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Wound-induced TGF-β1 and TGF-β2 enhance airway epithelial repair via HB-EGF and TGF-α

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ABSTRACT

The abundance of transforming growth factor-beta (TGF-β) in normal airway epithelium suggests its participation in physiological processes to maintain airway homeostasis. The current study was designed to address the hypothesis that TGF-β1 and TGF-β2 might contribute to normal reparative response of airway epithelial cells (AECs). Treatments with exogenous TGF-β1 or TGF-β2 significantly enhanced wound repair of confluent AEC monolayers. Mechanical injury of AEC monolayers induced production of both TGF-β1 and TGF-β2. Wound repair of AECs was significantly reduced by a specific inhibitor of TGF-β type I receptor kinase activity. We investigated whether the TGF-β-enhanced repair required epidermal growth factor receptor (EGFR) transactivation and secretion of EGFR ligands. Both TGF-β1 and TGF-β2 enhanced EGFR phosphorylation and induced production of heparin-binding EGF-like growth factor (HB-EGF) and transforming growth factor-alpha (TGF-α) in AECs. Moreover, treatment with a broad-spectrum metalloproteinase inhibitor or anti-HB-EGF and anti-TGF-α antibodies inhibited the wound repair and the EGFR phosphorylation by TGF-β1 and TGF-β2, indicating that the TGF-β1 and TGF-β2 effects on wound repair required the release of HB-EGF and TGF-α. Our data, for the first time, have shown that both TGF-β1 and TGF-β2 play a stimulatory role in airway epithelial repair through EGFR phosphorylation following autocrine production of HB-EGF and TGF-α. These findings highlight an important collaborative mechanism between TGF-β and EGFR in maintaining airway epithelial homeostasis.

1. Introduction

The airway epithelium is continuously exposed to inhaled gaseous and particulate components and therefore is subject to injury. A rapid repair after injury is crucial for restoring epithelial barrier function. In response to environmental challenges, bronchial epithelial cells produce a number of growth factors [1–3], including transforming growth factor-beta (TGF-β), which is a multifunctional regulator of cell growth, differentiation, migration, extracellular matrix formation, and immune responses [4,5]. Among the three isoforms of TGF-β family members secreted by mammalian cells, both TGF-β1 and TGF-β2 have been implicated in the early stage of skin wound repair [6]. In reference to the airway, some studies demonstrated that TGF-β2, but not TGF-β1, was produced by damaged airway epithelial cells (AECs) [3,7,8], but others reported that TGF-β1, but not TGF-β2, was expressed in AECs of fibrotic lungs, which promoted the migration of AECs in damaged monolayers [9,10]. Thus, the role of TGF-β1 and TGF-β2 in wound repair of airway epithelium is still controversial.

Epidermal growth factor receptor (EGFR) signaling has been implicated in some effects of TGF-β1, such as accumulation of fibronectin in mesangial cells, regulation of the cell cycle in fetal rat hepatocytes, and migration of smooth muscle cells [11–13]. The main members of EGFR ligands potentially involved in wound repair include epidermal growth factor (EGF), heparin-binding EGF-like growth factor (HB-EGF), transforming growth factor-alpha (TGF-α), and amphiregulin (AR) [14–16]. All these ligands are synthesized as transmembrane precursors and proteolytically processed to release the biologically active mature protein. An autocrine activation of the EGFR signaling plays a key role in epithelial wound repair by increasing epithelial cell proliferation, migration, differentiation, and survival [15,17]. It have been suggested that the EGFR

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transactivation in response to TGF-β1 may be mediated by the release of a membrane-anchored EGFR ligand [13].

In the present study, we examined the involvement of TGF-β1 and TGF-β2 in airway epithelial repair and the contribution of EGFR transactivation. Our data, for the first time, show that mechanical injury induces secretion of both TGF-β1 and TGF-β2, which enhance EGFR activation and wound repair through production of HB-EGF and TGF-α in an AEC in vitro culture model. These findings suggest that both TGF-β1 and TGF-β2 play an important role in normal airway epithelial repair, which activates EGFR pathway by autocrine HB-EGF and TGF-α.

2. Materials and methods

2.1. Reagents

Recombinant soluble human TGF-β1 and TGF-β2 were from Peprotech (Rocky Hill, NJ, USA). Anti-TGF-β1, anti-phosphorylated EGFR (pY^{845}) antibodies, and neutralizing antibodies of EGF, HB-EGF, TGF-α, and AR were obtained from R&D Systems (Minneapolis, MN, USA). ELISA kits for TGF-β1, TGF-β2, EGF, HB-EGF, TGF-α, and AR were also from R&D Systems. Anti-TGF-β2 and anti-α-tublin antibodies, AG1478, SB431542, and Eagle's minimum essential medium (MEM) were from Sigma Chemicals (St. Louis, MO, USA). GM6001 was from Calbiochem (San Diego, CA, USA). Anti-EGFR antibody was from Upstate Biotechnologies (Lake Placid, NY, USA). Bronchial epithelial growth medium (BEGM) was purchased from Cambrex (East Rutherford, NJ, USA).

