AKI – CKD transition – is it inevitable?

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Acute kidney injury (AKI) can result in a complete recovery of renal function, but may also be a foundation for the development or progression of chronic kidney disease (CKD). To understand and foster kidney repair mechanisms one cannot rely solely on animal models. Studies on humans are necessary to fully comprehend the decisive events that lead to either positive or negative outcomes. Recent research on human material by Hinze et al. focused on the molecular composition of kidney tissue affected by AKI using post-mortem examination. Kidney biopsies were obtained from eight individuals who succumbed to critical illness associated with severe respiratory infections and systemic inflammation, and severe AKI (stage 2 or 3 within 5 days before sampling). Research also included control biopsies from tumor-adjacent 'normal' tissue of three patients without AKI and post-mortem specimens from one brain-dead individual without AKI taken at 15, 60, and 120 min after cessation of circulation. The tissues were subjected to single-nuclear RNA sequencing and bulk RNA sequencing, as well as immune histochemistry and mRNA expression studies. These technologies allowed the identification of the major cell types in the kidney, including cell sets from every part of the nephron, leukocytes, endothelial cells, and interstitial cell populations. Each of these cell types could be identified by marker genes that are characteristic of that particular cell type. The quality of the RNA obtained was high: analysis identified more than two thousand genes and more than four thousand unique transcripts per cell (median), and there was a clear distinction between the different cell types based on their gene expression pattern.

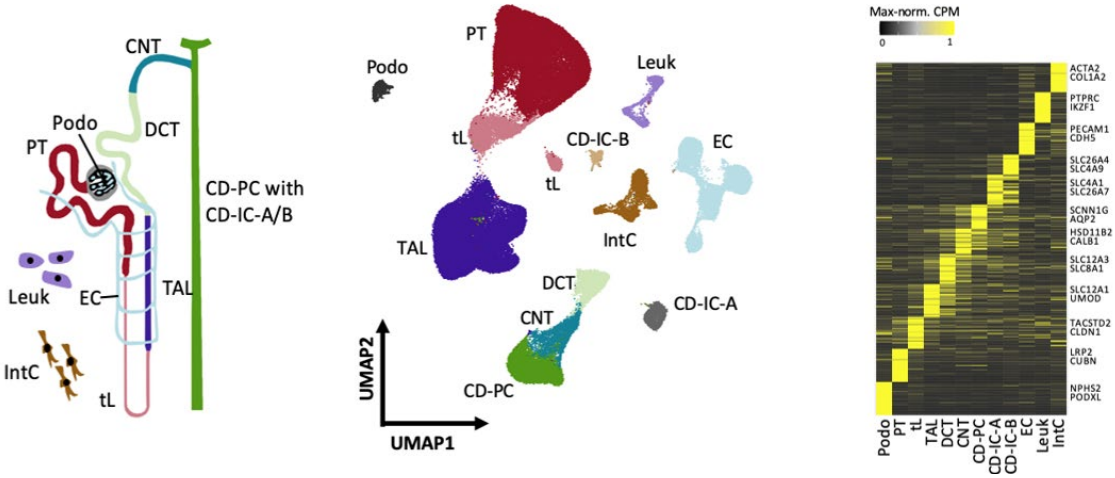

Figure 1. Identification of the major cell types in the kidney with clustering and marker gene expression; UMAP=Uniform Manifold Approximation and Projection

The comparison of kidney cell types between individuals with and without AKI showed that in both groups of samples the largest proportions of cells come from the proximal tubule and the thick ascending limb. Principal component analysis of gene expression in proximal tubular cells showed clear differences between control samples and AKI samples and marked heterogeneity between AKI samples, but no obvious difference between individuals with and without COVID-19.

