Interstitial renal fibrosis
Chronic kidney disease (CKD) prevalence approaches 10% in the western world. The rising incidence of diabetes and hypertension together with demographic changes (population aging) will lead to further increase of CKD and end-stage renal disease (ESRD) prevalence. CKD is accompanied by substantial changes in renal structure, notably matrix deposition and scar formation within the kidney interstitium. Structural changes in the renal interstitium (i.e. interstitial kidney fibrosis) correlate better with loss of function than glomerular changes.\textsuperscript{1-3} Inhibition of kidney fibrosis therefore has been proposed as a strategy to slow down progression of CKD. Remarkably, there is currently no approved drug to treat kidney fibrosis.\textsuperscript{4} While it has been widely accepted that myofibroblasts are the fibrosis causing scar forming cells, the cellular origin of these cells has been a matter of huge controversy over the last decades. While consensus is building that tubular epithelial cells do not contribute significantly (if at all) to the myofibroblast pool various, the role of other sources e.g. endothelial cells, resident stromal cells (pericytes and/or fibroblasts) and circulating bone marrow precursors (fibrocytes, mesenchymal stem cells - MSC) is still heavily debated. Kramann and colleagues have recently identified a novel myofibroblast progenitor population, which is defined by the expression of the Hedgehog transcriptional activator Gli1 in adult homeostasis, and their data indicates that these cells significantly contribute to the myofibroblast pool responsible for kidney fibrosis and that they are a novel therapeutic target.\textsuperscript{5,6}

\textbf{Did you know that} it has been estimated that 50% of all deaths in the industrialized world are associated with fibrotic disease? Matrix deposition and fibrosis plays an important role during disease in various tissues such as liver cirrhosis, heart failure, CKD, vascular sclerosis, atherosclerosis and myeloproliferative neoplasms (MPN), among others. While fibrosis also plays an important role in injury-repair, for example during wound healing and after myocardial infarction, it has been appreciated that overwhelming fibrosis might be a disease by itself, that slowly disrupts organ function. There is currently no approved drug to specifically treat fibrosis in major organs as kidney, heart and liver. Biotech and pharmaceutical companies have overlooked this disease space for many years, until very recently. In fact over the last couple of years fibrosis has become one of the hottest sectors in biotech with multiple acquisitions (e.g. Arresto by Gilead, Amira by BMA, Excaliard by Pfizer, Stromedix by Biogen). There is hope that this recent interest in fibrosis will lead to novel therapeutic options to slow down progression of CKD. Recent therapeutic developments in fibrosis have been reviewed previously. \textsuperscript{4,7,8}
Myofibroblasts (red) in a fibrotic mouse kidney 10 days after unilateral ureteral obstruction (UUO)

**Bibliography**

Meet the expert – discussion of two recent papers
Comment prepared by Rafael Kramann


**Background and rationale of the studies:**
While studying Hedgehog signalling we observed that the Hedgehog transcriptional activator Gli1 is specifically expressed in a perivascular cell population. These Gli1+ cells were residing in the pericyte niche with direct contact to endothelial cells, but also in the adventitia of arteries with distance to the endothelium. Over a century ago it has been described that after organ injury matrix deposition and scar formation emanates from the vasculature, and pericytes have been controversially discussed as a cellular origin of fibrosis. This prompted us to perform in vivo fate tracing and ablation experiments to study the role of Gli1+ perivascular cells in kidney injury and repair. In a second study we sought to unravel the role of Hedgehog-Gli signalling in this cell population as a therapeutic target in kidney fibrosis and CKD.

**Main results:**
We used transgenic mouse models to label Gli1 cells in the adult mouse. Our data indicates that Gli1 is specifically expressed in a sub fraction of the renal PDGFRβ+ interstitial cells with increased colony forming unit capacity, trilineage differentiation capability and a typical mouse mesenchymal stem cell (MSC) surface profile. Gli1+ cells reside in the pericyte niche and in the adventitia of large arteries across tissues studied (muscle, heart, kidney, lung, liver, bone-marrow) and they expand dramatically upon organ injury to become scar forming myofibroblasts. In two mouse kidney fibrosis models,
about 50% of the renal myofibroblast population was derived from Glil1+ MSC-like cells and up to 65% of myofibroblast in heart fibrosis were derived from this MSC population. Moreover, we also noted that a significant proportion of myofibroblasts during liver and lung fibrosis were derived from the Glil1+ perivascular cell-population.

We next performed bone-marrow transplantation and parabiosis experiments and demonstrated that resident and not circulating bone-marrow derived Glil1+ cells are the origin of myofibroblasts. Genetic ablation experiments using transgenic expression of the human diphtheria-toxin receptor (iDTR) on Glil1+ cells resulted in dramatically reduced fibrosis in kidney and heart and improved organ function.

To understand the role of Gli proteins in Glil1+ cells we performed knockout and overexpression experiments in a second study. We demonstrated that Gli2, but not Gli1 drives myofibroblast cell-cycle progression. Our data suggested that Gli2 could take over Glil1 proteins function after knockout of Glil1 but that Glil1 cannot compensate for Gli2 loss. In UUO mouse models conditional knockout of Gli2 in Glil1+ cells limited kidney fibrosis by induction of a myofibroblast specific G0/G1 cell cycle arrest. Pharmacologic targeting of this pathway with darinaparsin, a novel organic arsenic, or with GANT61, a small molecule Gli antagonist, showed reduced Gli protein levels (Gli1 + Gli2) with induction of a myofibroblast specific cell-cycle arrest and reduced kidney fibrosis. We demonstrated that this novel therapeutic strategy also reduces acute kidney injury (AKI) to CKD progression and improves kidney function late after AKI. Finally, we have demonstrated that Glil1 and Gli2 are up regulated in human kidney fibrosis.

Key messages:

- Glil1+ cells are an MSC-like subpopulation of the PDGFRβ+ interstitial cells.
- Resident but not circulating bone-marrow derived Glil1+ cells are a major source of myofibroblasts in fibrosis
- Glil1+ cell ablation ameliorates fibrosis and rescues organ function
- Knockout of Gli2 but not solely Glil1 in Glil1+ cells induces a cell cycle arrest with subsequently reduced fibrosis
- Pharmacologically targeting Gli proteins halts myofibroblast cell-cycle progression with subsequently reduced fibrosis and CKD progression, and therefore represent a novel therapeutic strategy in kidney fibrosis

Limitations:

Our in vitro and in vivo data was generated using mouse cells and mouse models of CKD and kidney fibrosis. In our recent paper we have demonstrated that the Hedgehog pathway is also activated in human kidney fibrosis, however, it remains unresolved whether Glil1 marks a similar MSC
like myofibroblast progenitor population in human kidneys. Future studies are needed to elucidate whether pharmacologically targeting Gli proteins in human kidney fibrosis reduces interstitial matrix deposition and halts progression of CKD.

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