Disruption of β-catenin/CBP signaling inhibits human airway epithelial–mesenchymal transition and repair

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ABSTRACT

The epithelium of asthmatics is characterized by reduced expression of E-cadherin and increased expression of the basal cell markers ck-5 and p63 that is indicative of a relatively undifferentiated repairing epithelium. This phenotype correlates with increased proliferation, compromised wound healing and an enhanced capacity to undergo epithelial–mesenchymal transition (EMT). The transcription factor β-catenin plays a vital role in epithelial cell differentiation and regeneration, depending on the co-factor recruited. Transcriptional programs driven by the β-catenin/CBP axis are critical for maintaining an undifferentiated and proliferative state, whereas the β-catenin/p300 axis is associated with cell differentiation. We hypothesized that disrupting the β-catenin/CBP signaling axis would promote epithelial differentiation and inhibit EMT. We treated monolayer cultures of human airway epithelial cells with TGFβ1 in the presence or absence of the selective small molecule ICG-001 to inhibit β-catenin/CBP signaling. We used western blots to assess expression of an EMT signature, CBP, p300, β-catenin, fibronectin and ITGβ1 and scratch wound assays to assess epithelial cell migration. Snai-1 and -2 expressions were determined using q-PCR. Exposure to TGFβ1 induced EMT, characterized by reduced E-cadherin expression with increased expression of α-smooth muscle actin and EDA-fibronectin. Either co-treatment or therapeutic administration of ICG-001 completely inhibited TGFβ1-induced EMT. ICG-001 also reduced the expression of ck-5 and -19 independent of TGFβ1. Exposure to ICG-001 significantly inhibited epithelial cell proliferation and migration, coincident with a down regulation of ITGβ1 and fibronectin expression. These data support our hypothesis that modulating the β-catenin/CBP signaling axis plays a key role in epithelial plasticity and function.

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1. Introduction

The airway epithelium is the interface between the inhaled environment and the sub-mucosa and forms the structural barrier against inhaled exogenous agents. Damage to the epithelium triggers a cascade of inflammatory and cell signaling events that can lead to regeneration and/or repair. Regeneration is the outcome of processes that returns the tissue to its normal structure

and function. By contrast, repair regulates the stability of a tissue, but fails to restore full structural or functional capacity, and in some cases results in excessive wound healing that can lead to pathological remodeling and fibrosis. Thus, full regeneration is critical in maintaining barrier integrity and normal function of the epithelium.

We and others showed that the airway epithelium of both children and adults with asthma is relatively undifferentiated characterized by a significantly increased proportion of progenitor and basal cells and reduced expression of key junction proteins (Hackett et al., 2008a,b, 2009; Kicic et al., 2006; de Boer et al., 2008). This altered phenotype (de Boer et al., 2008; Stevens et al., 2008) has potential implications for many aspects of airway epithelial homeostasis including compromised barrier function (Xiao et al., 2011) and wound repair capacity (Stevens et al., 2008; Kicic et al., 2010). Importantly, these abnormalities are maintained when the cells are grown at air–liquid interface (ALI) culture (Hackett et al., 2011).

It is likely that this phenotypic immaturity also facilitates cell plasticity. In this context we have demonstrated that epithelial cells from asthmatics exhibit a greater capacity to undergo epithelial–mesenchymal transition (EMT) in response to TGFβ1 (Hackett et al., 2009). EMT is a process by which epithelial cells lose polarity and intercellular contacts, and assume a mesenchymal phenotype (Hackett, 2012; Kalluri, 2009). These phenotypic changes are paralleled by transcriptional repression of epithelial genes such as E-cadherin and ZO-1 coordinated with increased expression of mesenchymal proteins, including EDA-fibronectin (EDA-FN), vimentin, and α-smooth muscle actin (Kalluri, 2009). Thus, increased EMT would have a disruptive effect on the capacity to regenerate an effective epithelial barrier, which in turn facilitates a chronic cycle of inflammation and airway remodeling (Knight et al., 2011).

β-catenin signaling is integral to many facets of epithelial development and differentiation (Ritchie et al., 2001; Schmidt-Ott and Barasch, 2008; Driskell et al., 2007; Filali et al., 2002; Zhang et al., 2008). When not bound to E-cadherin or activated, β-catenin is associated with a degradation complex, which keeps it phosphorylated and targeted for ubiquitination and proteolytic destruction. Disruption of this complex prevents β-catenin phosphorylation and promotes its nuclear translocation, where it forms a complex with members of the TCF/LEF family of transcription factors (Hart et al., 1998). The specific gene program induced by β-catenin depends on the recruitment of transcriptional co-activators, including CREB binding protein (CBP) or its closely related homolog p300. Transcriptional programs driven by β-catenin/CBP are critical for maintenance of an undifferentiated and proliferative state. Inhibiting the β-catenin/CBP interaction facilitates the binding of β-catenin to its alternate co-activator, p300, resulting in expression of β-catenin/p300-dependent target genes associated with cell differentiation (Lenz and Kahn, 2014).

