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β-Catenin/T-cell factor-mediated transcription is modulated by cell density in human bronchial epithelial cells

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Abstract

The embryonic Wnt/β-catenin ('canonical') pathway has been implicated in epithelial regeneration. To investigate the role of Wnt signal transduction in the airways, we characterised the expression of key pathway components in human bronchial epithelial cells (HBEC) and studied the influence of cell density on pathway activity, using sub-confluent cells in log-phase growth as a simple model of repairing epithelium. Primary HBEC and H292 bronchial epithelial cells were found to express TCF-4, TCF-3 and isoforms of LEF-1, transcription factors that are regulated by Wnt signalling. The cells also had the potential to respond to Wnt signalling through expression of several members of the Frizzled receptor family, including FZD-5 and -6. In confluent H292 cells, 20 mM lithium and 25% v/v Wnt-3a conditioned medium induced 4.5-fold ($p = 0.008$) and 1.4-fold ($p = 0.006$) increases in TOPflash activity, respectively. Under conditions of reduced cell density, TOPflash activity increased 1.8-fold ($p = 0.002$) in association with increased nuclear localisation of hypophosphorylated (active) β-catenin and increased cell proliferation. This up-regulation in reporter activity occurred independently of EGF receptor activation and could not be recapitulated by use of low-calcium medium to disrupt cadherin-mediated cell–cell adhesion, but was associated with changes in FZD-6 expression. We conclude that reactivation of this embryonic pathway may play an important role in bronchial epithelial regeneration, and that modulation of Fzd-6 receptors may regulate Wnt signalling at confluence. Recognising that many chronic inflammatory disorders of the airways involve epithelial damage and repair, altered Wnt signalling might contribute to disease pathogenesis or progression.

Keywords: Catenin; EGF receptor; Bronchial epithelial cells; TCF; LEF-1; Cell density

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1. Introduction

Bronchial epithelial repair plays an important role in the pathogenesis of chronic inflammatory disorders of the airways. In asthma, the bronchial epithelium appears to be abnormally susceptible to oxidant-induced apoptosis (Bucchieri et al., 2002), with shedding of columnar cells and failure to proliferate during repair (Demoly et al., 1994). Thus, the asthmatic epithelium is persistently stressed and activated, releasing cytokines that induce chronic airways inflammation and airway wall remodelling (Holgate, Lackie, Davies, Roche, & Walls, 1999). Investigating the processes that regulate epithelial repair could provide new insights into airways diseases. In this respect, recent interest has focused on the role played by reactivation of embryonic signalling pathways (Watkins et al., 2003).

In fetal lung, paracrine factors between epithelial and mesenchymal cells, including Wnt proteins, orchestrate branching morphogenesis (Chuang & McMahon, 2003; Shannon & Hyatt, 2004). Wnts are secreted signalling proteins that act to influence cell fate (Brandon, Eisenberg, & Eisenberg, 2000; Moon, Brown, & Torres, 1997; Nelson & Nusse, 2004; Wilson et al., 2001), and are thought to provide spatial information for tissue modelling (Christian, 2000; Hecht & Kemler, 2000). The canonical Wnt pathway is mediated by β-catenin (Behrens et al., 1996; Miller, Hocking, Brown, & Moon, 1999) and is required for the specification and differentiation of lung epithelial cells (Eberhart & Argani, 2001; Mucenski et al., 2003).

β-Catenin is a dual function protein with a role in cell–cell adhesion, linking classical cadherins to the actin cytoskeleton, and in regulation of gene expression through direct interaction with members of the T-cell factor/lymphoid enhancing factor-1 (Tcf/Lef-1) family of transcription factors (Behrens et al., 1996; Miller et al., 1999). In the absence of a Wnt signal, β-catenin is maintained at low cytosolic levels by the glycogen synthase kinase-3β/adenomatous polyposis coli/axin (Gsk-3β/Apc/axin) destruction complex (Behrens et al., 1998; Liu et al., 2002; Polakis, 2002) while Tcf/Lef-1 members are transcriptionally repressed through association with co-repressors, including the transducin-like enhancer of split (TLE) family (Brantjes, Roose, van De, & Clevers, 2001). When Wnts interact with Frizzled (Fzd) receptors, the destruction complex is inactivated. This allows β-catenin to accumulate and translocate into the nuclear compartment, where it binds to Tcf/Lef-1 proteins and activates target gene transcription (Miller et al., 1999).

Evidence suggests that the Wnt/β-catenin pathway also plays a role in adult tissues. Transient nuclear localisation of β-catenin in human colorectal epithelial cells following radiation-induced injury (Hardy et al., 2002) and human endometrial cells during the proliferative phase of the menstrual cycle (Nei et al., 1999), suggest a role in epithelial regeneration. Target genes identified in human colorectal cancer include genes for cell migration (*MMP7*, *CD44*) (Crawford et al., 1999; Wielenga et al., 1999) and proliferation (*cyclin-D1*, *c-myc*) (He et al., 1998; Shtutman et al., 1999), key processes in epithelial restitution. Therefore, we tested the hypothesis that adult human bronchial epithelial cells (HBEC) express components of the Wnt/β-catenin signalling pathway, and are capable of transducing a canonical Wnt signal. We investigated the influence of cell density on pathway activity, using sub-confluent cells in log-phase growth as a simple model of repairing epithelium, with relatively quiescent cells in confluent monolayer, modelling intact epithelium. Low-density cells were <20% confluent and high-density were >80% confluent at the commencement of experiments.

Significantly, we found transcriptional activity mediated by β-catenin to be altered in response to changes in cell density. Increased activity under conditions of reduced cell density correlated with increased cell proliferation and a nuclear distribution of β-catenin whereas down-regulation at confluence was found to correlate with decreased proliferation and increased expression of mRNA for Fzd-6 receptors. This provides evidence that activation of this embryonic pathway may play a role in repair processes in adult bronchial epithelial cells and raises the possibility that Fzd-6 receptors are involved in subsequent modulation of signalling at cell confluence.

2. Materials and methods

2.1. Primary bronchial epithelial cell cultures

Primary epithelial cell cultures were established using bronchial brushings from seven volunteers (Bucchieri et al., 2002). All were non-atopic, non-