μm pore nitrocellulose, DC Protein Assay, N,N',N'-tetramethylthelylenediamine, and sodium dodecyl sulfate (SDS) were purchased from Bio-Rad Laboratories (Hercules, CA), and cesium chloride was from ICN (Costa Mesa, CA). Pepstatin was purchased from Roche Molecular Biochemicals (Indianapolis, IN). Cytotox 96 nonradioactive cytotoxicity assay kit was purchased from Promega (Madison, WI). Tris, ammonium persulfate, and glycine were from EM Sciences (Gibbstown, NJ). Ammonium acetate was purchased from Mallinkrodt Baker (Paris, KY). Biotinylated SDS molecular weight standards, Ponceau S stain, Tween 20, sodium deoxycholic acid, Triton X-100, retinoic acid, bovine pancreatic trypsin, guanidine thiocyanate, methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone (AAPV-CMK), and all other chemicals were from Sigma (St. Louis, MO). Immunochemistry reagents and retrieval solution, LINK solution, and dianinobenzidine (DAB) substrate were obtained from Dako (Carpinteria, CA). NS1 was provided by the Developmental Studies Hybridoma Bank, Department of Biological Sciences, University of Iowa (Iowa City, IA).

Cell culture. NHBE were seeded in six-well plates or 60-mm tissue culture dishes in a serum-free 1:1 mixture of bronchial epithelial cell basic medium, and Dulbecco's modified Eagle's medium with SingleQuot supplements, bovine pituitary extract (0.13 mg/ml), EGF (0.5 ng/ml), BSA (1.5 μg/ml), and all trans retinoic acid (5 × 10−8 M) in place of SingleQuot retinoic acid and grown to confluence.

Cell stimulation. All studies were carried out on confluent plates of NHBE cells in serum-free growth factor-supplemented medium. Cells were exposed to NE or trypsin at doses and times specified in figure legends. Control conditions included cells treated with 50 μM sodium acetate, pH 5, 100 μM sodium chloride (NE buffer), 1 μM hydrochloric acid (HCl; trypsin buffer), or boiled NE.

RNA isolation and Northern analysis. RNA was isolated from cell cultures as previously described by the guanidinium thiocyanate/cesium chloride method (15, 53). Total RNA (10 μg) was separated by 1.2% agarose-formamide gel electrophoresis and transferred by capillary blot to a nylon filter (Nytrel Supercharged) in 1 M ammonium acetate. Under UV cross-linking, the filters were hybridized at 58°C with 32P-end-labeled oligomer probe (specific activity >106 counts·min−1·μg−1) for MUC4 (tandem repeat region) (5'-GTCGGTGACGTAAGAGGGGTGTCGCTGTGGGATGCTGAGGAAGT-3') (38) and at 62°C for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe (53) and 28s rRNA oligomer probe (18). Filters were washed twice with 2× SSC at 58°C for 30 min and then with 0.1× SSC and 0.1% SDS at 58°C for 5 min. Filters were exposed for autoradiography at −80°C. Band density on autoradiographs was determined by digitalization with Fotolook and Photoshop softwares and quantitation using NIH Image software.

RNA stability assay. Transcription in NHBE cells that were resting or stimulated with NE (100 nM, 17 h) was stopped by treatment with actinomycin D (4 μg/ml) for 0, 2, 4, and 8 h after NE treatment (53). For each time point, total cellular RNA was extracted and MUC4 mRNA and 28s rRNA levels were evaluated by Northern analysis and quantitated as described above. Results are plotted as a percentage of starting mRNA levels after the addition of actinomycin D. On the basis of linear regression and plot extrapolation using SigmaPlot software, the half-life is estimated to be at the time point where 50% of the original mRNA level remains.

