The different facets of myofibroblasts in kidney

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Over 10% of the global population suffers from chronic kidney disease (CKD). The course of the illness is characterized by renal fibrosis, and its severity is inherently linked to clinical outcomes. Unfortunately, there are no available antifibrotic treatments at the moment, largely due to the lack of knowledge about the origin, functional heterogeneity, and regulation of the involved cells. Kuppe et al. systematically mapped the complete human kidney by profiling the transcriptomes of the proximal tubule and non-proximal tubule cells in healthy and fibrotic organs using single-cell RNA sequencing. They characterized approximately 135,000 human and mouse kidney cells during homeostasis and fibrosis, allowing the dissection of heterogeneity of extracellular matrix (ECM)-producing cells at high resolution. The group also developed a single-cell ECM Expression Score based on the expression of genes encoding collagens, glycoproteins, and proteoglycans, and verified a distinct shift toward cells with high ECM expression among patients with eGFR<60mL/min.

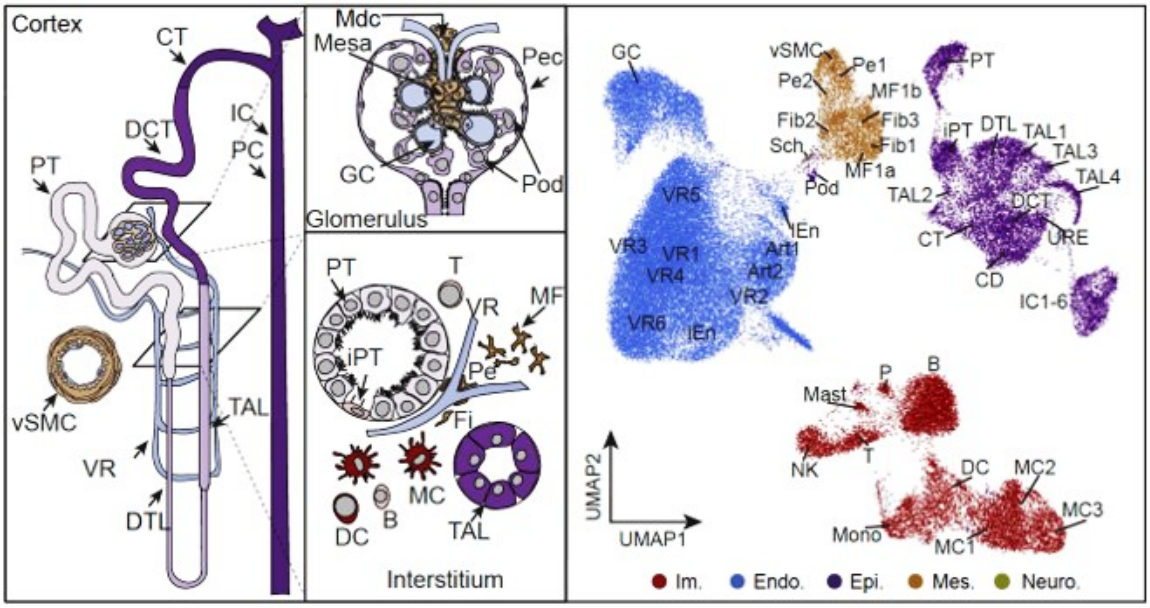

Figure 1. Single-cell map of human kidneys

Mesenchymal cells displayed the highest level of ECM expression, which further increased in CKD. The number of fibroblasts and myofibroblasts also increased in CKD. Myofibroblasts were historically identified by Acta2, but nowadays it is known that these cells express the vast majority of ECM genes. Researchers created a Uniform Manifold Approximation and Projection (UMAP) embedding of (myo)fibroblasts and pericytes to evaluate potential myofibroblast differentiation processes. This embedding was consistent with unsupervised graph clustering, demonstrating the heterogeneity of the renal mesenchyme. Diffusion mapping of mesenchymal cells with strong ECM expression revealed that myofibroblasts originate from distinct pericytes and fibroblast subsets. To structurally analyze these findings, they used genetic fate-tracing, time-course single-cell RNA-sequencing and ATAC-

sequencing experiments in mice, and spatial transcriptomics in human kidney fibrosis. These techniques shed new light at an unprecedented resolution on the origin, heterogeneity, and differentiation of human kidney myofibroblasts, as well as their fibroblast and pericyte precursors. By employing this approach, researchers were able to locate targets more easily, and they discovered naked cuticle homolog 2 (Nkd2) to be a myofibroblast-specific target in human kidney fibrosis. To validate Nkd2 as a therapeutic target, Kuppe et al. generated induced pluripotent stem cell (iPSC) derived kidney organoids containing all major compartments of a human kidney. They found that Nkd2 marks myofibroblasts in kidney fibrosis and is required for collagen expression.

