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Galectin-3: The Bridge over Troubled Waters

Respiratory diseases kill more than 400,000 Americans each year and significantly reduce quality of life for millions more. The National Heart, Lung, and Blood Institute estimated that in 2009 the annual cost of providing healthcare related to all respiratory conditions, excluding lung cancer, was $113 billion (1). The incidence of idiopathic pulmonary fibrosis (IPF) in the United States is estimated to be between 6.8 and 16.3 per 100,000 persons, but it is estimated that the incidence of IPF increased by 11% annually between 1991 and 2003 (2). It is unknown if the incidence and prevalence of IPF are influenced by geographic, ethnic, cultural, or racial factors, but chronic activation of inflammatory pathways (3), epithelial injury (4), and high expression of the pro-fibrotic factor transforming growth factor β (TGF-β) (5), are some of the mechanisms implicated in the pathogenesis of fibrosis dependent on the etiologic agent. Unfortunately, current therapies have no proven effect on function or survival.

Communication between different cell compartments requires mediators that interact with different biological components. For a long time, lectins have been known to build bridges that facilitate communication of the proteomics with the glycomics worlds. Lectins are a unique family of proteins that exhibit the ability to bind and transmit signals from various types of glycol-proteins, and multiple functions have been attributed. Lectins are classified into several families in which galectins represent the largest, with 16 different members (6). Galectin-3 is a 30-kD β-galactosidase–binding protein expressed and secreted by several cell types, in particular macrophages (7). Galectin-3 can be found simultaneously in multiple cell compartments, including nucleus, cytoplasm, cell surface, and within extracellular matrix and serum.

Biological activities of galectin-3 are many, and depend largely on the localization. In resting fibrolasts, for example, galectin-3 is mainly cytoplasmatic, while turning into nuclear localization during proliferation (8). Galectin-3 has been documented to induce re-epithelization of corneal injury by modulating integrin signaling required for wound healing (9). Effects of extracellular galectin-3 are even more diverse and complex, and involve several cellular effects, such as cell adhesion, immune reactions, and angiogenesis (10). Interestingly, in another type of connection, galectin-3 acts as bridge between different cell types. Secretion of galectin-3 by macrophages is critical in the transformation of renal myofibroblasts

into a profibrotic phenotype. The apparent mechanism is by galectin-3–induced activation of TGF-β receptor by cross-linking the receptor with modified glycans, resulting in a prolonged activation of the receptor (11).

In this issue of the *Journal*, MacKinnon and coworkers (pp. 537–546) present intriguing findings that link these general observations to IPF (12). The authors demonstrated that the inhibition of galectin-3 activity reduced TGF-β as well as bleomycin-induced lung fibrosis by targeting TGF-β signal activity.

The (myo)fibroblast in IPF represents a promising therapeutic target. Three different sources of myofibroblasts have been proposed: recruited fibroblast progenitor cells or fibrocytes (13), proliferation and transformation of local fibroblasts, and epithelial–mesenchymal transformation or EMT (14). These mechanisms depict a comprehensive approach to pan-organ fibrosis. In the current issue, MacKinnon and colleagues report that galectin-3 is critically involved in some of these processes in human and experimental lung fibrosis (12). The authors have described similar findings in liver and renal fibrosis, highlighting galectin-3 as a main mediator in pan-organ fibrosis (11). This publication adds to the previous reports the critical role for galectin-3 in fibroblast activation, which occurs independent of the severity of the inflammation. In addition, in all investigated models thus far, galectin-3 did not interfere with canonical TGF-β–induced Smad signaling. In the current issue, the authors expand their findings and addressed the question whether galectin-3 is involved in TGF-β–induced EMT in the lung. The EMT process, *in vivo*, is controversially discussed as a pivotal mechanism of fibrosis (15). Here, cell culture studies have been used to demonstrate that EMT, as measured by alterations in epithelial and mesenchymal marker expression, is induced in primary isolates of alveolar type II (ATII) cells upon TGF-β treatment and diminished in galectin-$3^{-/-}$ cells. These findings need further corroborating studies showing that this event is relevant *in vivo*. Further, the authors postulate that galectin-3 modifies TGF-β signaling via activation of a Smad-independent pathway, by phosphorylation an activation of β-catenin. Interaction between TGF-β and β-catenin is well described in the literature; what is particularly interesting is the new association in the context of EMT in the lung (16). Given that growing evidence that suggests that the Wnt family of secreted glycoproteins associates with fibrosis by their possible connection with Smad3 pathways (17), it opens the possibility that galectin-3 acts as another important bridge, communicating these two paths, which would open novel routes for the development of cell-specifc therapeutic options.