Western analysis. The mouse monoclonal antibody 1G8 (J. Zhang and K. L. Carraway, unpublished results) was used to detect MUC4 protein expression. The antigen for this antibody was purified rat ascites dialyoglycoprotein-2 (rat Muc4b; ASGP-2) protein from an ascites tumor 13762 MAT-C1 subline grown in rats. Hybridomas were screened with rat ASGP-2 from ascites tumor cells (19) and recombinant human MUC4b/ASGP-2 expressed in Cos7 and HC11 mouse mammary epithelial cells. 1G8 was selected because of its strong reaction with human MUC4b/ASGP-2 compared with rat ASGP-2. The antibody was purified using the HiTrap Protein G HP column.

Confluent cultures of NHBE cells (lot 8P1805; Clonetics) were treated with 50 nM NE or control vehicle (50 μM sodium acetate, pH 5, 100 μM sodium chloride) for 8 h. NE activity was stopped by the addition of 1 μM AAPV-CMK. Medium was then collected, and the cells were washed with fresh medium and changed to medium without NE or vehicle. Cells were incubated for an additional 16 h (chase period) before collection of cell lysates. At the conclusion of the chase period, the cells were washed once with phosphate-buffered saline containing 0.1 mM phenylmethylsulfonyl fluoride (PMSF), scraped, and collected by centrifugation. The cell pellet was subsequently lysed in 50 μl of lysis buffer (50 mM Tris-HCl, pH 8.0, 120 mM NaCl, 1% Na deoxycholate, 1% Triton X-100, 0.1% SDS, 0.5 mM PMSF, 2.7 mM EDTA, 10 μg/ml leupeptin, 40 μg/ml aprotinin, 10 μg/ml pepstatin, 10 μg/ml antipain, 10 μg/ml chymostatin, and 10 μg/ml benza-
Trifugation at 13,000 rpm for 10 min at 4°C. Cell debris was removed by centrifugation at 13,000 rpm for 10 min at 4°C. Total protein concentrations were determined by Bio-Rad DC Protein Assay. Cell lysates (50 μg of total protein) were diluted in SDS-PAGE sample buffer and separated under reducing conditions by electrophoresis using 6% SDS-polyacrylamide gels. Prestained or biotinylated molecular weight markers were loaded on each gel. Proteins were transferred to nitrocellulose membranes. We stained membranes with water-soluble Ponceau S to confirm equivalent loading between samples. Membranes were blocked with 5% (wt/vol) nonfat dry milk in Tris-buffered saline-0.5% Tween 20. After a 1-h incubation with anti-MUC4 monoclonal antibody 1G8, diluted in 1% BSA/Tris-buffered saline/0.5% Tween 20 (BSA plus TBST) (1:3,000 dilution), the membranes were incubated with horseradish peroxidase-conjugated sheep antimouse IgG diluted 1:50,000 in BSA plus TBST. Control myeloma supernatant, NS1, was used in place of 1G8 as a negative control. Protein bands and biotinylated molecular weight markers on membranes were detected with the Enhanced Chemiluminescence Plus kit and then exposed for autoradiography.

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue blocks including normal cartilaginous airway from lung cancer patients (n = 6) were sectioned (5 μm), mounted on slides, and rehydrated. After antigen retrieval with Retrieval solution at 97°C for 20 min and incubation with 3% hydrogen peroxide, slides were incubated with 1G8 (1:100 dilution) at 37°C for 30 min. After two washes in phosphate-buffered saline, LINK solution (biotinylated secondary antibody) was applied to the slides at room temperature for 30 min. After two washes in phosphate-buffered saline, streptavidin peroxidase solution was then applied for 15 min, followed by DAB substrate. After development, slides were counterstained with hematoxylin.

Cytotoxicity assessment. We assessed cytotoxicity by lactate dehydrogenase (LDH) release using a commercially available colorimetric assay for LDH according to the manufacturer's instructions. NHBE cells were exposed to NE (50 or 100 nM NE, 24 h) or control vehicle. Both supernatants and cell lysates were collected and assessed for LDH content. We calculated the percentage of LDH release by taking the ratio of LDH released into the supernatant to the total LDH in the supernatant and the cell lysate.

Statistical analysis. Analysis of data was performed by the Kruskal-Wallis one-way nonparametric analysis of variance and post hoc comparisons by the Wilcoxon-Mann-Whitney rank sum test (47). Differences were considered significant at P < 0.05.

