Roles of epidermal growth factor receptor activation in epithelial cell repair and mucin production in airway epithelium

P-R Burgel, J A Nadel

The epithelial cells lining the airways serve protective functions. The “barrier function” of the epithelium protects the individual from damage by inhaled irritants. The epithelium produces mucins which become hydrated and form a viscoelastic gel which spreads over the epithelial surface. In healthy individuals inhaled foreign materials become entrapped in the mucus and are cleared by mucociliary transport and by coughing. In many chronic inflammatory airway diseases, however, excessive mucus is produced and is inadequately cleared, leading to mucous obstruction and infection. At present there is no specific treatment for hypersecretion. However, the discovery that an epidermal growth factor receptor (EGFR) cascade is involved in mucin production by a wide variety of stimuli suggests that blockade may provide specific treatment for hypersecretory diseases. EGFR pathways have also been implicated in the repair of damaged airway epithelium. The roles of EGFR in airway epithelial cell hypersecretion and epithelial damage and repair are reviewed and future potential treatments are suggested.
resulted in MUC5AC mucin gene expression and protein production. They also showed that addition of EGFR ligands (EGF and TGF-α) to the densely cultured epithelial cells upregulates MUC5AC gene and protein expression in airway epithelial cells in vitro. Using selective inhibitors of EGFR tyrosine kinase phosphorylation, they reported that EGFR ligand induced mucin MUC5AC synthesis is dependent on EGFR activation.  

Roles of EGFR in airway epithelium

The role of EGFR activation in mediating epithelial repair has been shown in various cell types in vitro including keratinocytes, mammary epithelial cells, and alveolar epithelial cells. In 1993 Barrow et al hypothesised that EGFR ligands and other growth factors mediate bronchial epithelial repair. They showed that administration of aerosolised EGF plus PDGF for 2 weeks enhanced repair of sheep airway epithelial cells in vitro.  

**Box 1 Examples of stimuli that induce mucin synthesis in vitro or in vivo by EGFR activation in airways**

**In vitro experiments**

- **Bacterial products:**
  - *P. aeruginosa* supernatant
  - Lipopolysaccharide (LPS)
  - Lipoteichoic acid (LTA)

- **Phorbol 12-myristate 13-acetate (PMA)**

- **Cigarette smoke**

- **Inflammatory cells:**
  - Neutrophils
  - Eosinophils

- **Serine proteases:**
  - Human neutrophil elastase
  - Human airway trypsin-like protease

**In vivo experiments**

- **Th2 cells**
  - Antigen (ovalbumin)
  - IL-13

- **Mechanical damage of epithelium**

- **Cigarette smoke**

- **Leukotrienes**

EFGF activation may involve two different pathways—ligand dependent and ligand independent EGFR tyrosine phosphorylation. In ligand dependent EGFR tyrosine phosphorylation, EGFR ligands bind to EGF receptors in the extracellular domain and activate them (fig 3, left side) while, in ligand independent EGFR tyrosine phosphorylation, EGFR tyrosine phosphorylation occurs in the absence of exogenous EGFR ligands (fig 3, right side). Ligand independent EGFR phosphorylation is reported in response to oxidative stress that can be produced by cigarette smoke and by activated neutrophils. However, it was later realised that airway epithelial cells, in addition to expressing EGFR, express EGFR proligands on their surface. As will be seen below, some of these stimuli (such as cigarette smoke) can induce shedding of EGFR proligands from the epithelial cell surface, leading to ligand binding to the receptor and ligand dependent EGFR activation. Neutrophils are present in the airways of patients with hypersecretory diseases such as COPD, acute severe asthma, and cystic fibrosis and could promote ligand independent EGFR activation and mucin synthesis via the release of oxygen free radicals. Other inflammatory cells (such as macrophages and eosinophils) recruited to the airway epithelium in inflammatory respiratory diseases express EGFR ligands, raising the possibility that interactions between these cells and epithelial cells could result in ligand dependent activation of EGFR signalling cascades and mucin production. Borchers et al have recently shown that exposure of mice to acrolein, a product of cigarette smoke, results in goblet cell metaplasia. They suggested that the effect was due to macrophage elastase. Kim et al have recently shown that macrophages induce mucin production in cultured airway epithelial cells. Burgel et al showed that isolated human eosinophils, when activated, induce mucin synthesis in cultured airway epithelial cells by EGFR activation. Soluble TGF-α was increased in cell culture.
medium of epithelial cells stimulated with activated cosinophils, and a blocking antibody to TGF-α reduced mucin synthesis. These results implicated an EGFR cascade and suggested that TGF-α is involved in the response.

