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Join or Leave the Club

Kuchibhotla, Virinchi N. S.; Heijink, Irene H.

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Join or Leave the Club: Jagged1 and Notch2 Dictate the Fate of Airway Epithelial Cells

Asthma and chronic obstructive pulmonary disease (COPD) are both chronic inflammatory respiratory disorders with a worldwide increase in incidence. Chronic mucus hypersecretion is prevalent in both diseases (1, 2) and is associated with more severe symptoms and exacerbations in asthma (3) and lower quality of life, accelerated lung function decline, increased risk of exacerbations, and higher mortality in COPD (1). Within the airway epithelial layer, goblet cells are responsible for the secretion of the mucin proteins MUC5AC and MUC5B, the main constituents of mucus in the respiratory tract. Processes that contribute to chronic mucus hypersecretion are goblet cell metaplasia, excessive mucus production, and impaired mucociliary clearance. Goblet cell metaplasia and loss of columnar ciliated cells represent key features of the chronically remodeled airways in both asthma and COPD.

Environmental risk factors for asthma and COPD include allergens, viral and bacterial infections, cigarette smoke, and air pollutants, which encounter the airway epithelial lining of the respiratory tract and disturb cellular homeostasis. This leads to increased mucosal permeability and is associated with increased numbers of mucus-producing cells (4, 5). Moreover, disruption of epithelial barrier function may propagate chronic inflammation and airway remodeling (4, 6). Indeed, many of the susceptibility genes for asthma and COPD are expressed in the airway epithelium, highlighting the importance of events at the mucosal layer for the pathogenesis of both diseases (4, 6). Nevertheless, the precise molecular mechanisms that underlie abnormalities in the airway epithelium of asthma and COPD are yet to be elucidated.

The pseudostratified airway epithelial layer consists of various epithelial cell types, of which basal cells, secretory club cells, mucus-producing goblet cells, and columnar ciliated cells are the major cell types. Basal cells serve as progenitors that can differentiate into an intermediate state of secretory club cells, which differentiate subsequently into either mucus-secreting goblet cells or mucus-clearing ciliated cells (7). The Notch signaling pathway plays a crucial role in controlling the fate of airway epithelial cells in the developing airways, regulating stem/progenitor cell renewal and differentiation. In the adult lung, the mechanisms by which Notch signaling components modulate cellular homeostasis and responses to environmental insults are less well understood (8). Notch2 (Notch receptor 2) signaling has been shown to mediate cytokine-driven goblet cell hyperplasia of human airway epithelial cells (9), whereas Notch1 and Notch3 were reported to drive airway basal cell differentiation toward secretory cells (10). During airway

epithelial differentiation, Notch3 can prime a pool of basal cells into club cells that can later undergo differentiation into goblet or ciliated cells through Notch1/Notch2 downstream signaling, for which Notch ligand Jag1 (Jagged1) and Jag2 availability is required (11, 12). Specifically, Jag1 has been implicated in human airway epithelial cell differentiation toward secretory cells (13), whereas Notch3 has been shown to regulate goblet cell hyperplasia in airway epithelial cells from patients with COPD in response to rhinovirus infection (11). Furthermore, a recent study showed higher expression of Notch3 expression, but lower expression of Notch1, in asthmatic airway epithelium compared with control epithelium (15). Inhibition of Notch3 blocked MUC5AC production upon *in vitro* differentiation (15). Although expression of Notch target genes was found to be similar in club cells from patients with asthma and control subjects (9, 10), recent single-cell sequencing data in bronchial brushings have shown that the Notch target gene signature is lost in goblet cells and mucous ciliated cells in asthma (16). Collectively, this reveals an intricate interplay between Notch signaling components, encompassing balanced expression and timing during airway epithelial differentiation.

