Dysregulated balance of lung macrophage populations in idiopathic pulmonary fibrosis revealed by single-cell RNA seq: an unstable "ménage-à-trois"

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Using single-cell RNA sequencing, Morse and co-workers have identified profound modifications within pulmonary macrophage populations during the course of idiopathic pulmonary fibrosis and propose a new therapeutic strategy against this deadly disease http://bit.ly/30Hb0Gl

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Unravelling the cellular and molecular mechanisms associated with the initiation and progression of idiopathic pulmonary fibrosis (IPF) represents a huge challenge. A better knowledge of these issues is not only imperative for elucidating the pathogenic mechanisms of IPF but also to provide new therapeutic strategies as well as new options for diagnosis and prognosis. The pathogenesis of IPF is particularly complex and involves multiple cell types associated within a dynamic pathobiological process, involving excessive wound healing with chronic inflammation, apoptosis of alveolar epithelial and endothelial cells, formation of honeycomb cysts composed of pseudostratified and bronchiolar-like epithelium, activation of myofibroblast effector cells with the formation of fibroblasts foci, and, finally, excessive deposition of extracellular matrix resulting in the destruction of the lung architecture and the loss of lung functions. Genetic susceptibility, ageing, and exposure to inhaled toxins all contribute to the initiation of the fibrotic process [1–3].

In the current issue of the *European Respiratory Journal*, Morse *et al.* [4] used single-cell RNA sequencing (scRNA-seq) to provide a detailed map of the changes in cell populations and associated gene expression in fresh samples of normal lungs compared with lungs from IPF patients.

Recent advances in single cell analyses and associated computational methodologies now provide the unique opportunity to characterise the cellular heterogeneity in a specific pathophysiological context. Droplet-based scRNA-seq systems such as the one used in this study [5] enable the capture and gene-expression profiling of several thousand cells per run. Unlike conventional bulk approaches, scRNA-seq elucidates precise cellular compositions, and developmental and activation states [6–9], and has also been used to identify new rare cell types, such as the recently described ionocyte in the human airway wall [10, 11]. An intense collaborative effort, led by the Human Cell Atlas consortium, aims to characterise all human cell types in the human body in terms of their specific gene expression pattern,

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physiological state, developmental trajectories or spatial distribution in tissue, and has recently highlighted lung tissue as one of its priority projects [12]. This complete atlas of the healthy lung will thus represent a unique reference set for the study of chronic lung diseases, such as IPF, by revealing the changes in cellular states and the altered cell–cell interactions associated with a specific disease. It should also point out mechanistic clues indicating whether these cellular changes are linked to reactivation of developmental pathways at the epithelial–mesenchymal interface or to the acquisition of new pathological programmes, and lead to the identification of new biomarkers as well as progresses in therapeutic strategies.

The current publication by Morse *et al.* [4] already provides several important new insights on the pathogenesis of IPF. Interestingly, their strategy was to select two specific locations in the lung of the same IPF patient, corresponding to the severely fibrotic lower and less fibrotic upper lobes in order to capture disease at different points in evolution. The authors compared gene expression from these distinct locations to normal lungs by scRNA-seq using freshly digested lung samples from three IPF patients and three healthy controls, providing a wide set of single cell transcriptomes of 47 771 cells, representing 17 231 and 30 540 cells from healthy and IPF lungs, respectively. Computational analyses allowed the identification of more than 20 cell clusters, which were then subsequently identified as discrete cell types, including inflammatory, epithelial, vascular and mesenchymal cell types using previously described markers. The authors then comprehensively identified changes in cell populations and associated gene expression in IPF, according to the status of the disease (normal, upper and lower lobes). They could confirm, in fibrotic lower lobes, a marked loss of alveolar type I and type 2 epithelial cells and a reciprocal increase in basal, goblet, ciliated and club cells, which comprised honeycomb cysts, as previously described [13]. As expected, the proportion of fibroblasts also significantly increased in fibrotic lobes, with increased expression of multiple matrix genes, in agreement with histological analyses showing increased fibroblast foci.

Remarkably, their data also captured a profound modification within pulmonary macrophages populations during the course of the disease. The importance of specific macrophages subpopulations during fibrogenesis in multiple organs such as heart, skin and lung has been recently highlighted, using both mice models and human samples [14–19]. Morse *et al.* [4] notably show that both normal and IPF lungs contain three discrete macrophages subsets, only one of which has markers of monocyte-derived macrophages, such as FCN1 (FCN1^{hi}). The two other populations correspond to resident-like macrophages, one highly expressing FABP4 and INHBA (FABP4^{hi}) and the other highly expressing SPP1/osteopontin and MERTK (SPP1^{hi}) (figure 1). While the population of monocyte-derived macrophages remained stable between IPF and control lung samples, a switched situation was observed for the two

