The concept of Sfrp1$^+$ transitional fibroblasts: the key to dissociating lineage heterogeneity and fate of invasive fibroblasts in pulmonary fibrosis?

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To the Editor:

One of the significant advances in the biology of idiopathic pulmonary fibrosis (IPF) has been the recognition of fibroblast heterogeneity in the lung. Fibroblast heterogeneity can be interpreted as fibroblast subtypes, probably derived from distinct mesenchymal lineages, as well as various activation states, such as proliferation, matrix production and invasiveness. With great interest, we read the original work by Mayr *et al.* [1] presenting a concept that the Sfrp1$^+$ transitional fibroblasts with low invasive capacity emerge early after bleomycin-induced injury and ultimately transit to Spp1/Cthrc1$^+$ matrix-producing (myo)fibroblasts with the driving force of transforming growth factor (TGF)β1 signalling from myeloid and epithelial lineages. This study largely aligns with our recent publication proposing that multiple fibroblast subtypes from IPF lungs contribute to the invasive phenotype of fibroblasts and the matrix deposition in pulmonary fibrosis [2].

IPF is a progressive lung disease of unknown cause without effective therapeutic approaches and is histopathologically characterised by destruction of gas-exchanging regions in the lung with excessive fibroblast accumulation and extracellular matrix (ECM) production [3]. The mechanisms that regulate severe pulmonary fibrosis in IPF remain incompletely understood. One of the latest conceptual advances is the emergence of an invasive mesenchymal phenotype that appears to drive severe fibrosis [4–6]. We have identified new mechanisms that regulate the function of IPF invasive fibroblasts. Importantly, intervening at several distinct points with the common theme of inhibiting fibroblast invasion ameliorates progressive pulmonary fibrosis [4, 7, 8]. Recent studies utilising single-cell RNA sequencing (scRNA-seq) identified a metastatic signalling parallel between IPF fibroblast invasion and cancer cell metastasis, opening additional potential therapeutic strategies in IPF [2, 8].

SFRP1 is a biphasic modulator of WNT signalling [9] and recent studies have identified additional functions unrelated to WNTs [10]. This may theoretically address the potential caveats Tsoyi and Rosas [11] raised, that SFRP1 exhibited significant complexity in either pro-fibrotic or anti-fibrotic cellular processes in different models of organ injury and fibrosis. To complement the data concerning the expression of SFRP1 in invasive fibroblasts from human IPF lungs, we revisited the scRNA-seq [8] and found significantly increased RNA levels of SFRP1 in primary fibroblasts from IPF compared to those from healthy lungs (figure 1a), while in the IPF lungs SFRP1 is notably downregulated in the invasive fibroblasts (figure 1b and c). These data suggest a striking accumulation of SFRP1$^+$ transitional fibroblasts in human IPF lungs, and in a high TGFβ1 microenvironment [12] the SFRP1$^+$ noninvasive fibroblasts may have the potential to undergo transition into invasive fibroblasts.

Another valuable resource this study shares is a longitudinal scRNA-seq database on murine lung fibroblasts at different injury stages, including healthy, early injury, fibrogenic and resolution phases. Leveraging this database, the investigators observe a transient peak of the rates of the Sfrp1$^+$ transitional fibroblasts early after injury (day 3). This timeframe, traditionally believed to be a stage at which epithelial cell injury and acute inflammation occur [13], has rarely been reported for changes in mesenchymal components. Expanding on these concepts, this study brings forth another point of interest: what are the

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The novel concept of Sfrp1$^+$ transitional fibroblasts has sparked novel points of interest: the mechanisms under which the Sfrp1$^+$ transitional fibroblasts emerge and the *in vivo* functions of Sfrp1 and Sfrp1$^+$ transitional fibroblasts in pulmonary fibrosis https://bit.ly/4aq6iAI