Differential gene expression analysis showed a substantial change in the gene expression patterns with both downregulation and upregulation of genes in AKI compared to controls. These changes were more profound in the cortex and outer medulla (proximal tubule, thick ascending limb, distal collecting tubule, and cortical collecting tubule) and much less in the inner medulla. The cortex and the outer medulla are very susceptible to hypoxia and ischemic injury, whereas the inner medulla is chronically exposed to hypoxia, and therefore adapted to it. Also, more genes were upregulated rather than downregulated, which pertains to differentially expressed single genes as well. Looking at the list of genes that were found to be either upregulated or downregulated provides additional information on what occurs in the kidney. Upregulated genes frequently related to inflammation, the cellular response to hypoxia and epithelial-mesenchymal transition, whereas the downregulated genes stand for molecular transport and metabolism, which were impaired under the conditions of injury.

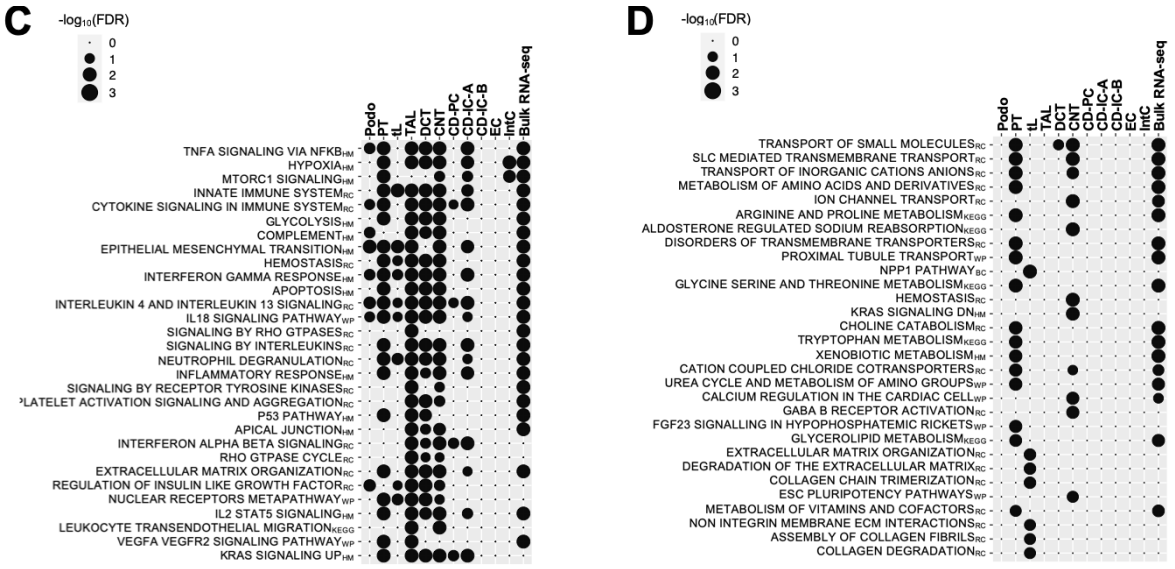

Figure 2. Pathway enrichment analyses on genes upregulated (left) and downregulated (right) in AKI versus control. FDR – false discovery rate.

Considering tubular cells in all parts of the nephron, cell populations that were depleted or enriched in individuals with AKI were identified. In particular there was a marked depletion of cells from the proximal tubule, thick ascending limb, and distal tubule, and new cell types were found that were associated with the same tubular segments and to a lesser extent with other nephron segments. These findings suggest that many cells along the nephron underwent a transformation, which changed their repertoire of gene expression so that they eventually changed to another cell state or cell type. Interestingly, very little change was observed in the interstitial cells, although over time, AKI leads to an expansion of interstitial fibroblasts and inflammatory cells; however, these results could be attributed to the timing of the biopsies.

The new cell populations showed a marked downregulation of canonical marker genes of the respective major cell types. Moreover, they expressed characteristic new marker genes associated with specific activated pathways. For the proximal tubule these new cell populations were significantly enriched in AKI samples. Using selected marker genes and the existence of injured cell states could be validated in proximal tubules and thick ascending limbs.

