Strapline: Inflammation

Tracing the origins of lung fibrosis

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Standfirst
A lung specific fibroblast that normally provides a niche for alveolar cells is found to be the predominant source of emergent inflammatory and fibrotic fibroblast subsets with significant implications for the treatment of lung diseases.

Main text
Fibrosis is a form of wound healing in which connective tissue and extracellular matrix replaces the normal functional parts of an organ. Unchecked it can lead to organ failure that in the case of the lung, heart, kidney and liver can only be cured by organ transplantation. Until now a detailed description of cellular interactions and pathways by which fibrosis starts and becomes established have remained elusive. Writing in Nature, Tsukui et al [1] show how two long suspected culprits in the development of lung disease contribute to pulmonary inflammation and fibrosis.

The lung, like all organs, illustrates how form follows function. It is comprised of distinct cell types, some of which are unique to the lung, for example alveolar epithelial cells, and others like fibroblasts and endothelial cells are found in all organs. The specific cell types that are unique to each organ are collectively termed the parenchyma. The mesenchyme describes embryonic connective tissue shared between organs that gives rise to all other connective tissues of the body during early development. Within the lung, distinct anatomical zones or niches exist whose role is to facilitate the specific functional features of the lung, such as the movement of air from the larynx via the bronchus to the alveolus where pulmonary gas exchange takes place. However, it has remained unclear precisely what role each of the individual cell types within the lung (for example endothelial, epithelial and hematopoietic cells) play in driving inflammation and fibrosis and how a long-suspected cell type (fibroblast) and growth factor (TGFβ) are mechanistically involved in driving inflammation and fibrosis in the lung (Fig 1A).

To address this question two technical advances had to occur. The first was the ability to analyze cell subsets from digested tissue one cell at a time (single cell RNA sequencing: scRNA). The second was an ability to identify and specifically delete genes and cell types in a lung specific manner using genetic mouse models. This way, the organ specific drivers of fibrosis and inflammation that predominantly affect parenchymal tissue can be separated from those in the mesenchyme.

By using scRNA sequencing techniques this same research group previously found that in the normal healthy mouse lung not all fibroblasts are the same, with distinct subsets of fibroblasts located at different anatomic locations. For example, one subset was found close to alveolar epithelial cells, while others were located between the blood vessels and the surrounding space (adventitial fibroblasts) or in the parabronchial region [2]. As has been observed at other sites, such as the synovial joint [3], this suggested to the authors that not all fibroblast subsets in the lung have the same function. The problem was how to identify and track the linage relationships between these different subsets since all the current mouse models could not discriminate between lung specific, or indeed alveolar specific, subsets.

Using standard mouse transgenic approaches, the research team generated a new alveolar fibroblast specific reporter mouse (Scube2-CreER) that they used to conditionally fate map or delete alveolar as opposed to other lung fibroblasts. They found that Scube2 marks alveolar fibroblasts but no other fibroblast subsets in the lung, and that this fibroblast subtype co-located with alveolar type 2 epithelial (AT2) cells. When the Scube2 alveolar fibroblasts were ablated using a genetic cell deletion strategy, the alveolar supportive niche was disrupted. Following a bleomycin lung challenge, the alveolar fibroblast ablated mice died of IL17A-mediated lung disease.

Next, the researchers wanted to explore the fate of these alveolar fibroblasts after lung injury. By crossing their Scube2-CreER mice with mice that expressed tdTomato in which they induced injury, they noticed the emergence of new subsets of fibroblasts most of which originated from the Scube2 alveolar fibroblasts. Further characterization of these emergent fibroblasts suggested that they differentiated into profibrotic fibroblasts marked by Cthrc1. To ensure that these observations were not just a specific feature of the bleomycin challenge, the team did similar experiments using a model of intratracheal silica administration. Reassuringly, they found that alveolar fibroblasts were again the major source of both inflammatory as well as fibrotic fibroblasts that formed around the silica nodules. As the research team had discovered the gene modules that define inflammatory compared to fibrotic fibroblast cell states, they next tested if a range of proinflammatory cytokines could induce the expression of inflammatory fibroblast markers in vitro. While the proinflammatory IL1a induced an inflammatory fibroblast state treatment with TGFβ1 antagonized the expression of inflammatory markers, and in contrast drove a fibrotic fibroblast signature.