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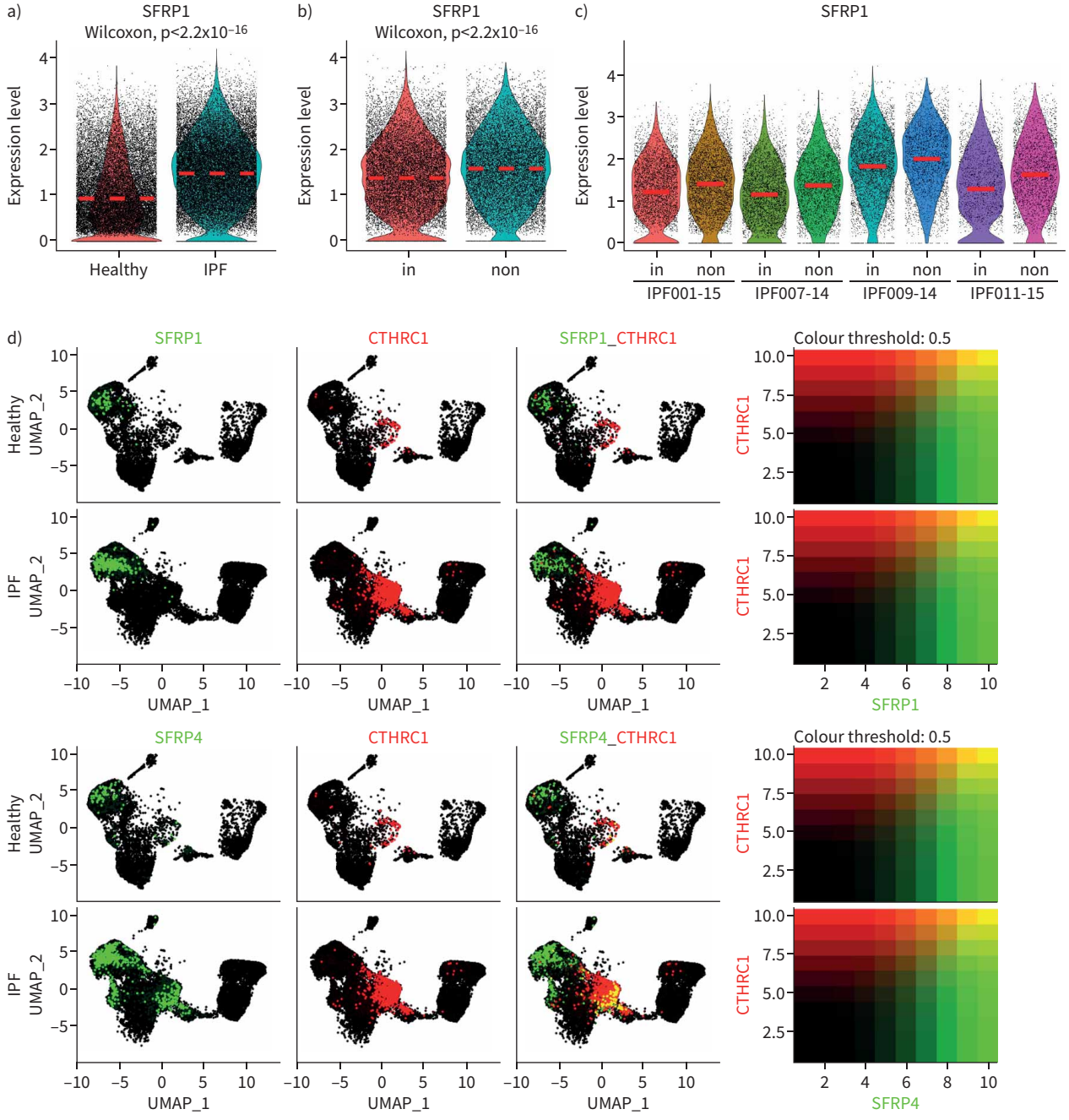
a)
SFRP1
Wilcoxon, p<2.2x10−16
Expression level
Healthy
IPF
b)
SFRP1
Wilcoxon, p<2.2x10−16
Expression level
in
non
c)
SFRP1
Expression level
in non in non in non in non
IPF001-15 IPF007-14 IPF009-14 IPF011-15
d)
SFRP1
CTHRC1
SFRP1_CTHRC1
Colour threshold: 0.5
Healthy UMAP_2
IPF UMAP_2
UMAP_1
CTHRC1
SFRP1
SFRP4
SFRP4_CTHRC1
e) Lineage tracing: Sfrp1-CreER^{T2}; ROSA$^{mT/mG}$
Bleomycin
Immune
Endothelium
Epithelium
Tgfβ1
Adventitial fibroblast
Lipofibroblast
Peribronchiolar fibroblast
Pericyte
Sfrp1+ transitional
Cthrc1+ fibroblast
Tamoxifen
Invasion
ECM
Normal
Early injury
Fibrogenesis
Resolution

FIGURE 1 Downregulated SFRP1 expression in invasive fibroblasts from idiopathic pulmonary fibrosis (IPF) lungs. Transcriptional levels of SFRP1 in primary fibroblasts from lungs of IPF patients and healthy donors (a) and in invasive (in) *versus* noninvasive (non) fibroblasts from IPF lungs (b and c) by single-cell RNA sequencing (scRNA-seq) analysis. d) Transcriptional levels of SFRP1/CTHRC1 and SFRP4/CTHRC1 in published integrated scRNA-seq analysis (integration of GSE136831, GSE157376, GSE128169, GSE135893, GSE128033, GSE122960 and GSE132771) on fibroblasts from lungs of IPF patients and healthy donors by blended feature plots. e) Graphical illustration of the lineage tracing on Sfrp1^{+} transitional fibroblasts in murine lung at different stages of bleomycin-induced injury/fibrogenesis. TGF: transforming growth factor; ECM: extracellular matrix.

signals driving the emergence of the Sfrp1^{+} transitional fibroblasts? Do they require TGFβ signalling? Or WNT signalling from injured epithelial or inflammatory components, given the role of Sfrp1 in modulating canonical WNT signalling?

