ABC Transporters: An Overlooked Mechanism of Drug Failure in Our Preclinical Models?

Pulmonary fibrosis (PF) is a progressive, fatal, interstitial lung disease that is initiated by damage to the alveolar epithelium and is often diagnosed as idiopathic PF (IPF) (1, 2). Patients with IPF have a median survival of 2–3 years after diagnosis, a reduced quality of life, and limited effective therapeutic options (3). This calls for the continued use and further characterization of animal models to study and design new therapeutics. Animal models are commonly used to study PF; however, none of these models completely recapitulate the complex and heterogeneous nature of the PF observed in humans. Despite these limitations, they still provide valuable insights into the mechanisms of disease development and fibrosis resolution, as well as methods to test the efficacy of preclinical therapeutics (4). In that context, the data presented by Park and colleagues (pp. 178–190) in this issue of the *Journal* indicate the need to refine our systems for preclinical drug screening to maximize both efficiency and accuracy (5). The authors introduce the concept that the well-characterized bleomycin model of PF in mice has an additional important confounder we need to consider when using it to test potential therapeutic compounds (5).

ABC (ATP-binding cassette) transporters play an important physiologic role in protecting cells against xenobiotics and endogenous metabolites, and also play a significant role in determining the efficacy and toxicity profile of many drugs. In this article, the authors pose a fundamental question concerning the mechanism by which drug levels are regulated in the lungs through the upregulation of two well-known ABC transporters: P-gp/MDR1/*Abcb1* (permeability glycoprotein) and BCRP/*Abcg2* (breast cancer resistance protein) (5). Although the investigators found that neither P-gp nor BCRP was present in the lungs of patients with IPF, extensive staining for both transporters was observed in murine lungs after bleomycin-induced fibrosis. Given its mechanism of action and use as a chemotherapeutic agent, it is not surprising that exposing the lungs to bleomycin may induce cellular defense mechanisms, including the upregulation of drug transporters, that are unrelated to the outcome of fibrosis (6). The authors provide evidence that delivery of bleomycin, by either the oropharyngeal or subcutaneous route, induces the upregulation of both P-gp and BCRP in alveolar type II cells, fibroblasts, endothelial cells, and macrophages (5). Therefore, efforts to gain a better understanding of how cellular transporters are altered in the lungs during fibrotic disease, and more specifically in our animal models of disease, are warranted.

The main function of P-gp is to protect against exposure to exogenous toxic substances and endogenous metabolites, which explains its high expression in hepatocytes, the apical surface of epithelial cells in the proximal tubules of the kidneys, the columnar epithelium in the intestine, epithelial cells of the placenta, and the luminal surface of capillary endothelial cells in the brain (7, 8). P-gp and BCRP have been well studied in association with lung cancer, where their upregulation confers multidrug resistance to tumor cells, resulting in a poor outcome for patients (9). However, reports about P-pg and BCRP expression in the normal human lung are conflicting. Several studies demonstrated low transcriptional and protein expression of these transporters in normal lung (5, 7, 8, 10). Other reports suggested moderate to high levels of P-gp and BCRP RNA and protein in the ciliated epithelium and trachea in both mouse and human lungs at baseline (11–13). A comprehensive assessment of the transcript expression of all 48 ABC transporters in tracheal and large- and small-airway epithelial cells from healthy subjects showed only low basal levels of P-pg and BRCP (10). In the same study, an analysis of smokers or patients with chronic obstructive pulmonary disease or asthma did not show upregulation of these two transporters. However, smoking did alter the expression of *Abca13, Abcb4, Abcc1*, and *Abcc3*, indicating that future studies to examine the effect of these transporters on current and future therapeutics for other lung disorders are also necessary. A detailed examination of these transporters has not yet been conducted in patients with IPF, but expression data (datasets GES2052 and GSE44723) obtained from whole-lung tissue from patients with IPF and control subjects or isolated normal and IPF fibroblasts did not indicate significant differences (14, 15). Consensus regarding the baseline expression of P-gp and BCRP and how they are altered during fibrosis in humans will be important, as several of the new therapeutics in clinical trials are known substrates of P-gp (5), and a better understanding of these transporters will also shape how future animal studies are conducted. Furthermore, the use of P-gp– and BCRP-deficient animals, which are viable and show alterations in pharmacological functions (16–18) in the bleomycin model, will advance our knowledge about how these transporters affect the development of bleomycin-induced fibrosis and the efficacy of preclinical compounds.

Another important finding in the work presented by Park and colleagues is that both the sex and age of the mice may also play a role in the expression of P-pg and BCRP (5). P-pg immunostaining was higher in young male mice than in female mice, and both P-pg and BCRP levels were higher in male mice that were 6 months of age than in younger mice (5). The authors hypothesize that this increase of P-pg in the lungs of male mice resulted in a reduced efficacy of nintedanib, which is a known P-gp substrate, compared with previous findings in female mice (5, 19). In studies assessing ABC transporter function in the lungs of mice, it will be important to examine mice of both sexes and, when possible, of advanced age, for a better correlation with human disease (4, 20, 21).

As is always the case with the development of animal models, it is important to return to human samples to best gauge their

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accuracy. Given the importance of other ABC transporters that play a significant role in lung disease, such as CFTR/*Abcc7* in cystic fibrosis (22), mutations in *Abca3* that lead to a fatal surfactant deficiency in newborns (23), deficiency in *Abca1* that leads to Tangier disease (24), and the association of pulmonary alveolar proteinosis with *Abcg1* deficiency in alveolar macrophages (25), further studies of the location and role of P-gp and BCRP in the lungs during homeostasis and fibrosis are necessary. Regardless, if our animal models lead to under- or overpredictions of drug efficacy, the results will be less than ideal. The presence of protein efflux transporters, such as P-gp, BCRP, and the other members of the ABC transporter family, as well as their respective roles in limiting drug absorption through the pulmonary epithelium, endothelium, macrophages, and fibroblasts, should be an important consideration during the development of novel therapeutic interventions. In addition, harnessing published single-cell RNA-sequencing datasets along with careful immunohistochemical analyses of normal and diseased lungs may help shed light on the similarities and differences between the cellular localization and modulation of ABC transporters in mice versus humans. This in turn may help direct the development and/or interpretation of preclinical therapeutic studies and aid in their translation into clinical trials. ■

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