Although Notch signaling has been shown to contribute to mucous cell differentiation under homeostatic conditions, its involvement in allergen-induced goblet cell metaplasia has not been studied. In this issue of the *Journal*, Carrer and colleagues (pp. 46–56) (8) describe novel prophylactic and therapeutic effects of antisense oligonucleotide (ASO) downregulation of Jag1 and Notch2 on goblet cell metaplasia in a house dust mite (HDM) mouse model of allergen-induced asthma. The authors show that ASO-mediated knockdown of either Jag1 or Notch2, but not Jag2, Notch1, or Notch3, results in reduced expression of the secretory cell marker Scgb1a1 (secretoglobulin family 1A member 1) and increased expression of the ciliated cell marker FoxJ1 (forkhead box J1) in unchallenged and HDM-exposed mice, as well as reduced mucus production with lower mRNA expression of Muc5ac, Muc5b, and their transcriptional regulator Spdef (SAM pointed domain-containing Ets transcription factor) upon HDM exposure (Figure 1). This was accompanied by a robust amelioration of airway hyperresponsiveness to methacholine as measured by whole-body plethysmography and a trend toward improved lung function parameters, with a significant effect on forced vital capacity as measured by flexiVent (SCIREQ), whereas proinflammatory cytokines were not significantly affected.

The findings of Carrer and colleagues are of particular interest, given the emerging role of disturbances in the airway

