in circulation, whereas more substantial staining can be detected in the polymorphonuclear fraction. These technical issues are critically important when interpreting presumably contradictory study results from different groups.

Thus, the question of exactly what cell types constitute fibrocytes *in vivo,* and whether a fibrocyte is a distinct cell, a cell type in transition, or a group of cells, as well as the importance of their collagen synthetic function in humans, is still unclear. However, it is becoming increasingly clear from the current study on HPS and earlier reports about IPF (2, 3) that the measurement of fibrocytes in the circulation of patients with fibrotic lung disease may have clinical use in the future, either for clinical trial design or clinical management. Even though the study by Trimble and colleagues found no correlations between fibrocyte counts and pirfenidone treatment (2), this does not rule out that fibrocytes could be useful as markers of response to other therapies. However, the episodic nature of the alterations in fibrocyte numbers longitudinally and the inability of fibrocyte numbers to predict changes in pulmonary function tests indicates they may not be very useful as predictors of drug action. Perhaps the next realm of fibrocyte research should be two-directional: to identify the paracrine factors that promote disease progression and attract fibrocytes along chemokine gradients to the site of putative injury, making them particularly well suited to deliver a pathologic payload, and to generate consensus on a standardized set of fibrocyte markers and a readily accessible protocol for identification of fibrocytes by flow cytometry that could be used in multicenter studies. Ideally, protocols could be developed that may allow analysis of peripheral blood buffy coats collected during future clinical trials and submitted to well-annotated biorepositories such as the type that have been called for in IPF research (13). These goals are not mutually exclusive, and the major insights that will be gained will inevitably help to improve the management of patients with fibrotic lung disease. ■

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Bethany B. Moore, Ph.D.
Department of Internal Medicine
and
Department of Microbiology and Immunology
University of Michigan Medical School
Ann Arbor, Michigan

Martin Kolb, M.D., Ph.D.
Firestone Institute for Respiratory Health
McMaster University
Hamilton, Ontario, Canada

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Live and Let Die: Targeting Alveolar Epithelial Cell Proliferation in Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is the most common interstitial lung disease, with a medium survival of 3–5 years. Although this year two drugs are becoming available for patients with mild to moderate IPF, there is still an unmet clinical need for the development of targeted and specific therapies for this devastating disease (1). Repetitive injury and alterations of the alveolar epithelium, including alveolar epithelial cell proliferation and hyperplasia, have been described and linked with the development of IPF (2–4). It is thought that abnormal alveolar epithelial cell activation leads to impaired epithelial–mesenchymal crosstalk and, ultimately, to fibroblast accumulation, as well as enhanced extracellular matrix (ECM) deposition. Development of novel

therapeutic targets has been majorly focusing on the effectiveness of potential drugs on fibroblast accumulation and distorted ECM turnover (1).

In this issue of the *Journal*, Weng and colleagues (pp. 1402–1412) highlight the importance and suitability of targeting abnormal alveolar epithelial cell proliferation as a relevant therapeutic option (5). The authors report that deoxycytidine kinase (DCK), which is a key enzyme providing deoxynucleotides for DNA replication, is increasingly expressed in proliferating and hyperplastic alveolar epithelial cells in experimental and human IPF. DCK is regulated by hypoxia-inducible factor (HIF)-1α under hypoxic conditions, which are apparent in the IPF lung. Using dyC, an enzymatic inhibitor of DCK, Weng and colleagues demonstrate significant attenuation of established experimental lung fibrosis, which was accompanied by reduced proliferation of alveolar epithelial cells and fibrotic mediator release (5).

The finding that alveolar epithelial cell proliferation and hyperplasia contribute to the profibrotic milieu in IPF is supported by several studies in the field (3, 6, 7). Key regulators of alveolar epithelial cell proliferation, such as the Wnt inducible signaling protein 1 or dimethylarginine dimethylaminohydrolase, have been identified, and inhibition of these mediators led to attenuated experimental lung fibrosis (6, 7). Moreover, epithelial cell–derived matrix metalloproteinase-1 (MMP-1) is up-regulated in fibrosis and has been shown to induce a proliferative alveolar epithelial cell phenotype (8). Notably, Herrera and colleagues demonstrated that MMP-1 is a potent inducer of HIF-1α, which raises the possibility that MMP-1 might be involved in the mechanisms proposed by Weng and colleagues (8).