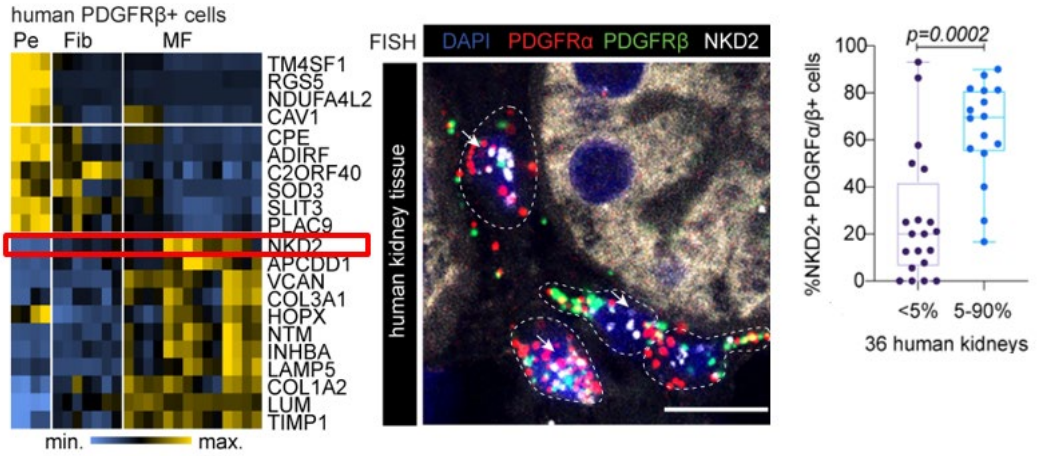

Figure 2. Nkd2 as a potential therapeutic target in kidney fibrosis

Kidney fibrosis and COVID-19

Recent evidence indicates that kidneys are often affected in COVID-19 patients, regardless of the severity of the disease. Even more so, patients who develop AKI are more likely to retain chronic kidney functional impairment after severe disease. The research group led by Jitske Jansen reported that SARS-CoV-2 directly infects kidney cells and is associated with increased tubule-interstitial kidney fibrosis in patient autopsy samples. Furthermore, data indicate that COVID-19 patients show increased tubulointerstitial fibrosis compared to patients with non-COVID-19-related acute respiratory distress syndrome (ARDS) or age-matched non-SARS-CoV-2-infected individuals, independent of pre-existing CKD prevalence. This finding may suggest that patients with severe COVID-19 may be at risk of chronic renal function decline in the future, as kidney fibrosis is a hallmark of CKD. Based on the observation that the infected proximal tubule cells secrete pro-inflammatory ligands that activate fibroblasts, it was attempted to model this process in vitro to exclude immune cell effects. To study direct viral effects on the kidney independent of systemic effects of COVID-19, researchers infected human-induced pluripotent stem-cell-derived kidney organoids with SARS-CoV-2. Using immune-based correlative light and electron microscopy (CLEM), viral particles were detected based on the corresponding SARS-CoV-2 nucleocapsid protein fluorescent signal. Single-cell sequencing confirmed that infected proximal tubule epithelial cells activate mesenchymal cells with pro-inflammatory pro-fibrotic ligands. The fibrosis observed in the organoids is transforming growth factor β (TGF-β)-dependent, and using TGF-β superfamily type 1 receptor inhibitors (ALK5, ALK7) contributes to the cessation of fibrosis caused by the virus. To test the prevention of viral uptake by kidney epithelial

cells, researchers analyzed a novel non-covalent inhibitor of the SARS-CoV-2 main protease concluding that it might reduce SARS-CoV-2 viral replication in kidney cells.

