CRTH2 in Pulmonary Fibrosis: Friend or Foe?

The current paradigm of pulmonary fibrosis development is based on aberrant epithelial cell injury/expansion and dysregulated repair by profibrotic monocyte-derived alveolar macrophages (MoAMs) and activated fibroblasts (1). CHI3L1 (chitinase3-like 1) is a chintinase-like protein that has been found to play a role in both injury/inflammation and repair. *Chi3l1*$^{-/-}$ macrophages exhibit attenuated bacterial killing and augmented apoptosis (2). In contrast, recombinant CHI3L1 can attenuate bleomycin-induced apoptosis in primary type II alveolar epithelial cells (3). Serum and bronchoalveolar lavage fluid (BALF) CHI3L1 levels are elevated in idiopathic pulmonary fibrosis (IPF) subjects and in subjects with Hermansky-Pudlak syndrome with pulmonary fibrosis (3–6). Additionally, IPF subjects with high serum and/or BALF CHI3L1 levels demonstrate significantly poorer survival (4).

Prior work using a yeast two-hybrid system demonstrated that CRTH2 (chemoattractant receptor-homologous molecule expressed on T-helper type 2 cells) is a target for CHI3L1 and that use of a CRTH2 chemical inhibitor abrogated development of pulmonary fibrosis in CHI3L1^{Tg} mice (3). CRTH2 is widely expressed in multiple pulmonary cells, including myeloid cells, lymphoid cells, and fibroblasts (7, 8). While *in vitro* studies suggested that interactions between CHI3L1 and CRTH2 in monocytes/macrophages potentially modulate macrophage differentiation (3), levels of CRTH2 and its effects in pulmonary fibrosis have not been evaluated *in vivo*.

In this issue of the *Journal*, Cao and colleagues (pp. 201–214) extended their previous work by showing that CRTH2 levels are elevated in pulmonary fibrosis *in vivo* (9). CHI3L1 acts on CRTH2 in macrophages to promote macrophage profibrotic differentiation. Moreover, genetic silencing of CRTH2 attenuated pulmonary fibrosis. The authors found that CRTH2 is elevated in BAL macrophages using three different models of pulmonary fibrosis: bleomycin-induced, TGF-β transgenic overexpression (TGF-β^{Tg}), and IL-13 transgenic overexpression (IL-13^{Tg}). Confocal microscopy further confirmed that CRTH2 is expressed on CX3CR1^{+} MoAMs. Furthermore, they demonstrate that CRTH2 global knockout (KO) mice exhibit attenuated bleomycin-induced pulmonary fibrosis. Similar protective effects of CRTH2 knockout were observed in two new strains of mice: TGF-β^{Tg}/CRTH2^{KO} and IL-13^{Tg}/CRTH2^{KO} as these mice exhibited diminished pulmonary fibrosis. Mechanistically, genetic deletion or chemical inhibition of CRTH2 *in vivo* led to reduced expression of several profibrotic differentiation markers in the lung, such as CD206, arginase-1, YM-1, and FIZZ-1, and a reduction in the number of MoAMs.

The translational significance of this study is strengthened by their demonstration that CHI3L1 levels are correlated with CD206 expression in primary peripheral blood mononuclear (PBMCs) from IPF subjects. Stimulation of PBMCs with CHI3L1 increased CD206 expression, which correlated with CRTH2 expression. Chemical inhibition of CRTH2 attenuated CHI3L1-mediated CD206 expression. More importantly, plasma levels of CRTH2, rather than plasma levels of CHI3L1 or monocyte CD206 expression, negatively correlated with baseline percentage forced vital capacity and percentage diffusing capacity of the lung for carbon monoxide. However, when examined longitudinally for survival (or using transplant as the endpoint), no correlation between CRTH2, CHI3L1, or monocyte CD206 expression and survival was found. In summary, the authors generated two mouse strains to validate the importance of CRTH2 in the pathogenesis of pulmonary fibrosis, and they provide translational significance of CRTH2 as a biomarker and potential target for treatment.

