News & views

Immunology

Fibroblast cells reveal their ancestry

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Cells called fibroblasts can boost health yet also drive disease. Cell-lineage analysis has unveiled the first comprehensive atlas of fibroblasts from various healthy and diseased tissues, a result that has major clinical implications. See p.575

Fibroblasts are cells that are easily identified by their distinctive spindle shape, a characteristic that delineates them from other structural cells of tissues, such as epithelial cells. They are a diverse group of cells with a multifaceted role in health and disease: they help to define tissue architecture by producing the extracellular-matrix material that surrounds cells, they aid the functioning and positioning of other cell types and, after injury, they promote healing or drive inflammation and scarring.

Innovations in single-cell RNA-sequencing technology have provided evidence that fibroblasts consist of functionally distinct populations, which differ according to their tissue of origin and the disease with which they are associated[1,2]. Moreover, even in a single tissue, not all fibroblasts are the same. Discrete, non-overlapping subtypes drive different aspects of the many biological functions assigned to these cells[3]. On page 575, Buechler *et al.*[4] report a cross-tissue comparative atlas of fibroblast gene expression that reveals the general organizing principles of the fibroblast cellular lineage within and across organs. This work indicates the existence of universal, specialized and disease-specific subsets of fibroblast, and points to a shared ancestry for these three subtypes.

The family relationships between fibroblasts isolated either from the same or from different tissues has long been an enigma. One reason is that, until the advent of methods to profile RNA in single cells, it was hard to classify fibroblasts into distinct subtypes. All fibroblasts perform similar functions consistent with their lineage, such as making and modifying molecules of the extracellular matrix. Yet they can also execute specialized programs that are suited to the needs of the particular tissues in which they reside. For example, specialized fibroblasts support the development of haematopoietic (blood and immune) cells in the bone marrow. How fibroblasts achieve both general and specialized functions has been unclear.

Haematopoietic cells, such as macrophages of the immune system, solve the problem of being both generalists and specialists by adopting a shared, lineage-wide, core pattern of gene expression, which is then supplemented with tissue-specific gene expression driven by microenvironmental cues[5–7]. Macrophages are replenished from a cell type called a monocyte, which circulates in the blood and acts as a universal reservoir for the production of tissue macrophages. The question unanswered until now is whether fibroblasts follow this macrophage approach, or whether there is an alternative scenario for fibroblasts in a given tissue, in that a 'universal', pan-tissue fibroblast-precursor subset exists alongside a more mature, tissue-specific subset of these cells.

To address this question, Buechler and colleagues first took a bioinformatics approach to generate a cross-tissue atlas of gene expression in mouse fibroblasts, using single-cell RNA studies from data sets across 16 tissues. The authors thereby identified ten distinct clusters of gene-expression profiles, of which two (named the *Pi16* and *Col15a1* clusters) were found in certain cells across nearly all organs surveyed, suggesting that these clusters might represent the transcriptional profiles of universal, pan-tissue fibroblasts (Fig. 1). A more limited set of tissues had fibroblasts corresponding to the eight remaining clusters, raising the possibility that these are hallmarks of specialized fibroblasts, or particular fibroblast states, that are specific for certain tissues.

The ubiquity of the two *Pi16* and *Col15a1* clusters, which included high levels of expression of genes such as *Cd34* and *Ly6a* that are

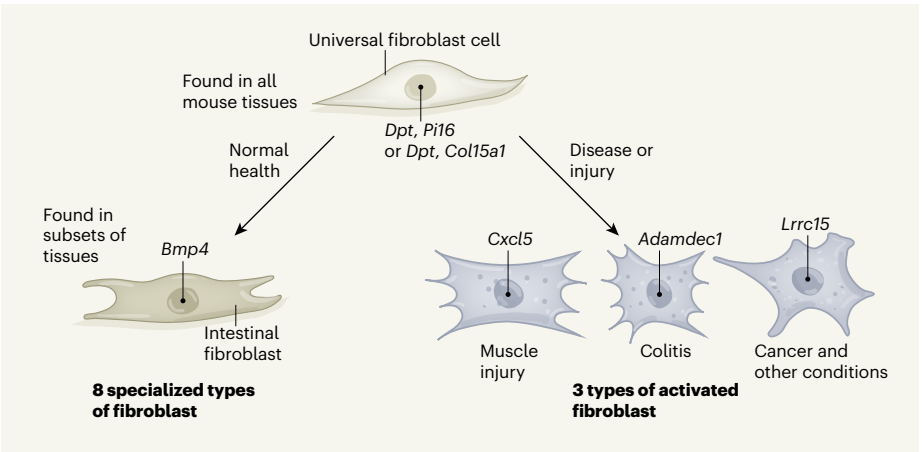

Figure 1 | The organization of fibroblast cells across various organs. Fibroblasts are structural cells that have key roles in the body. Buechler *et al.*[4] report their analysis of the relationship between fibroblasts found in various mouse organs. By analysing gene-expression data sets and using other approaches, the authors report the identification of two 'universal' types of fibroblast cell (that express either the genes *Dpt* and *Pi16* or the genes *Dpt* and *Col15a*), which were found across the different tissues examined. The authors' work indicates that these cells give rise to eight types of specialized fibroblast that are found only in certain normal tissues, such as intestinal fibroblasts that express the gene *Bmp4*. Buechler *et al.* report that the universal fibroblasts also give rise to three types of activated fibroblast in the context of disease or injury. Fibroblasts that express the gene *Cxcl5* are associated with muscle injury, fibroblasts associated with the gut disease colitis express the gene *Adamdec1*, and fibroblasts found in tumours and at sites of inflammation express the gene *Lrrc15*.