Nevertheless, the majority of recent studies focused on alveolar epithelial cell apoptosis as a main driver of the disease (9). For example, it has been shown that induction of alveolar epithelial cells apoptosis is a potent initiator of pulmonary fibrosis development (10). On the basis of the collective evidence, it appears that both ongoing alveolar epithelial apoptosis and (subsequent) proliferation with the attempt to repair are characteristic of IPF progression. It remains unclear, however, why the proliferative response does not lead to proper wound repair, but ongoing distortion of the alveolar epithelium and the lung architecture. Heat shock proteins (HSPs) might represent a possible link between cellular apoptosis and proliferation. For instance, several studies have shown that HSPs are induced during the process of apoptosis and inhibit apoptosis by interacting with proteins involved in programmed cell death. Importantly, HSPs take part in cancer cell proliferation, and recent studies have reported that HSPs are involved in uncontrolled epithelial cell proliferation in fibrotic lungs (11, 12).

Notably, the relevance of the HIF–DCK–2'-deoxyadenosine 5'-triphosphate (dATP) axis for chronic lung disease has been recently demonstrated by the same group (13). Similar to IPF, Weng and colleagues found that hypoxia-mediated DCK activity, along with increased deoxyadenosine and dATP level in chronic obstructive pulmonary disease (COPD), however surprisingly, was associated with the opposite cellular outcome, alveolar epithelial cell apoptosis. In the current publication, the authors observed DCK mainly in proliferating cells, and DCK inhibition by dyC led to decreased alveolar epithelial cell proliferation. In experimental models of both COPD and IPF, DCK inhibition also led to a reduction dATP level, suggesting that the inhibitory effect may be directly mediated by dATP. These findings suggest that the fine-tuned level of deoxyadenosine/dATP is the main determent of functional outcome. It remains open how dynamic and different these levels are in human disease *in vivo* and how these are further regulated by other factors that lead to disease-specific outcomes.

Accumulating evidence strongly suggests a genetic predisposition in familial as well as sporadic IPF, including a genetic variant in the genes of surfactant proteins, telomerase, or mucins (MUC5B) (14). Although, some mutations, for example, in surfactant proteins, have been linked to alveolar epithelial dysfunction and increased endoplasmic reticulum stress in the alveolar epithelium (15), a causal link to alveolar epithelial cell proliferation and/or hyperplasia needs to be addressed in future studies. One plausible way to reconcile the different outcomes of DCK expression and activity observed in IPF compared with those in COPD is to consider the differences in genetic susceptibility as well as disease-specific epigenetic alterations underlying IPF and COPD (16).

Weng and colleagues further report increased hypoxia-driven HIF-1α in IPF, thereby confirming previous studies (17). Hypoxic conditions can be found in many chronic lung diseases, as well as other conditions, such as ischemic heart disease or cancer (18). As such, there is growing interest in DCK inhibitors as novel therapeutics. Adverse effects of DCK inhibitors still need to be accurately evaluated. For example, the inactivation of DCK induces replication stress and DNA damage in erythroid, B lymphoid, and T lymphoid lineages, thereby leading to a dramatic decrease in the number of B lymphocytes in circulation (19). This role on replication stress, particularly on lymphocytes, may also be involved in the protection of the fibrotic process beyond the mechanism found by Weng and colleagues. Moreover, one could speculate that DCK inhibition also affects fibroblast proliferation, which alongside reduced alveolar epithelial cell proliferation results in reversal of experimental lung fibrosis.

Altogether, Weng and colleagues provide substantial evidence that alveolar epithelial cell proliferation and hyperplasia might actively contribute to progression and worsening of pulmonary fibrosis. Notably, the authors present promising data that this proliferating cell population might be a suitable target for the development of potent antifibrotic agents. This is of particular interest, as direct antiapoptotic strategies exert only limited clinical feasibility because of their possible potential for cancer-enabling mechanisms. Future in-depth analysis of the genotypes/phenotypes, as well as functionality of alveolar epithelial cell subpopulations in IPF, are clearly needed to further identify suitable therapeutics that target these cells. Together with existing promising compounds in (pre)clinical trials, which primarily affect fibroblast function and/or ECM deposition, we thus might be able to successfully reverse the intriguing and complex face of IPF. ■

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Melanie Königshoff, M.D., Ph.D.
Comprehensive Pneumology Center
Helmholtz Center Munich and University Hospital of the Ludwig Maximilians University
Munich, Germany
and
German Center for Lung Research (DZL)
Munich, Germany

Philippe Bonniaud, M.D.
INSERM U866 Équipe Labellisée Ligue Contre le Cancer
Dijon, France
Faculty of Medicine and Pharmacy
University of Burgundy
Dijon, France
and
Service de Pneumologie
Dijon, France

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