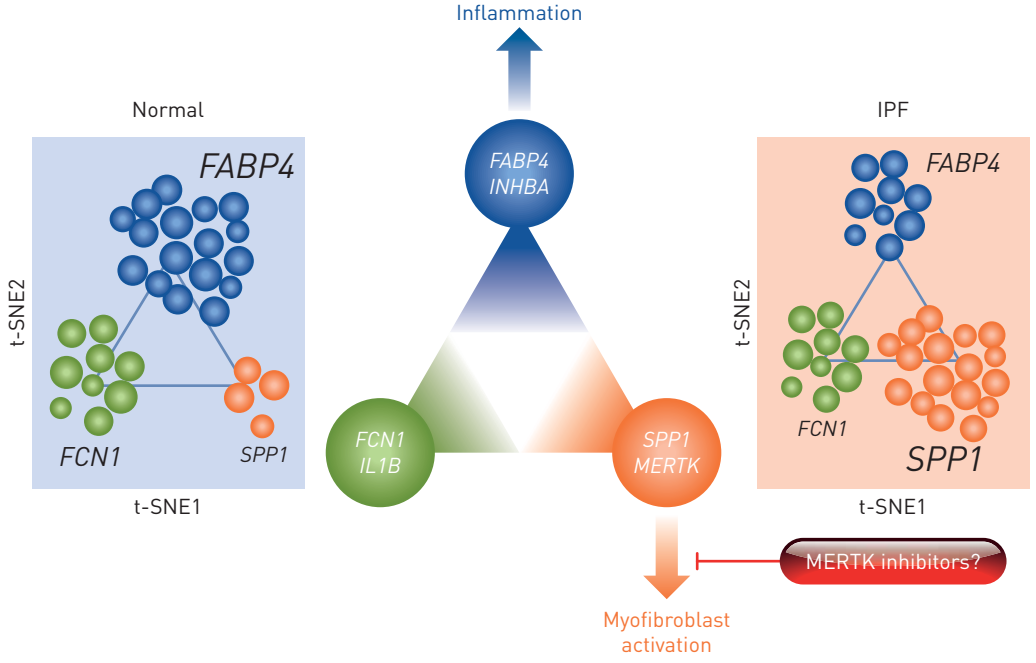

FIGURE 1 Lung macrophage subpopulations in healthy and idiopathic pulmonary fibrosis (IPF) patients. Three macrophage subpopulations identified in lungs from normal or IPF patients are visualised as t-SNE (t-distributed stochastic neighbour embedding) plots. Dysregulated balance of the three populations are associated with disease progression. The authors thank Franck Aguila for artwork.

resident-like subsets during the course of the disease, with a strong increase of $SPP1^{hi}$ macrophages in fibrotic lower lobes (figure 1). Based on several previous studies showing the key profibrotic function of osteopontin in IPF and mice model of pulmonary fibrosis [20, 21], these data strongly point $SPP1^{hi}$ macrophages to a profibrotic myeloid cell subset. This hypothesis is supported by additional immunofluorescence imaging data showing a marked deposition of osteopontin in the IPF matrix and co-localisation of highly proliferative $SPP1^{hi}$ macrophages with fibroblast foci in lower, fibrotic lobes. Moreover, the elevated expression of MERTK, a member of the MER/AXL/TYRO3 receptor kinase family, in $SPP1^{hi}$ cells suggest a specific key function of this kinase in cell activation and survival. As MERTK inhibition has been associated with myeloid cells survival [22], the authors suggest that MERTK inhibitors might deplete profibrotic macrophage and fibroblast activation in IPF lungs (figure 1).

The study reported by Morse *et al.* [4] is partly overlapping another very recent study published by Reyfman *et al.* [16]. Both studies pointed to an increase in $SPP1^{hi}$ profibrotic macrophages in the IPF lung, but their conclusions differ regarding the presence of this subpopulation in healthy lungs. Indeed, while Reyfman *et al.* [16] present the SPP^{hi} macrophages as a novel population of profibrotic alveolar macrophages arising during fibrogenesis, the data of Morse *et al.* [4] support the notion that this subpopulation is part of the natural heterogeneity of lung macrophages, already present in normal lungs. A comparative analysis of the two datasets, that are both publically available, as well as additional similar studies, should allow a resolution of this important question. Indeed, the presence of SPP^{hi} macrophages in healthy lungs may have important therapeutic implications, indicating that therapeutic strategies targeting monocyte/macrophage migration into IPF lungs are likely to fail, while therapeutics targeting MERTK-mediated macrophage survival would likely show promise for this disease.

As noted by the investigators, further work should be also performed to answer to several key points, notably to decipher the molecular mechanisms responsible for tissue-resident macrophage proliferation in IPF, which may involve CSF-1, IL-4 and/or IL-13. Another important issue concerns the relative low proportion of some specific cell types in scRNA-seq datasets from IPF patient samples, notably mesenchymal cells, due to the selective loss of these cells during tissue digestion. This pitfall is currently preventing a comparison with data obtained from mice models [23, 24] and a deep analysis of the plasticity of specific mesenchymal subpopulations associated with disease progression, including myofibroblasts or lipofibroblasts [25]. Such an analysis may indeed reveal new therapeutic targets involved in myofibroblast activation, including for instance mesenchymal-specific regulators of the TGF-β pathway, as recently proposed [26]. Regarding this specific issue, the use of novel technologies, such as spatial transcriptomics [27], may provide an additional layer of precision by catching the modulation of gene expression in specific niches, including honeycomb cysts and fibroblast foci.

Overall, the outstanding work performed by Morse *et al.* [4] is already contributing to a better understanding of the pathogenesis of this deadly disease and has paved the way for further single cell studies that will expand our knowledge about the multiple changes in cellular states and altered cell–cell interactions occurring during the progression of the disease.

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