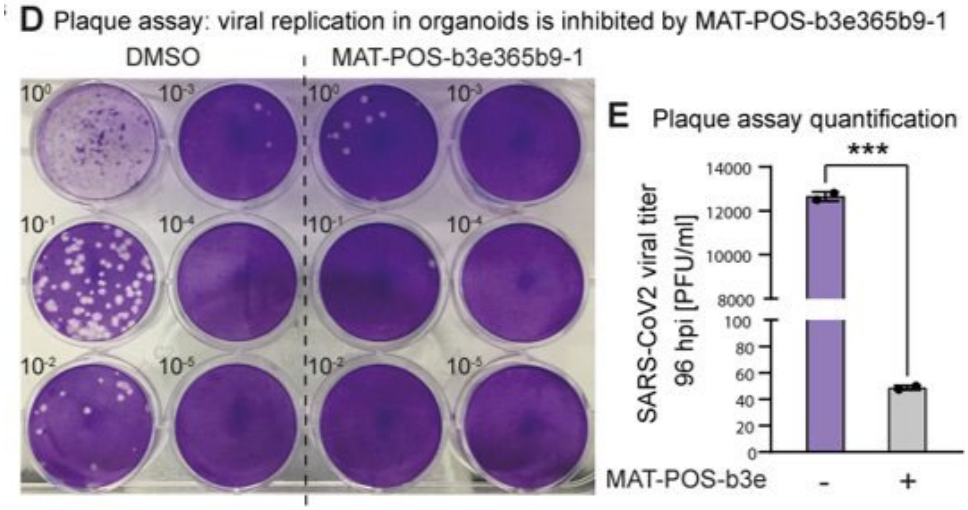

Figure 3. Novel SARS-CoV2 protease inhibitor can block the infection of kidney organoids

Fibroblasts and myofibroblasts differentiation in the heart

Current research is also directed on myofibroblasts differentiation in organs other than kidneys. Kuppe et al. generated an integrative high-resolution map of human cardiac remodeling after myocardial infarction using single-cell gene expression, chromatin accessibility, and spatial transcriptomic profiling of multiple physiological zones at distinct time points in myocardium from patients with myocardial infarction and controls. Results showed cell populations similar to those in the kidney. Interestingly, fibroblasts represent the vast majority of myofibroblasts, while pericytes almost have very little contribution to cardiac scarring. The underlying mechanisms, that pertain both to the kidney and the heart, show an increased expression of transcription factor associated with hematopoiesis, Runx1, in cells differentiating towards myofibroblast population. The assay for transposase-accessible chromatin with sequencing (ATAC-Seq) shows an increased transforming growth factor beta (TGF-β) signaling and a significant Runx1 activity, similar to the kidney. Experiments on mouse kidneys demonstrate that Runx1 is the most active transcription factor in the fibroblast's differentiation to myofibroblasts. To validate this, researchers used isolated and immortalized human platelet-derived growth factor receptor beta (PDGFR-β) kidney and overexpressed Runx1 with the lentivirus. Interestingly, the overexpression of Runx1 slows down the cycling, and cells begin to differentiate towards myofibroblasts. Using machine learning tools that analyze the spatial transcriptomic data, the research found that, in the spatial context, the presence of certain macrophage subsets predicts the presence of specific myofibroblasts. This spatial data can be used to understand cellular crosstalk, and therefore gain more insight into the mechanisms behind the disease.

Key points

1. Distinct pericyte and fibroblast populations are the major source of myofibroblasts in the human kidney.
2. Naked cuticle homolog 2 (Nkd2) was found to be a myofibroblast-specific target in human kidney fibrosis.
3. Severe covid-19 is associated with kidney fibrosis; SARS-CoV2 infects the human kidney and drives fibrosis in organoids.

Further reading

(1) Kuppe C, Ibrahim MM, Kranz J, et al. Decoding myofibroblast origins in human kidney fibrosis. Nature. 2021;589(7841):281-286. doi: 10.1038/s41586-020-2941-1.
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(3) Bowe B, Xie Y, Xu E, Al-Aly Z. Kidney Outcomes in Long COVID. J Am Soc Nephrol. 2021;32(11):2851-2862. doi: 10.1681/ASN.2021060734.
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(5) Kuppe C, Ramirez Flores RO, Li Z, et al. Spatial multi-omic map of human myocardial infarction. Nature. 2022;608(7924):766-777. doi: 10.1038/s41586-022-05060-x.
(6) Li Z, Kuppe C, Ziegler S, et al. Chromatin-accessibility estimation from single-cell ATAC-seq data with scOpen. Nat Commun. 2021;12(1):6386. doi: 10.1038/s41467-021-26530-2.