RESULTS

NE increased MUC4 mRNA levels in a dose- and time-dependent manner. As illustrated in Fig. 1, MUC4 mRNA expressed in NHBE cells is >9.5 kb. NE treatment (0–100 nM) increased MUC4 mRNA levels in NHBE cells in a concentration-dependent manner (Fig. 1A). At a concentration of 100 nM for 24 h, NE increased MUC4 mRNA levels approximately sevenfold compared with vehicle alone (Fig. 1C). MUC4 expression in NHBE cells was also regulated by NE in a time-dependent manner (Fig. 2). MUC4 transcript levels were increased after 1 h of exposure to NE, with 100 nM NE increasing MUC4 mRNA levels in a concentration-dependent manner (Fig. 2A) or with 100 nM (2.6 U/ml) NE (●) for 1, 8, and 24 h. RNA was isolated and evaluated by Northern analyses and autoradiography for MUC4 expression. The graph summarizes the densitometry data from 3 separate experiments (C). The ratio of MUC4/28s rRNA is expressed as a percentage of control levels (means ± SE, n = 5–7). *Significantly different from control, P < 0.05.
significant increases in MUC4 expression exhibited at both 8 and 24 h of continuous NE exposure. NE treatment under these conditions caused <5% LDH release and was considered to be noncytotoxic at these concentrations (data not shown). Heat-inactivated NE (boiled) had no effect on MUC4 expression in NHBE cells (data not shown).

**MUC4 mRNA expression was differentially regulated by another serine protease, trypsin.** To determine whether other serine proteases regulated MUC4 expression in a similar manner as NE, we treated NHBE cells with bovine pancreatic trypsin and examined the regulation of MUC4 expression. Trypsin stimulated a significant increase in MUC4 mRNA expression in NHBE cells but required 10-fold higher protease activity (13 U/ml) than NE (1.3 U/ml ≈ 50 nM; Fig. 3). At equivalent enzymatic activity (1.3 U/ml), trypsin had no significant effect on MUC4 mRNA expression (Fig. 3). Similar to NE, trypsin did not affect 28s rRNA levels (Fig. 3B).

**NE increased MUC4 mRNA expression by a posttranscriptional mechanism.** To evaluate whether NE regulated MUC4 gene expression by a posttranscriptional mechanism, we performed mRNA stability assays. RNA stability assays revealed that NE treatment prolonged MUC4 mRNA half-life from 5 h in control cells to 21 h (Fig. 4A). In contrast, NE did not prolong the half-life of GAPDH mRNA or 28s rRNA, demonstrating that the effect on MUC4 mRNA stability was specific (Fig. 4, B and C). These experiments are consistent with the concept that NE regulates MUC4 expression, at least in part, by enhancing mRNA stability.
NE treatment stimulated increased MUC4 glycoprotein production in NHBE cells. Western analysis of MUC4 glycoprotein was performed to determine the effect of NE on MUC4 glycoprotein production. Using the mouse monoclonal anti-MUC4 antibody 1G8, Western analyses of NHBE cell lysates revealed a predominant band at ~147 kDa, similar to that previously observed for the transmembrane subunit ASGP-2/MUC4⁸ of human MUC4 (Fig. 5) (36) and six smaller bands between 116 and 45 kDa representing MUC4 glycoprotein (Fig. 6). Treatment with NE increased MUC4 glycoprotein levels in NHBE cell lysates compared with control cell lysates (Fig. 6A). These protein bands were not detected following incubation with NS1, a control myeloma supernatant (Fig. 6B), demonstrating the specificity of the antibody-antigen complex. Ponceau S staining demonstrated equivalent sample loading for each gel (data not shown).

MUC4 glycoprotein is expressed in pseudostratified columnar airway epithelium. Using the mouse monoclonal anti-MUC4 antibody 1G8, we performed immunohistochemistry to determine the localization of MUC4 glycoprotein in vivo. The MUC4 protein was expressed throughout the normal pseudostratified columnar epithelium from non-CF, non-CB patients (Fig. 7A). Protein detection was specific because immunostaining was negative with control myeloma supernatant, NS1 (Fig. 7B). Localization of the protein agrees with previous reports of MUC4 mRNA expression by in situ hybridization (2, 7).