2.2. Cell culture

1HAEo$^-$ cells are SV40-transformed normal human airway epithelial cells that have been characterized previously [18]. Primary normal human bronchial epithelial (NHBE) cells were purchased from Cambrex. Cells were grown on collagen I-coated flasks or plates (Asahi Techno Glass, Japan). 1HAEo$^-$ cells were cultured in MEM containing 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 μg/ml of streptomycin and 100 U/ml of penicillin G and incubated at 37 °C in 5% CO_2. NHBE cells were cultured in complete BEGM, which consists of bronchial epithelial basal medium (BEBM) supplemented with insulin (5 μg/ml), hydrocortisone (0.5 μg/ml), transferrin (10 μg/ml), triiodothyronine (6.5 ng/ml), epinephrine (0.5 μg/ml), human EGF (0.5 ng/ml), retinoic acid (0.1 ng/ml), gentamycin (50 μg/ml), and bovine pituitary extract (52 μg/ml). Twenty-four hours before and throughout the conduct of each experiment, the 1HAEo$^-$ cells and NHBE cells were cultured in growth factor-free and serum-free medium. Pretreatment with recombinant soluble human TGF-β1 (0.1–100 ng/ml), TGF-β2 (0.1–100 ng/ml), SB431542 (10 μM), AG1478 (1 μM), GM6001 (50 μM), dimethyl sulfoxide (DMSO) as vehicle and neutralizing antibodies of EGF (6 μg/ml), HB-EGF (3 μg/ml), and TGF-α (3 μg/ml) is described in the text.

2.3. Monolayer wound repair assay

We have established this method previously [14]. Briefly, NHBE and 1HAEo$^-$ cells were grown in 6-well plate and then placed upon confluence in the growth factor-free BEBM or serum-free MEM, respectively. A circular wound (~2.0 mm^2) was made in the confluent monolayer using a 20-μl pipette tip (4 wounds per well). In each experiment, one well was used as a negative control with no treatment. The wounds were imaged 0, 12 and 24 h after wound creation using a Nikon Eclipse TE200 inverted microscope equipped with a Nikon Coolpix E995. Corresponding wound areas were determined using ImagePro Plus and the remaining wound areas were calculated as a percentage of area at time 0.

2.4. Preparation of cell lysates and Western blotting

To determine the production of TGF-β1 and TGF-β2 after mechanical injury, confluent monolayers of NHBE in the growth factor-free BEBM and 1HAEo$^-$ cells in the serum-free MEM were subjected to multiple linear injuries (7 × 7 linear scratches in each well) using a 20-μl pipette tip. Monolayers with no scratch wounds were used as the control. In other experiments, to determine the production of EGFR ligands and EGFR phosphorylation, confluent monolayers of NHBE and 1HAEo$^-$ cells were treated with 10 ng/ml of recombinant human TGF-β1 or TGF-β2. Whole cell lysates were prepared at the indicated time points using RIPA buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate, 25 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin and 1 μg/ml leupeptin). Protein concentration was determined by the Bradford assay. Equal amounts of whole cell lysates (20–40 μg) were separated by 8% or 15% SDS–PAGE and blotted onto polyvinylidene difluoride

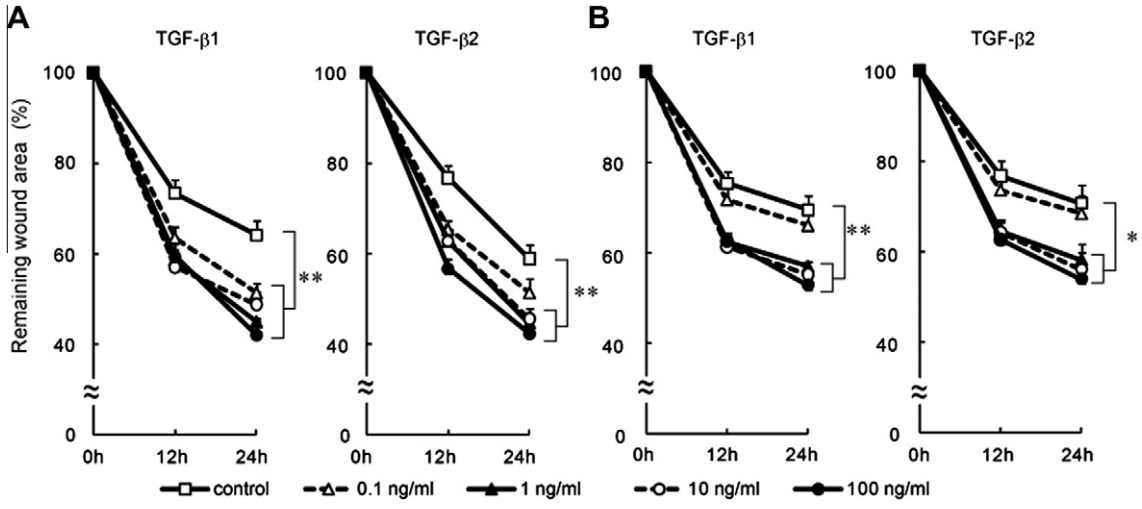

Fig. 1. TGF-β1 and TGF-β2 enhance airway epithelial repair. Injured monolayers of NHBE (A) and 1HAEo$^-$ (B) cells were treated with the indicated concentrations of TGF-β1 or TGF-β2 immediately after the injury, and wound areas were determined at 12 and 24 h. Data are represented as the mean ± SEM of triplicate samples. $^*p < 0.05$, $^{**}p < 0.01$ compared with the absence of TGF-β (control). Similar results were obtained in three independent experiments.