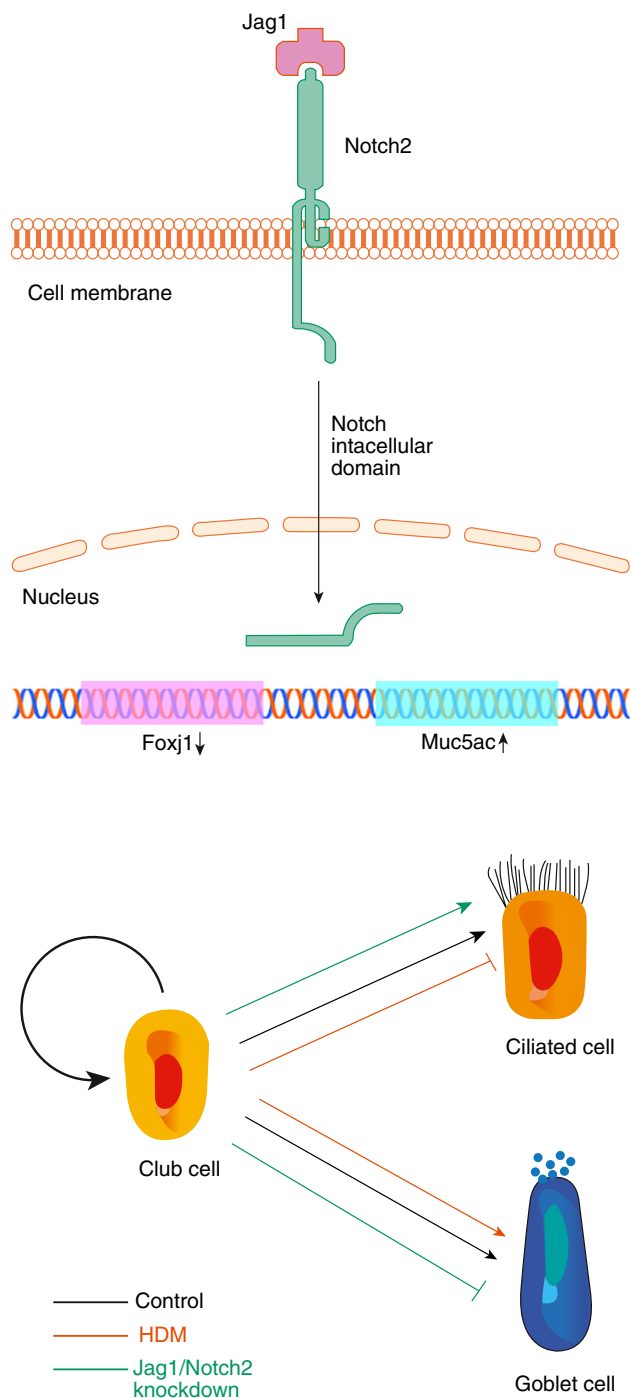


Figure 1. Proposed role of Jag1 (Jagged1)/Notch2 (Notch receptor 2) signaling in airway epithelial cell fate. Activation of Notch2 by ligation with Jag1 and subsequent nuclear translocation of the Notch2 intracellular domain in murine airway epithelium downregulates the expression of ciliated cell marker Foxj1 (forkhead box J1) and upregulates the expression of goblet cell markers (e.g., Muc5ac [mucin 5AC]). Upon house dust mite (HDM) exposure, this activation of Notch2 and Jag1 leads to increased differentiation of club cells into goblet cells and decreased ciliated cell differentiation, which is reversed by specific downregulation of Jag1 or Notch2.

epithelial layer as a driver of obstructive lung disease (4, 6). In addition to the role of epithelial barrier dysfunction in airway inflammation and remodeling, goblet cell hyperplasia and MUC5AC-mediated plugging may play a critical role in airway hyperresponsiveness, mechanically contributing to increased reactivity to methacholine (8). However, the ASOs Carrer and colleagues administered to suppress Jag1 in the epithelium could conceivably have lessened bronchoconstriction by affecting on smooth muscle cells if the ASOs also entered smooth muscle cells, although they argue against this possibility on the basis of historical evidence that ASOs do not efficiently target smooth muscle (8).

Seemingly in contrast to the role of Notch2 in HDM-induced goblet cell hyperplasia, HDM exposure resulted in downregulation of Notch2, which was accompanied by a downregulation of Scgb1a1 (8). The authors explain this HDM-induced downregulation by a reduction in the fraction of club cells upon HDM exposure and argue that only a robust downregulation of Notch2, such as by ASO treatment, will serve to prevent goblet cell metaplasia. It will be of high relevance to further elucidate the role of Notch signaling in epithelial cell fate upon injury (e.g., by allergens such as HDM), including effects on epithelial-to-mesenchymal transition and changes of epithelial barrier function related to disrupted differentiation, which may play a key role in airway remodeling in obstructive lung disease. The authors show that Jag1/Notch2 signaling, but not Notch1 and Notch3, are critical in determining the fate of club cell differentiation, an effect that may be mediated by regulation of Spdef expression (8). However, it is unclear from their study whether Spdef is a specific target of Notch2 signaling or if it is also regulated by Notch 1 and Notch3. It is rather unexpected that the authors have not observed any effects with knockdown of Notch1 or Notch3, given the previously described the role of both receptors in differentiation of human basal airway epithelial cells to secretory cells *in vitro* (10). It will be imperative to confirm their findings in a human setting, using primary bronchial epithelial cells from healthy donors as well as from patients with asthma. This is of particular importance because there have been contradictory findings on the role of Notch receptors in humans and mice (9, 14, 15). Moreover, it will be important to repeat the experiment with lower concentrations of HDM, because 2 mg/ml may not reflect the real-life situation. In addition, investigating the mechanism of Foxj1 regulation by Notch2 would be of interest. Nevertheless, the findings of Carrer and colleagues suggest that airway epithelial Jag1/Notch2 signaling may be a novel therapeutic target in allergic asthma, as well as in other respiratory diseases associated with excessive mucus secretion. Blockade of this pathway may serve to promote ciliary differentiation at the expense of goblet cell metaplasia, restoring the normal composition of the mucociliary epithelium, thereby also improving lung function. ■

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Virinchi N. S. Kuchibhotla, M.Sc.
 Department of Pathology and Medical Biology
 University Medical Center Groningen
 Groningen, the Netherlands
 School of Biomedical Sciences and Pharmacy
 University of Newcastle
 Callaghan, New South Wales, Australia
 and
 Priority Research Centre for Healthy Lungs
 Hunter Medical Research Institute
 New Lambton Heights, New South Wales, Australia

Irene H. Heijink, Ph.D.
 Department of Pathology and Medical Biology
 Department of Pulmonology
 and
 GRIAC Research Institute
 University of Groningen, University Medical Center Groningen
 Groningen, the Netherlands

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