DISCUSSION

In this study, we demonstrate that NE increased MUC4 mRNA expression in NHBE cells by a concentration- and time-dependent mechanism. NE proteolytic activity was required for this response, and the concentrations of NE activity used in this study correlate with elastase activity levels found in airway secretions of patients with CF (32) and CB (49). Interestingly, another serine protease, bovine pancreatic trypsin, also increased MUC4 mRNA expression but required higher enzymatic activity compared with NE. Using a monoclonal antibody that detects an MUC4 nontandem repeat protein sequence, we demonstrate that MUC4 glycoprotein is present in superficial airway epithelial cells in control, non-CF, non-CB airways. Importantly, following NE exposure, MUC4 glycoprotein expression increased corresponding to the increase in MUC4 mRNA expression.

Although the functions of MUC4 in the lung are not yet known, its high level of expression in the lung and its structural features suggest that the molecule may play important roles in airway homeostasis and repair. The deduced size of the MUC4 protein backbone is larger than MUC1 (14, 31). Furthermore, the major protein domain in MUC4 is a tandemly repeating sequence of 16 amino acids enriched in serine and threonine that is the major site for O-linked glycosylation and that shares no homology with other MUC molecules or with the rat homolog of MUC4, the sialomucin complex (SMC) (19). Because MUC1 extends 250–500 nm above the apical membrane (5) and beyond the glycocalyx (50), MUC4 likely extends a unique complex of carbohydrate structures into the airway lumen beyond other cell-associated glycoproteins (31). In addi-
ASGP-2, and a member of the EGFR family ErbB2 (8). Furthermore, Muc4/SMC potentiates the receptor tyrosine kinase activity of ErbB2. Moreover, anti-ErbB2 antibody binding to human melanoma cells and human breast carcinoma cells was reduced by overexpression of Muc4/SMC (40). As ErbB2 is a major heterodimerization partner of the EGFR (16, 17), a major growth factor receptor in the lung (11), MUC4 may regulate growth, differentiation, and repair processes in normal airway tissues.

In this report, we used a monoclonal antibody that recognizes a unique nontandem repeat region of MUC4 to detect MUC4. The epitope used to develop the monoclonal antibody is a portion of ASGP-2 and has been used to develop other anti-MUC4 antibodies. By immunohistochemistry, MUC4\(\beta\) subunit expression is localized to the same cells that express MUC4 mRNA by in situ hybridization (2, 7). Anti-MUC4 antibody specificity is further supported by the lack of staining when the antibody is replaced by control myeloma supernatant. In an SDS-PAGE system for Western analysis, the MUC4\(\beta\) moeity likely dissociates from the amino-terminal portion of MUC4, thus resulting in a smaller size than what would be expected for the full-length molecule. By Western analysis, we detected the MUC4\(\beta\) glycoprotein with an approximate size of 147 kDa and several smaller proteins. The size of MUC4\(\beta\) in NHBE cell lysates is similar to the size of MUC4\(\beta\) detected by Western analysis in human corneal epithelium and tears (36) and similar to the size of ASGP-2 (120–140 kDa) in rat epithelial tissues (20, 29). Because our samples are from cell lysates, the smaller proteins detected by the antibody may be precursor proteins or products of alternatively spliced transcripts (30). Importantly, in NHBE cells, increases in MUC4 glycoprotein expression correlated well with increased MUC4 mRNA levels.