While most of the work the team describe was conducted in mice models, the obvious issue remains of how generalizable these findings are to human disease where exposure to environmental toxins and stimuli are clearly different to those in caged inbred mice colonies. Using scRNA sequence data from human lungs the authors evaluated whether a similar linage of alveolar, inflammatory and fibrotic fibroblasts could be inferred. They identified similar but not identical inflammatory and fibrotic subsets and showed using histological methods where these subsets were located human fibrotic lung disease. Finally, in an elegant bioinformatic exploration of the lineage relationship between alveolar, inflammatory, and fibrotic fibroblasts, the authors

suggest that, when injured, alveolar fibroblasts develop into inflammatory fibroblasts from which fibrotic fibroblasts subsequently emerge (Fig 1B).

Many studies have shown a key role for the Collagen triple helix repeat containing 1 protein (Cthrc1) and the cytokine TGFβ in fibrosis and remodeling [4]. Therefore, to support the central conclusions of their work that Scube2 alveolar fibroblasts are the origin of both inflammatory and fibrotic fibroblast subsets in the lung, the authors next explored the profibrotic function of Cthrc1 fibroblasts and the cytokine TGFβ. They created mice in which Cthrc1 cells could be deleted and fate mapped, as well as mice in which TGFB signaling could be prevented specifically in alveolar fibroblasts. Attempts to fully delete Cthrr1 cells were not as successful as the researchers hoped (about 50% efficiency), which meant that it was not possible to fully exclude a role for Cthrc1 positive fibroblasts in the development of lung fibrosis. However, preventing TGFβ signaling in alveolar fibroblasts abrogated fibrosis but exacerbated lung inflammation. This provides further support for the idea that alveolar fibroblasts differentiate into inflammatory fibroblasts during the initial phase of lung injury, which is normally reversed by TGFβ. If this transition back to homeostasis is not achieved, the excessive exposure of inflammatory fibroblasts to TGFβ results in the establishment of persistent fibrosis.

As the authors acknowledge, some caveats apply to their findings. Their data suggests that an organ specific parenchymal alveolar fibroblast (Scube2), rather than cross organ universal fibroblast, gives rise to most of the emergent fibroblast subsets following lung injury. Recent work has proposed that a universal fibroblast subset marked by high expression of the gene encoding for peptidase inhibitor 16 (Pi16), located in the perivascular space, is responsible for fibrosis across many organs [5]. One interesting possibility that might reconcile these discrepancies is that there are at least two fibroblast origins for fibrosis; one that is tissue specific and arises from an organ specific fibroblast (parenchymal fibrosis) and another that is shared across many organs and arises from perivascular universal fibroblasts (mesenchymal fibrosis). Whether fibrotic fibroblasts in other organs also have their origins from tissue specific parenchymal organs and whether pan organ universal mesenchymal fibroblast subsets can also contribute to fibrosis will require further investigations.

Perhaps the two most exciting implications of these findings lie in the clinical implications that flow from these observations. The first is the suggestion that the sequential linage transition from resting to profibrotic fibroblast subsets occurs via an inflammatory intermediate. If correct, this is an important finding, as it provides an elegant cellular explanation for the link between inflammation and fibrosis in the lung. It also predicts that the early aggressive treatment of the inflammatory phases of lung disease might prevent the subsequent development of fibrosis. The second is that not all fibrosis is the same, such that there may be forms of fibrosis that are shared across all organs originating from common perivascular subsets of fibroblasts, as well as those that are organ specific and relevant to lung, kidney heart and liver only. If these tissue-specific fibroblast subsets could be targeted without affecting other universal fibroblast subsets, it might be possible to treat fibrosis in a completely organ specific manner, a holy grail in the field of fibrosis.

Competing interests
CDB declares that he is a founder of Mestag Therapeutics. KM has no competing interests

Reference list:
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3. Croft A et al Nature *Nature* **570**, 246–251 (2019).
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Figure 1. The cellular basis of lung inflammation and fibrosis
(A) Changes in cell types and structure between the healthy and diseased lung. (B) Tsukui et al show that alveolar fibroblasts specific to the lung are the source of both inflammatory and fibrotic fibroblasts. How this new parenchymal pathway intersects with the established pan organ mesenchymal route to fibrosis from the universal fibroblast found around blood vessels remains to be discovered.