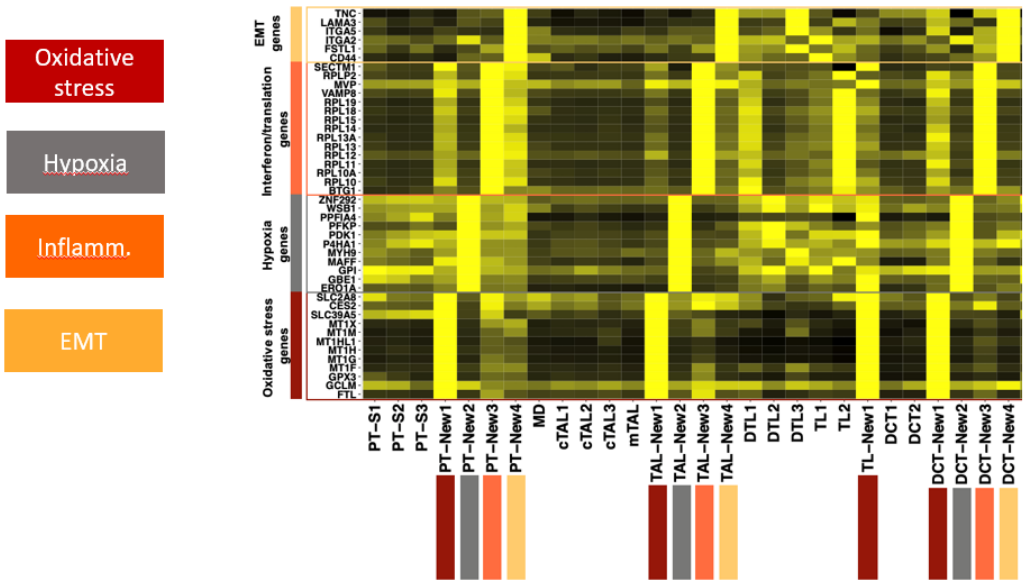

Figure 3. Expression of overlapping marker genes in the injured AKI-associated “New” cell states in PT, TAL and DCT.

Further analysis of the gene expression patterns revealed four main clusters that define four sets of mechanisms: oxidative stress, hypoxia, inflammation and epithelial-to-mesenchymal transition. Comparing these different clusters across different tubular segments revealed marked similarities in marker gene expression, suggesting a rather uniform response to acute injury along the nephron.
In summary this research shows that AKI is a condition that affects tubular cells along the entire nephron. Four response patterns were identified, and the degree and magnitude to which these pathways are activated differ from patient to patient. In the future such information may be helpful in terms of understanding the individual prognosis and guiding personalized AKI management.

Key points

1. mRNA expression is sufficiently stable for up to 120 min post-mortem to allow an analysis of gene expression at single cell resolution in the human kidney.
2. AKI of a maximum of 5 days duration in critically ill patients is associated with profound changes in gene expression along the whole nephron ("pan-nephron insult").
3. Molecular changes associated with AKI are more marked in the cortex and outer medulla.
4. Four dominant injury/response patterns can be delineated: oxidative stress, hypoxia, inflammation, and EMT/failed repair.
5. There were no differences between AKI associated and not associated with COVID-19.

Further reading

(1) Hinze C, Kocks C, Leiz J, et al. Single-cell transcriptomics reveals common epithelial response patterns in human acute kidney injury. Genome Med. 2022;14(1):103. doi: 10.1186/s13073-022-01108-9.
(2) Klocke J, Kim SJ, Skopnik CM, et al. Urinary single-cell sequencing captures kidney injury and repair processes in human acute kidney injury [published online ahead of print, 2022 Aug 29]. *Kidney Int*. 2022;S0085-2538(22)00688-3. doi:10.1016/j.kint.2022.07.032