Cigarette Smoke–induced Effects on Airway Basal Cells: Taking It Up a NOTCH

The role of the conducting airway is to deliver clean, warm, humidified air to the alveolar region where gas exchange takes place. To fulfill this function, the airway is lined with a pseudostratified epithelium that contains secretory and ciliated cells. The former produces a thin layer of hydrated mucus that helps trap particles and pathogens. The latter move mucus up and out of the airway toward the digestive tract for expulsion. Collectively, these functions are termed mucociliary clearance, and their normal operation is crucial for the proper function of the airway (1).

An imbalance in the abundance or function of secretory and ciliated cells, as seen in genetic and acquired diseases, can lead to mucociliary clearance malfunctions. The result is that mucus, pathogens, and particles can accumulate, causing obstruction that leads to infection and inflammation, which, if not alleviated, can lead to the development of lung disease (2, 3).

In patients with chronic obstructive pulmonary disease (COPD), environmental pollutants strongly associated with the development of the disease, including cigarette smoke, result in both dysfunctional ciliated cells (4, 5) and a dysfunctional mucus layer (6), leading to mucociliary clearance defects (7). An important driver of this dysfunction is goblet cell metaplasia and hyperplasia (8, 9). However, the underlying molecular mechanism that drives this response remains unclear. In this issue of the *Journal*, Bodas and colleagues (pp. 426–440) describe their use of primary human bronchial epithelial cells grown at an air–liquid interface (ALI) to investigate the molecular response of the differentiated mucociliary epithelium to cigarette smoke (10). They found that activation of NOTCH3, one of the four mammalian receptors of the Notch signaling pathway, is a nonredundant step in the aberrant response of basal cells to cigarette smoke that results in goblet cell metaplasia or hyperplasia (GCMH). Knockdown of the expression of NOTCH3 in basal cells reduces the GCMH that is induced by treatment of the cultures with cigarette smoke extract (CSE), suggesting its value as a target for therapeutic intervention.

The Notch signaling pathway is an evolutionarily conserved pathway, the ligands and receptors of which are transmembrane proteins. Notch signaling is activated when a ligand on one cell binds to the receptor on a neighboring cell. This interaction induces a series of two proteolytic cleavages that release the intracellular domain (ICD) of the receptor, which then translocates to the nucleus where it functions as a transcription factor (11). In the airway, NOTCH1 has been shown previously to be important for basal cell differentiation (12), whereas NOTCH2 has been shown to be crucial for secretory cell fate maintenance (13, 14). NOTCH3, on the other hand, has been implicated in the maintenance of a progenitor cell population in a quiescent state (15, 16). The involvement, however, of individual receptors and ligands in the response to cigarette smoke remains poorly understood.

To interrogate the activation of the Notch signaling pathway following CSE exposure, Bodas and colleagues quantified the activated fraction of the Notch receptors by measuring the amount of ICD. This approach proved pivotal to their findings because it demonstrated the increase in active NOTCH3 despite the fact that expression of the receptor did not increase. Importantly, the slight reduction in NOTCH1 and JAG2, and the lack of change in the levels of NOTCH2, suggest a role for the specific activation of NOTCH3 in the early response of basal cells to CSE.

The susceptibility of all secretory cells to Notch inhibition makes experiments with DBZ, a pan-Notch inhibitor, challenging to interpret at the cell type–specific level and to directly link Notch activation to the generation of new goblet cells due to CSE. To specifically inhibit NOTCH3 expression, the authors used siRNA to knock it down in basal cells, which produced a rescue of the CSE-induced phenotype. Furthermore, they demonstrate this response in human primary basal cells from both healthy nonsmoker donors and patients with COPD, suggesting a preservation of the mechanisms in the disease state. These results are consistent with previous reports focused on NOTCH3 but demonstrate a previously unappreciated link to the initial challenge imposed by CSE.

It is worth stating that much of the regulation that gives different Notch receptors cell-type specificity happens through the expression pattern of ligands and receptors. The use of siRNA can often result in only transient effects that are likely to be lost over the course of ALI culture. A better understanding of the cell-type specificity of the knockdown of NOTCH3 could also help reconcile the apparent disconnect between these results and a recent report in which a NOTCH3-blocking antibody was used. In that study, NOTCH3 blockade resulted in an increase of goblet cells, consistent with previous reports on the role of NOTCH3 in the mouse airway (15, 16). One way to reconcile these results is to consider the possibility that the downstream consequences of NOTCH3 activation are cell-type specific. Thus, N3ICD may have a different effect in an intermediate cell type between basal and secretory cells (the main cell type expressing NOTCH3 in the study by Carraro and colleagues) versus in basal cells exposed to CSE.

A better understanding of the role of the NOTCH receptors in the individual cell types along the basal cell to secretory and ciliated cell differentiation axis will certainly help shed light on this discrepancy. This can also help answer the reasonable question relating to the mechanism by which N3ICD abundance is increased. As the authors discuss, a possible explanation is the prolonged stability of the N3ICD due to reduced turnover rather than an

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increase in ligand-dependent activation. Elucidation of this aspect has obvious implications when thinking about therapeutic interventions.

The study by Bodas and colleagues extends previous observations about the involvement of NOTCH3 in GCMH by placing the increase in NOTCH3 signaling as a consequence of cigarette smoke exposure and highlights NOTCH3 as a potential therapeutic target to alleviate GCMH in COPD (17, 18). There are a number of questions that stem for this work. For instance, whether aberrant activation of NOTCH3 is the persistent driver of the patient GCMH phenotype, especially after cigarette smoking cessation, is unclear. When the authors withdraw CSE from the ALI cultures, MUC5AC and N3ICD are reduced. Is there a mechanism by which NOTCH3 remains aberrantly active in patients' airways, or is the pathway only involved in the early stages and is subsequently replaced by a different pathway or pathophysiological mechanism? If it is the former, what is the molecular mechanism that drives aberrant NOTCH3 activation? A closer interrogation of samples from patients with COPD may help answer some of these mechanistic questions and highlight a path toward therapeutic intervention. ■

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