associated with stem-cell properties, combined with a bioinformatics technique that can infer developmental relatedness by what is called trajectory analysis, led the authors to propose the following model: that universal and specialized fibroblasts exist side by side in normal, 'steady state' mouse tissues, and that these fibroblasts might be developmentally linked. The locations of fibroblasts expressing the *Col15a1* and *Pi16* clusters suggested that the *Col15a1* cells, which reside in the internal region of a tissue, might regulate the extracellular matrix, whereas the *Pi16* cells, which are found near blood vessels, might act as reservoir cells from which tissue fibroblasts originate.

To test this hunch, Buechler *et al*. set out to find cell-surface markers that could be used to identify *Pi16* and *Col15a1* fibroblasts. This work revealed a set of genes whose expression correlated inversely with fibroblast specialization and that was highly enriched in *Pi16* fibroblasts, and to a lesser extent in *Col15a1* fibroblasts. Of these genes, the authors focused on *Dpt* as a possible marker for *Pi16* and *Col15a1* universal fibroblasts.

Buechler and colleagues engineered mice to express a fluorescent version of the protein encoded by *Dpt*. Genetic analysis and cell-tracking experiments that compared the progeny of universal fibroblasts and tissue-specific fibroblasts provided compelling experimental evidence supporting the bioinformatics data. The authors' results point to the existence of a set of two universal fibroblast populations from which all specialized fibroblasts originate, in a wide range of mouse tissues under physiological conditions.

The authors next investigated whether the fibroblast populations they had discovered changed when tissues were injured or were damaged in disease. They found that, in perturbed tissues, the *Pi16* and *Col15a1* fibroblasts seemed to be universally present and expressed high levels of *Dpt*, consistent with the idea that the *Pi16 Dpt* cell type serves as a reservoir cell. Excitingly, they identified three clusters of gene expression (marked by the genes *Cxcl5*, *Adamdec1* and *Lrrc15*, respectively) that seem to represent perturbation-specific, activated states of fibroblasts not observed in the steady state. Each of these fibroblast clusters was associated with certain organ-specific injuries. For example, the *Cxcl5* cluster was characteristic of muscle injury. Importantly, when *Dpt* reservoir cells in mice were genetically marked before a tumour was transplanted into the animals, they developed into *Lrrc15* cancer-associated fibroblasts, supporting the idea that the universal fibroblasts give rise to activated fibroblasts after injury and inflammation.

Finally, Buechler *et al*. investigated whether human tissue contains universal and activated fibroblast clusters, similar to those identified in mice. The authors investigated a range of tissues (such as pancreas and lung) and diseases (cancer, infection and inflammation). Their results, limited by the number of relevant data sets for human fibroblasts that are publicly available, suggest that, as in mice, an equivalent *Pi16* universal human fibroblast subset exists, as well as five activation subsets, or states, found in disease. Interestingly, although this work in humans confirmed aspects of the authors' results in mice, such as the findings relating to *Lrrc15* fibroblasts, Buechler *et al*. discovered some activated fibroblast subsets not observed in mice, such as those marked by expression of *COL3A1* (observed in COVID-19) or *CCL19* (associated with the gut disease colitis). Of note, *CCL19* and *COL3A1* clusters of gene expression in fibroblasts were found to be associated with disease in another study[8] that focused on common fibroblast subsets across four inflamed human tissues.

Buechler and colleagues' landmark study has far-reaching implications. It establishes the key organizing principles of the fibroblast lineage in health and disease. Unlike macrophages, which, like fibroblasts, act as sentinel cells looking for signs of danger in tissues, the fibroblast lineage is compartmentalized into three major subtypes — universal and specialized (steady-state) subsets, as well as activated (perturbed state) subsets — all of which exist together in the same tissue. The concordance between certain fibroblasts in mice and in humans is particularly relevant, because it indicates that mechanistic studies in mice might have direct relevance for human disease. Moreover, this work provides a resource that will help to clarify nomenclature and boost the precision of identification of specific fibroblast subtypes across tissues. Such progress is urgently needed to standardize the fibroblast subsets that are currently used as cellular therapies to repair tissues in clinical studies.

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As the authors acknowledge, more studies remain to be done. These investigations include defining the anatomical location of fibroblast subtypes, searching for the existence of other subtypes, particularly in human tissue, and trying to identify the cells (possibly structural or immune cells) that might promote the development and differentiation of different types of fibroblast. It is unclear why two universal fibroblast subtypes exist in mice, compared with the single universal haematopoietic progenitor cell. But the authors speculate that having two universal subtypes might be a necessary division of labour for the fibroblast lineage in tissues.

Perhaps the most exciting implications of these findings lie in the clinical dividends that might result from the observation that non-overlapping disease-associated subsets of fibroblasts exist in specific tissues, and that these cells are distinct from other subtypes involved in healthy tissue repair and tissue specialization. If these disease-causing fibroblast subsets could be targeted without affecting the fibroblast subsets associated with health, it might be possible to treat certain inflammatory and malignant diseases in a more targeted and less immunosuppressive manner. Being able to target harmful fibroblasts while sparing beneficial ones and haematopoietic cells would revolutionize the treatment of many chronic diseases.

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