Airway epithelial cells express several EGFR ligands—for example, EGF, TGF-α, HB-EGF, and amphiregulin. Various stimuli have been reported to increase the expression of selected ligands in experimental models in vitro and in vivo, but mechanisms of inducing this expression are unknown. Among these stimuli are those that induce mucin production—for example, IL-13, cigarette smoke, and acrolein—or stimuli used in studies of airway remodelling and repair—for example, vanadium, bleomycin, naphthalene, and compression of bronchial epithelial cells in vitro.

In the epithelium, EGFR proligands are synthesised as membrane anchored molecules that are cleaved by proteases to become activated. Metalloproteases cleave EGFR proligands in response to activation by G-protein agonists in mammary epithelial cells. Lemjabbar et al. showed that lipoteichoic acid (LTA), a component of Gram positive bacterial cell walls, induces mucin synthesis by activating EGFR in airway epithelial cells. Mechanisms of EGFR activation in this model are reported to involve recognition of LTA by platelet activating factor receptor (PAFR), a G-protein coupled receptor that activates a membrane anchored metalloprotease, ADAM 10, resulting in cleavage of proHB-EGF, EGFR activation, and mucin synthesis. Shao et al. showed that TNF-α converting enzyme (TACE/ADAM 17), another member of a disintegrin and metalloprotease (ADAM) family, is an important regulator of EGFR activation leading to mucin synthesis in airways. Using cultured human airway epithelial cells, these authors showed that phorbol 12-myristate 13-acetate (PMA), an activator of TACE, and pathophysiological stimuli (such as lipopolysaccharide (LPS), supernatant of the Gram negative bacteria P aeruginosa, and cigarette smoke) induce mucin synthesis. Importantly, knockdown of TACE by specific small interfering RNA prevented EGFR activation and mucin synthesis by these stimuli. Mechanisms involved are cleavage of epithelial membrane anchored proTGF-α by TACE, binding of soluble TGF-α to EGFR, and subsequent phosphorylation of EGFR leading to mucin synthesis. Thus, bacterial products of Gram positive and Gram negative bacteria induce mucin synthesis in airway epithelial cells in vitro by shedding of EGFR proligands leading to autocrine activation of an EGFR cascade. Cleavage of epithelial proligands and autocrine activation of EGFR can also be promoted by neutrophil proteases: Kohri et al. reported that induction of mucin synthesis by the serine protease human neutrophil elastase (HNE) causes EGFR activation: HNE causes the cleavage of membrane anchored proTGF-α from the epithelial surface resulting in the release of mature TGF-α which binds to EGFR, causing EGFR activation and mucin synthesis. An EGFR blocking antibody inhibited the response to elastase, implicating a ligand dependent process. Voynow et al. reported that the increase in MUC5AC mRNA following exposure to human neutrophil elastase could be due to increased mRNA stability.

These results implicate EGFR activation by a wide variety of stimuli, and various paracrine interactions among cells and molecules are responsible for the effects on the airway epithelium.

**FUTURE CLINICAL STUDIES**

Mucous hypersecretion contributes to morbidity and mortality in various airway inflammatory diseases (such as asthma, COPD, cystic fibrosis, and nasal polyposis), but no treatment to prevent hypersecretion currently exists. Airway mucus
Roles of EGFR in airway epithelium

Figure 3. Examples of ligand dependent and ligand independent EGFR activation leading to mucin synthesis. Binding of EGFR ligands (triangles) in the extracellular domain in airway epithelial cells is followed by phosphorylation of tyrosine residues (P) in the intracellular domain (ligand dependent activation). Active (soluble) ligands (exemplified by TGF-α) may be released by recruited inflammatory cells (such as eosinophils and macrophages) or produced by cleavage of membrane anchored proligand (exemplified by proTGF-α). The proligand may be cleaved by neutrophil proteases (such as human neutrophil elastase) or by epithelial membrane anchored proteases (such as "a disintegrin and metalloprotease" (ADAM) family exemplified by ADAM 17/TNF-α converting enzyme) in response to stimuli such as P aeruginosa bacteria. Alternatively, activation of EGFR may occur in the absence of ligand binding (ligand independent activation) by phosphorylation of tyrosine residues in the intracellular domain directly (no ligand) in response to stimuli (such as cigarette smoke, activated neutrophils producing oxidative stress). Oxygen free radicals have also been shown to activate shedding of EGFR proligands in epithelial cells resulting in EGFR activation. Regardless of the mechanisms (ligand dependent or ligand independent) of EGFR activation, phosphorylation of tyrosine residues in the intracellular domain triggers a downstream cascade leading to mucin gene and protein synthesis.