As mechanistic events driving EMT, Twist and Snail are two transcription factors that have been identified to be essential in the induction of EMT alveolar epithelial cells, the link between the TGF-β/β-catenin and these two transcription factors in a bridge that needs to be built to connect all these finely tuned mechanisms.

Interestingly, the authors shed light into how galectin-3 modifies TGF-β signaling in alveolar epithelial cells. Here, galectin-3 led to a change in the surface expression of TGFR2. It has been recently reported that activation of TGFR2 by TGF-β contributes to the development of pulmonary fibrosis in a murine model of bleomycin-induced pulmonary fibrosis, making this receptor a target for potential rational therapeutic strategies (18).

Despite nearly two decades of research on TGF-β signaling promoting the activation, proliferation, and differentiation of collagen-producing myofibroblasts, there are still currently no FDA-approved drugs that specifically target any proposed mechanism of pulmonary fibrosis (19). The closest candidate is pirfenidone, which in 2011 was approved for use in Europe for patients with IPF.

MacKinnon and coworkers show that TGF-β led to an increased expression of galectin-3 on primary isolates of alveolar epithelial cells; however, the overall relevance of the mechanisms by which galectin-3 induces the activation of TGF-β receptor in the alveolar epithelial cell needs further study, as well as the interaction with different cell types. As literature data indicate, Galectin-3 is expressed and secreted by several cell types, mainly in macrophages. Interestingly, galectin-$3^{-/-}$ mice did not depict any alteration in the initial inflammatory response in the lung, which is a controversial issue that needs to be expanded in future research. Cell-specific depletion of galectin-3 would be useful to further clarify the role of macrophage-derived galectin-3. The authors used a similar approach to investigate the role of macrophage-bound galectin-3 in renal fibrosis, showing that depletion of galectin-3 in macrophages had a strong influence on fibrosis.

Bronchoalveolar lavage fluids of patients with IPF did exhibit high levels of galectin-3, probably secreted mainly by macrophages and epithelial cells, and high galectin-3 levels have been measured in serum samples of stable IPF, which was even higher in patients with acute exacerbation. The presented results are, by far, based on a too-small sample size, and future studies are warranted to indicate the significance of galectin-3, before it can join the crescent number of biomolecules that had been identified as possible biomarkers for IPF (20).

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Biological Insights from Clinical Trials and Networks

Translational research seeks to bridge the gap between the remarkable progress made in basic science discoveries and their "translation" to improved diagnostics and therapeutics for patients. A bedside-to-bench (and back to the bedside) approach is likely to be more successful than the traditional bench-to-bedside approach; unfortunately, the latter has most often led to failed clinical trials and meant a return to the "drawing board," and usually it is back to the *bench*. While our understanding of biology will continue to grow from the essential and vital research conducted at the bench, it is the relevance (to human disease) of the questions asked that will be better informed if one starts this iterative endeavor *at the bedside*.