The Cthrc1^{+} collagen-producing fibroblast subpopulation was initially identified by the Sheppard group and is suggested to be the major contributor of pathological ECM in fibrotic lungs [14]. RNA velocity and pseudotime trajectory analysis suggested Cthrc1^{+} fibroblasts might principally differentiate from alveolar fibroblasts, characterised by Col1a1^{low}/Acta2^{-} [14], the similar features of Sfrp1^{+} transitional fibroblasts. Thus, it would be intriguing to determine whether the Sfrp1^{+} transitional fibroblasts correspond to or partially overlap with the pathogenetic alveolar fibroblasts. *In silico* analysis is helpful to predict fate probability of the transition from Sfrp1^{+} transitional fibroblasts to Cthrc1^{+} collagen-producing fibroblasts. A more robust approach to validate this is to search for shared signatures of these two subpopulations, for example SFRP4 (figure 1d), that have been preserved during the development of fibrosis in IPF lungs [2]. Future studies will greatly benefit from verifying this transition using lineage-tracing murine models (figure 1e). In addition, given the critical role of Sfrp1 in maintaining the low invasive properties of lung fibroblasts, it would be interesting to investigate the *in vivo* functions of this gene in pulmonary fibrosis *via* targeted mutated or conditional knockout murine models.

In summary, the investigators present compelling evidence regarding potential roles of injury-induced Sfrp1^{+} transitional fibroblasts in murine lungs and their capacities to transit into highly invasive ECM-producing Cthrc1^{+} fibroblasts. We are particularly intrigued by and support the investigators' additional studies aiming at delving into the mechanisms under which the Sfrp1^{+} transitional fibroblasts emerge right after injury and the *in vivo* functions of these transitional fibroblasts in fibrosis. Further investigations may enhance our understanding of the relationship between Sfrp1^{+} transitional fibroblasts and the development of the invasive fibroblasts within the fibrotic lungs, and thus provide more precise approaches to regulating fibroblast invasion and impacting progressive lung fibrosis.

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References

1 Mayr CH, Sengupta A, Asgharpour S, *et al.* Sfrp1 inhibits lung fibroblast invasion during transition to injury-induced myofibroblasts. *Eur Respir J* 2024; 63: 2301326.
2 Liu X, Dai K, Zhang X, *et al.* Multiple fibroblast subtypes contribute to matrix deposition in pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2023; 69: 45–56.
3 Noble PW, Barkauskas CE, Jiang D. Pulmonary fibrosis: patterns and perpetrators. *J Clin Invest* 2012; 122: 2756–2762.
4 Li Y, Jiang D, Liang J, *et al.* Severe lung fibrosis requires an invasive fibroblast phenotype regulated by hyaluronan and CD44. *J Exp Med* 2011; 208: 1459–1471.

5 Ahluwalia N, Grasberger PE, Mugo BM, *et al.* Fibrogenic lung injury induces non-cell-autonomous fibroblast invasion. *Am J Respir Cell Mol Biol* 2016; 54: 831–842.

6 Chen H, Qu J, Huang X, *et al.* Mechanosensing by the α_6-integrin confers an invasive fibroblast phenotype and mediates lung fibrosis. *Nat Commun* 2016; 7: 12564.

7 Geng Y, Liu X, Liang J, *et al.* PD-L1 on invasive fibroblasts drives fibrosis in a humanized model of idiopathic pulmonary fibrosis. *JCI Insight* 2019; 4: e125326.

8 Liu X, Geng Y, Liang J, *et al.* HER2 drives lung fibrosis by activating a metastatic cancer signature in invasive lung fibroblasts. *J Exp Med* 2022; 219: e20220126.

9 Liang CJ, Wang ZW, Chang YW, *et al.* SFRPs are biphasic modulators of Wnt-signaling-elicited cancer stem cell properties beyond extracellular control. *Cell Rep* 2019; 28: 1511–1525.

10 Nathan E, Tzahor E. sFRPs: a declaration of (Wnt) independence. *Nat Cell Biol* 2009; 11: 13.

11 Tsoyi K, Rosas IO. Fibroblast heterogeneity in pulmonary fibrosis: a new target for therapeutics development? *Eur Respir J* 2024; 63: 2302188.

12 Frangogiannis N. Transforming growth factor-β in tissue fibrosis. *J Exp Med* 2020; 217: e20190103.

13 Liang J, Zhang Y, Xie T, *et al.* Hyaluronan and TLR4 promote surfactant-protein-C-positive alveolar progenitor cell renewal and prevent severe pulmonary fibrosis in mice. *Nat Med* 2016; 22: 1285–1293.

14 Tsukui T, Sun KH, Wetter JB, *et al.* Collagen-producing lung cell atlas identifies multiple subsets with distinct localization and relevance to fibrosis. *Nat Commun* 2020; 11: 1920.