Although this study provides novel data regarding the role of CRTH2 in pulmonary fibrosis and reveals a previously unidentified mechanism of how CHI3L1 signaling promotes pulmonary fibrosis, many questions remain unanswered. While CHI3L1 is known to be elevated in circulating blood and BALF, it is unexplored whether CHI3L1 is secreted by macrophages in an autocrine fashion or by alveolar type II (AT II) epithelial cells in a paracrine fashion. Single-cell transcriptomic analysis and immunohistochemistry studies previously found that CHI3L1 was highly expressed in both macrophages and AT II cells in patients with pulmonary fibrosis (6, 10). Moreover, epithelial cells demonstrate increased production of CHI3L1 in response to increasing tissue stiffness, the cardinal feature of pulmonary fibrosis (11). As AT II cell injury is considered to be the initial event in pulmonary fibrosis and MoAMs mediate fibrosis progression, identifying the source of CHI3L1 is necessary to utilize it as a therapeutic target. Together with their prior work (5), the authors show that serum CHI3L1 is elevated in IPF subjects. The authors acknowledge the demographic differences between normal subjects and IPF subjects recruited in this study. A recent study showed that serum CHI3L1 is elevated in older subjects (age ⩾50) (12), suggesting that CHI3L1 expression could be related to an aging process. Because senescence is considered to have a role in pulmonary fibrosis, it is important to further evaluate the relationship between CHI3L1 and cellular senescence.

CRTH2 is expressed in myeloid cells as well as lymphoid cells and fibroblasts. A study by Ueda and colleagues showed that CRTH2 global knockout (CRTH2^{KO}) mice exhibited increased inflammation, collagen deposition, and mortality after bleomycin exposure (13). Adoptive transfer of wild-type splenocytes protected CRTH2^{KO} mice from bleomycin-induced injury. The authors from that study concluded that CRTH2 in γδT cells have protective effects in bleomycin-induced lung injury. There are two main differences

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between these two studies. First, Ueda and colleagues used CRTH2 in a BALB/c background, whereas the current article used mice in a C57BL/6 background (9). Second, the dose of bleomycin (10 U/kg) used by Ueda and colleagues was significantly higher than the dose used by the authors of this manuscript (1.25 U/kg). Future studies using mice harboring a conditional deletion of CRTH2 are required to verify the role of CRTH2 in macrophages. A recent study using a fibroblast-specific CRTH2 conditional knockout mice driven by a tamoxifen-induced *Col1a2-Cre* showed that $CRTH2^{null}$ mice had exacerbated bleomycin-induced pulmonary fibrosis compared with $CRTH2^{flox/flox}$ mice (8). Mechanistically, those authors showed that CRTH2 relocated from the plasma membrane to endoplasmic reticulum to promote collagen mRNA degradation, and that CRTH2 deficiency augmented collagen synthesis. Using the publicly available scRNA-seq data (14), CRTH2 expression is also elevated in mast cells, dendritic cells, and fibroblasts in subjects with pulmonary fibrosis, suggesting it is crucial to examine intercellular crosstalk among other cell populations. Another study showed that chemical inhibition of epithelial CRTH2 could attenuate macrophage-mediated epithelial-mesenchymal transition (15), whereas the activation of CRTH2 in macrophages by its endogenous agonist, prostaglandin D2, increased neutrophil recruitment *in vivo* (16).

This article is the first to identify CRTH2 as a novel marker for pulmonary fibrosis and it provides new insights about CHI3L1 signaling in the development of pulmonary fibrosis. Furthermore, CRTH2 levels correlate with baseline pulmonary function, but not survival, in IPF subjects. Based on their observations, additional studies can further evaluate whether CRTH2-targeted therapy would be therapeutic in treating fibrotic lung diseases. ■

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