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Targeting Asporin in Lung Fibrosis: A New Approach to an Old Concept

TGF-βs (transforming growth factor βs) are pleiotropic cytokines that act through heterotetrameric TGF-β type II (TβRII) and TGF-β type I (TβRI) serine and threonine kinase receptor complexes. TGF-β ligands bind to the extracellular domain of TβRII, and the subsequent hetero-oligomerization of TβRII and TβRI causes a conformational change in the receptor complex, resulting in phosphorylation and activation of TβRI. Activation of TβRI leads to signal propagation through the well-characterized Smad-dependent canonical pathway and/or Smad-independent noncanonical pathway. The outcome of TGF-β signaling is context-dependent. In normal tissue, TGF-β signaling serves a role in the maintenance of tissue homeostasis. However, in many disease states, including fibrosis, the output of TGF-β signaling is diverted toward disease progression. There has been a concerted effort to block TGF-β signaling for therapeutic benefit (1).

PGs (proteoglycans) are a large family of proteins typically covalently attached to CAG (glycosaminoglycan) chains (2). Syndecan-2 and syndecan-4 of the cell surface PGs have been shown to play an important role in the development of pulmonary fibrosis (3, 4). Biglycan and decorin are small leucine-rich proteoglycans (SLRPs) and make up the largest group of PGs. In bleomycin-exposed rats, biglycan expression was increased and decorin expression was decreased in whole lung homogenates (5, 6). The discrepancy between biglycan and decorin levels in fibrogenesis remains unresolved but is believed to be related to the differential regulation of TGF-β signaling. Decorin has been proposed to be an endogenous TGF-β "neutralizing antibody." Serum decorin levels are lower in subjects experiencing idiopathic pulmonary fibrosis (IPF) acute exacerbation compared with subjects with IPF with stable symptoms and normal subjects (7). Either intratracheal injection of decorin or intranasal injection of adenoviral decorin vectors was able to attenuate fibrosis in animal models of bleomycin-induced pulmonary fibrosis (8, 9).

Asporin, initially isolated from human articular cartilage, is a novel member of the SLRP family. Previous studies have shown that TGF-β induces asporin and biglycan expression, whereas decorin expression is suppressed by TGF-β *in vitro* (10). Interestingly, asporin can suppress TGF-β–induced collagen production in chondrocytes (11), suggesting a complex regulatory mechanism between asporin and TGF-β signals. It has been recently found that asporin overexpression is common among different malignancies, including breast, prostate, gastric, and pancreatic cancers, and cytoplasmic asporin expression is associated with poor prognosis in prostate and colorectal cancers (12). Mechanistically, the cytoplasmic asporin binds to Smad2/3 and translocates to the nucleus where it modulates the transcriptional activity of the Smads. So far, little is known about the role of asporin in pulmonary fibrosis.

In this issue of the *Journal*, Huang and colleagues (pp. 158–170) identify asporin as one of the most upregulated genes in fibroblasts from subjects with IPF through a bioinformatics-based analysis of three publicly available microarray data sets (13). The authors experimentally verified the upregulation of asporin expression in two independent animal models of pulmonary fibrosis (bleomycin and amiodarone). Immunofluorescence staining showed that asporin was predominantly expressed by α-smooth muscle actin (SMA)$^+$ myofibroblasts intracellularly. Silencing of asporin expression inhibited Smad2 phosphorylation and α-SMA and collagen I production by primary murine lung fibroblasts.

Interestingly, in asporin-deficient fibroblasts, TβRI protein expression was reduced, but not mRNA expression. Overexpression of TβRI was able to rescue the profibrotic phenotype in asporin-deficient fibroblasts. Based on these findings, the authors hypothesized that asporin modulates TβRI via a posttranslational mechanism. Using biotinylation assays and immunofluorescent staining, the authors further demonstrate that asporin regulates TβRI recycling. In asporin-deficient fibroblasts, less TβRI was recycled back to the cell membrane, resulting in reduced activation of TGF-β signaling. Mechanistically, asporin promotes TβRI recycling through a small Ras-related GTPase, Rab11. Coimmunoprecipitation experiments showed that asporin interacts with Rab11, and immunofluorescent staining showed that asporin localized in Rab11$^+$ vesicles. Furthermore, overexpression of Rab11 could reverse the effects of asporin knockdown and reactivate the TGF-β–Smad pathway. A second approach using both constitutively active and dominant negative Rab11 vectors validated this observation.

Although the findings from Huang and colleagues suggest a novel role of asporin in lung fibrogenesis and uncover the potential mechanisms by which asporin regulates TGF-β signaling, many questions remain unanswered. It is unexplored whether asporin is expressed in other cell types critical for the development of pulmonary fibrosis, such as alveolar macrophages or alveolar epithelial cells (AECs). A recent study showed that overexpression of asporin inhibited TGF-β–Smad pathway activation in AECs and promoted AEC repair. AEC injury is considered to be an initial insult that leads to the development of pulmonary fibrosis. Intratracheal injection of a single dose of recombinant human asporin protein during either the acute injury phase (Day 3) or the initial fibrotic phase (Day 7) after bleomycin exposure was able to attenuate fibrosis development in mice (14). These different effects of asporin in fibroblasts and AECs warrant additional evaluation. It is also possible

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that asporin from other cell types can influence TGF-β–Smad pathway activation in fibroblasts. For example, syndecan-2 was overexpressed in IPF alveolar macrophages and promoted caveolin-1–dependent internalization of TGF-β1 and TβRI in AECs, which inhibited TGF-β1 signaling and attenuated fibrosis (3). In addition, the role of asporin *in vivo* was not validated. Future studies using either asporin knockout mice or fibroblast-conditional knockout mice are needed to confirm the *in vitro* findings. Although the data presented in this article highlight the role of intracellular asporin in regulating the TGF-β–Smad pathway, the authors did not examine the expression asporin in the extracellular matrix, as asporin is generally considered an extracellular/secretory PG.

This article is the first to identify asporin as a pulmonary fibrosis–associated gene by bioinformatics analysis and experimental verification. The study reveals a novel function of asporin in the regulation of TβR1 trafficking, thus providing mechanistic insights into how asporin regulates TGF-β signaling. The findings by Huang and colleagues provide strong rationale for targeting asporin as a novel pharmacological TGF-β blockade strategy in treating fibrotic lung diseases. ■

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