We previously demonstrated that the novel small molecule inhibitor ICG-001, which selectively inhibits the association between β-catenin and CBP (Ma et al., 2005) induces a mesenchymal–epithelial transition in human fibroblasts (Henderson et al., 2010). In this study, we aimed to elucidate whether this pathway was involved in EMT of human tracheobronchial epithelial cells. We show that ICG-001 ameliorates and therapeutically reverses TGFβ1-induced EMT in human airway epithelial cells, inhibits expression of the basal cell markers cytokeratin (ck)-5, ck-14 and ck-19, but reduces expression of β1 integrin (ITGβ1) and fibronectin and suppresses epithelial cell wound repair. These data highlight the complexity of β-catenin signaling in determining epithelial cell fate and differentiation and the therapeutic potential of inhibiting the β-catenin/CBP pathway.

2. Materials and methods

2.1. Human airway epithelial cells and cell culture

This study was approved by the Research Ethics Boards of the University of British Columbia and the University of Newcastle. Primary human airway epithelial cells (AECs) were isolated from non-asthmatic and asthmatic donor lungs deemed unsuitable for transplant, as described previously (Hackett et al., 2008a). In addition, cells were obtained by bronchial brushing of non-asthmatic and asthmatic subjects (Hallstrand et al., 2010). AECs were maintained in bronchial epithelial growth media (Lonza) and supplemented with antibiotics-antimycotics (Gibco, Burlington, ON, Canada). Experiments were conducted on cells at passage 2 or 3. Minimally-immortalized bronchial epithelial cells (HBEC6-KT) were generously provided by Dr. John Minna (Ramirez et al., 2004) and maintained in Keratinocyte Serum-Free Media (KSFM; Invitrogen) with growth supplements and antibiotics as described in on-line supplement. We have previously characterized these cells for E-cadherin and actin expression and show that they form confluent monolayers with defined intercellular junctions (Hirota et al., 2014). Experiments were conducted on cells at passage 12 and 13.

2.2. Induction of EMT

Cells were cultured in 6-well tissue culture plates (BD Falcon, NJ) to reach 60–70% confluence. ICG-001 (10 μM) obtained from Dr. Michael Kahn (University of Southern California, CA) was then added, followed by addition of recombinant human TGFβ1 treatment (10 ng/mL, Pepro Tech, NJ) 30 min later. Protein samples were obtained 72 h after treatment (Hackett et al., 2009).

Therapeutic administration experiments were carried out when cells were at 60–70% confluence. TGFβ1 exposure was carried out as described above, with addition of ICG-001 (10 μM) at 12, 18, 24, or 48 h after addition of TGFβ1. Protein samples were obtained 72 h after addition of TGFβ1.

2.3. Western blot

Cells were lysed in lysis-buffer (25 mM Tris, 150 mM NaCl, 5 mm EDTA, 1% triton X-100, 0.1% SDS, 1% Sodium deoxycholate, pH 7.6) containing a cocktail of protease inhibitors and centrifuged at 13,000 rpm for 10 min. The protein content of the supernatant was determined by the BCA protein assay method. Equal amounts of protein (10 μg) were added to SDS-PAGE buffer, boiled for 5 min and electrophoresed on a 4–15% gradient gel and then transferred to PVDF membranes. Membranes were incubated with Tris buffered saline with 0.5% tween-20 (TBST) containing 5% skim milk powder for 1 h to block non-specific binding and then incubated with primary antibodies over night at 4 °C. After washing in TBST, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody in TBST for 1 h, washed and developed using the ECL detection system. The following antibodies were used for immunoblotting: E-cadherin (mouse; Santa Cruz Biotechnology: sc-8426, 1 μg/mL), α-smooth muscle actin (mouse; Sigma–Aldrich: A5228), EDA-fibronectin (EDA-FN: mouse; Millipore: MAB 1940, 0.1 μg/mL), vimentin (rabbit; Abcam: ab-45939, 2 μg/mL), ZO-1 (rabbit; Santa Cruz Biotechnology: sc-10804, 1 μg/mL), p300 (rabbit; Santa Cruz Biotechnology: sc-585, 1 μg/mL), CBP (rabbit; Santa Cruz Biotechnology: sc-369, 1 μg/mL), ITGβ1 (mouse; R & D: MAB 17783), plasma fibronectin (mouse; R & D: MAB 1918), phospho-Smad3 S423 + S425 (Rabbit; Abcam: ab52903, 0.015 μg/mL), Stat3 (rabbit; Abcam: ab15523, 0.1 μg/ml), p-Stat3 (Tyr705) (rabbit: Cell Signaling: 9145, 56 μg/ml), p-Stat3 (Ser727) (rabbit; Abcam: ab30647, 0.5 μg/ml), cytokeratin 14 (mouse; Abcam: ab-7800, 0.008 μg/mL), cytokeratin 5