Mucin protein
Nucleus
Gene Transcription
INFLAMMATION
INFECTION

LIGAND DEPENDENT

Eosinophils
Macrophages
Neutrophils
Gram-negative bacteria
(LPS/P aeruginosa)

ProTGF-α
TACE/ADAM17
Soluble TGF-α

EGFR
EGFR
α

Mucin

LIGAND INDEPENDENT

Oxidative Stress

Cigarette smoke
neutrophils

H2O2

Damage to the airway epithelium has been described in asthma. EGFR expression is increased in asthmatic epithelium and activation of EGFR contributes to airway epithelial repair. Because recombinant EGFR has been reported to have beneficial effects in ulcerative colitis, a recent review suggests that the use of recombinant EGF may be beneficial in the treatment of asthma. In processes where the predominant abnormality is epithelial damage, activation of EGFR may therefore result in improved wound healing. However, in diseases where mucin hypersecretion predominates, inhibition of EGFR phosphorylation could result in reversal of the pathophysiological process.

In conclusion, there is increasing evidence that EGFR is an important player in regulating mucus production in airway epithelium and in the repair of epithelium after injury. Studies performed in recent years have contributed to a better understanding of cellular and molecular mechanisms.

hypersecretion can occur with limited clinical symptoms (especially in peripheral airways where cough receptors are absent and where extensive mucous plugging may be undetected by pulmonary function tests), and reproducible biological measurement of mucus production in airway secretions (for example, sputum and bronchoalveolar lavage fluid) in human diseases is difficult. Determining the outcomes in clinical studies of treatments that target mucous hypersecretion in humans is therefore complicated. In a recent study we assessed the effects of an intranasal corticosteroid on mucus production in nasal polyps. One nasal polyp was removed surgically before treatment and another was removed after 8 weeks of treatment with nasal polyps (400 µg/day) in nine subjects. The polyp tissues were examined morphometrically. Evaluation of alcian blue (AB)/PAS staining for mucus glycoconjugates and staining with a monoclonal antibody to MUC5AC mucin in the epithelium showed that steroids did not affect mucin protein expression. Similarly, MUC5AC mRNA, assessed by in situ hybridisation, was expressed in epithelium before and after treatment, suggesting that intranasal corticosteroids do not reduce mucus production in nasal polyps. We suggest that, because of their location and accessibility, nasal polyps provide a convenient “model” for evaluating various treatments in the suppression of mucin production in the respiratory system.

The finding that various pathophysiological stimuli converge in the EGFR pathway to induce mucin production and goblet cell metaplasia provides new therapeutic opportunities, using treatment targeting mechanisms of EGFR expression or EGFR activation. TNF-α is increased in airways in hypersecretory diseases and may contribute to EGFR expression. Inhibitors of TNF-α or TNF-α receptors are in clinical use for rheumatoid arthritis and should be evaluated for treating hypersecretion. Small molecules inhibiting EGFR tyrosine kinase phosphorylation or monoclonal antibody to EGFR are undergoing clinical trials in patients with non-small cell lung cancer with minimal toxicity. Clinical studies using molecules targeting EGFR activation in hypersecretory diseases will be of interest. Because various proteases (such as neutrophil elastase and members of the ADAM family of metalloproteases) have been implicated in the cleavage of EGFR proligands and in EGFR activation (see above), it is conceivable that treatments which inhibit these molecules might prevent mucus hypersecretion.
involved in EGFR expression and activation leading to mucin production in response to noxious stimuli. It is suggested that disrupting the EGFR cascade that leads to mucus production is beneficial in airway inflammatory (hypersecretory) disease. Proof of concept requires clinical trials evaluating new therapeutic opportunities opened by these discoveries.

Authors’ affiliations

P-Burgel, Service de Pneumologie, Université René Descartes, Hôpital Cochin, Paris, France

J A Nadel, Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco, USA

REFERENCES