The regulation of MUC4 gene expression is just beginning to be elucidated. In human pancreatic tumor cells, transforming growth factor-beta 2 (TGF-\(\beta_2\)) mediates the upregulation of MUC4 expression by retinoic acid (9). In the human pancreatic tumor cell lines CAPAN-1 and CAPAN-2, MUC4 promoter activity can be increased by protein kinase C activation, EGF, or transforming growth factor (TGF-\(\alpha\)) treatment (35). In addition, in CAPAN-2 human pancreatic cancer tumor cells, interferon-\(\gamma\) in combination with tumor necrosis factor-\(\alpha\) or TGF-\(\alpha\), but not each cytokine or growth factor independently, increases MUC4 promoter activity (35). In contrast to transcriptional regulation of MUC4, in rat mammary epithelial cells, TGF-\(\beta\) treatment decreases Muc4 glycoprotein levels by suppressing Muc4 glycoprotein production without changing Muc4 transcript levels (39). Furthermore, TGF-\(\beta\) treatment regulates Muc4/SMC expression by a posttranslational mechanism where the processing of the precursor protein is altered (41).

Our study demonstrates that human MUC4 is also posttranscriptionally regulated. In human bronchial epithelial cells, NE increased MUC4 expression by

![Figure 7. Immunohistochemistry of MUC4 glycoprotein in airway epithelium. MUC4 glycoprotein was detected in formalin-fixed, paraffin-embedded airway sections from a non-cystic fibrosis, non-chronic bronchitis patient by immunohistochemistry using the monoclonal, anti-MUC4 antibody 1G8. Sections (5 \(\mu\)m) were mounted on slides, rehydrated, and exposed to antigen retrieval solution, followed by 3% hydrogen peroxide. Slides were then incubated with primary antibody, 1G8 (A), or control myeloma supernatant, NS1 (B). Slides were then exposed to an avidin-biotin complex peroxidase-diaminobenzidine detection method. Photomicrographs are shown (\(\times 120\) magnification). These photomicrographs are representative of samples from 6 subjects.](http://ajplung.physiology.org/ by 10.220.33.1 on June 21, 2017)
enhancing mRNA stability. This observation adds to a growing number of reports demonstrating post-transcriptional regulation of mucin genes. We have previously reported that in A549 cells NE increases the expression of another major respiratory tract mucin gene, MUC5AC, also by enhancing mRNA stability (53). Similarly, in NCI-H292 cells, TNF-α treatment increases the half-life of MUC5AC mRNA (4). In HT29 colonic carcinoma cells, in response to phorbol ester treatment, MUC2 expression is regulated by a posttranscriptional mechanism (52). Collectively, these studies support the concept that inflammatory mediators amplify the expression of mucin genes by posttranscriptional mechanisms. These observations underscore the importance of understanding the molecular mechanisms required for posttranscriptional regulation of MUC gene expression.

The specific mechanism(s) of NE-mediated mRNA stability are not yet known. It is possible that NE interacts with a cell surface receptor resulting in activation of the receptor and its associated signaling cascade or that NE releases a secondary mediator that functions in a paracrine/autocrine manner. On platelets, NE cleaves a surface integrin important for the potentiation of platelet aggregation (46). In respiratory epithelial cells, NE upregulates IL-8 expression through a signaling cascade mediated by IL-1 receptor-associated kinase and tumor necrosis factor-associated factor 6 (TRAF6) (54). Signals from TRAF6 can be further relayed via MAP kinase kinase kinase downstream to activate other members of the MAP kinase cascades (42) and subsequently may regulate mRNA stability (22, 56). Similarly, NE treatment can promote release of growth factors that in turn can mediate posttranscriptional processes. NE treatment causes the release of TGF-β (51), which has been shown to be important in the regulation of SMC/Muc4 (20, 41). Similarly, another protease, thrombin, decreases the half-life of endothelial nitric oxide synthase through a Rho GTPase-mediated process (12). Stabilization of mammalian gene mRNAs are mediated by many intracellular signals, including protein kinases, growth factors, ions, and reactive oxygen species (44). However, the specific signaling cascades mediating NE-induced posttranscriptional regulation of MUC4 expression have not been delineated and warrant further investigation.

Our report is one of the first to show that MUC4 expression is regulated in normal human airway epithelium and that expression is increased by a major inflammatory protease. We now have a model system to study the function of MUC4 during the epithelial response to injury.

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REFERENCES


16. Graus-Porta D, Beerli RR, Daly JM, and Hynes NE. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. *EMBO J* 16: 1647–1655, 1997.