In this issue of the *Journal* (pp. 547–556), Xu and colleagues (1) utilized a bedside-to-bench approach to gain insights into the pathogenesis of pulmonary fibrosis in human subjects with a history of cigarette smoking. Cigarette smoking has been associated with a number of clinical syndromes characterized by distinct, sometimes overlapping, histopathologic and radiologic patterns, including pulmonary fibrosis, emphysema, and combined patterns (2). The reasons for these varied host tissue responses to smoking-induced lung injury are not well understood. Xu and colleagues probed a large cohort of former/active smokers enrolled in the COPDGene study (www.copdgene.org) and discovered that statin use is associated with a higher incidence of interstitial lung abnormalities (ILA) on high-resolution computed tomography after adjustment for relevant covariables, including a history of high cholesterol, coronary artery disease, diabetes, hypertension, and other cardiovascular medications (odds ratio [OR], 1.60; 95% confidence interval [CI], 1.03–2.50; $P = 0.04$). ILA identified in this cohort included radiologic features more typical of pulmonary fibrosis such as reticular abnormalities and traction bronchiectasis in addition to features commonly associated with inflammatory lung diseases (e.g., ground glass opacities). The risk of ILA in statin users was significantly higher in older subjects and in those taking hydrophilic statins, although the reasons for these important modifiers were not explored. To investigate the association between statins and pulmonary fibrosis, these investigators evaluated the effect of pre-treatment with a hydrophilic statin (pravastatin) on bleomycin-induced pulmonary fibrosis in mice. They demonstrate that statin administration aggravates lung fibrosis in this model in association with enhanced inflammatory cell recruitment, caspase-1 activation, and cleaved IL-1β expression, supporting a role for activation of the NLRP3 inflammasome in mediating this effect; further support for this mechanism is provided by demonstrating that statins enhance NLRP3-inflammasome activation via mitochondrial reactive oxygen species (*mt*ROS) generation in macrophages (Figure 1).

Inflammasome activation is an important aspect of innate immune signaling, and may be a critical link between environmental exposures and fibrotic responses in a number of clinical contexts (3–5). In the lung, inflammasome activation links extracellular danger signaling to activation of fibrotic responses (6), and is known to be critical in environmental lung diseases such as silicosis and asbestosis (3, 4). Although originally described in myeloid cells, it is increasingly clear that components of the inflammasome exist in fibroblasts and can mediate phenotypic alterations associated with fibrogenesis (7). Because lipid signaling is critical to inflammatory responses, statin modulation of inflammasome activation is not unexpected. However, statins are known to have pleiotropic effects on cell signaling independent of cholesterol (8). Statin modulation of the Rho kinases may be particularly relevant for wound healing and fibrotic responses. In fact, statins are known to have the potential to accelerate wound-healing responses (9).

An intriguing finding from this study is the influence of aging on statin-associated pulmonary fibrosis in current/former smokers. In subjects over 65 years old, statin users were more likely to have ILA after adjusting for relevant covariates (OR, 1.96; 95% CI, 1.06–3.61; $P = 0.03$), an effect that was completely lost in subjects 45 to 55 years old. In fact, there was a trend toward protection from ILA in statin users in this younger cohort (OR, 0.36; 95% CI, 0.07–1.78; $P = 0.21$). How might age affect the purported fibrotic susceptibility to lung injury in statin users? Does aging influence the macrophage phenotype and promote NLRP3 inflammasome activation? Inflammasome activation has been implicated in other aging-associated disorders (10). Is there a link between senescence of one or more lung cell types (including macrophages) and the potential "metabolic stress" induced by statins/cigarette smoke? Do age-associated epigenetic alterations affect the molecular responses to statins and/or smoking? Is the effect of statins on mitochondrial dysfunction and *mt*ROS generation sufficient to explain this age-associated phenotype? What are the effects of statins on structural lung cells implicated in the fibrotic process such as epithelial cells, endothelial cells, and mesenchymal cells?

The study by Xu and colleagues may well raise more questions than it answers. However, in doing so it creates excellent opportunities to better understand the biology of lung inflammation and