Identification of surrogate biomarkers reflecting tubular failed repair in CKD

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**Background**

Interstitial fibrosis, tubular atrophy and inflammation (IFTA) are common final pathways to end stage kidney disease (ESKD), contributing to progressive nephron loss and functional decline in most chronic kidney diseases (CKD), including those typically glomerular in origin. Disease-associated failed repair proximal tubule epithelial cells (FR-PTs) have been described in rodent models and are characterized by a proinflammatory and profibrotic phenotype that contributes to IFTA severity. We have recently demonstrated that accumulation of FR-PTs in human disease predicts reduced renal event-free survival in multiple CKD etiologies.

We used multomics analysis of patient-matched kidney biopsies and biofluids from the NURTuRE CKD cohort to discover biomarkers associated with an accumulation of FR-PTs to noninvasively identify patients at risk for disease progression.

**Study Design & Methods**

NURTuRE is a unique prospective cohort study involving 3500 CKD patients that is linked to a biobank of matched patient samples covering a broad range of diagnoses and kidney functional states. A unique multidimensional dataset was generated by combining clinical and histopathological records with multomics analyses of kidney biopsies and biofluids.

A data-driven selection of kidney biopsies (n = 332) from multiple CKD etiologies was analyzed via RNA-Seq and scored for a gene signature reflecting FR-PTs. Patient-matched serum (n = 67) and urine samples (n = 22) were assayed using the Clink and Biomax proteomics platforms, respectively. Correlation analysis of protein abundance with FR-PT patient biopsy scores and kidney mRNA expression suggested candidate noninvasive biomarkers for further validation (r ≥ 0.4 and p ≤ 0.05).

Kidney cell type-specific gene sets were derived from GSE171314 using FindAllMarkers in Seurat. A human failed repair gene signature was derived from marker genes for the FR -PT cluster.

A liver-specific gene set was derived from Human Protein Atlas.

**Results**

**Characterization of FR-PTs in human CKD and identification of a human FR-PT gene signature**

1. FR biopsy score + mRNA correlated, N = 9

**Multomics biomarker discovery from patient biopsy-matched NURTuRE biofluids**

2. Correlation with FR biopsy score

**Serum and urine proteins are correlated with kidney biopsy but not whole blood RNA**

3. Additional patient-matched samples available for validation of biomarker candidates

4. Patient-matched serum samples are strongly correlated with kidney biopsy (left) but not with whole blood mRNA (right).

5. Patient-matched urine samples are strongly correlated with kidney biopsy (left) but not with whole blood mRNA (right).

**Study Design & Methods**

**Goal**

To identify serum and urine biomarkers associated with an accumulation of FR-PTs to noninvasively identify patients at risk for CKD progression.

**Summary & Next Steps**

- 78 serum and 79 urine proteins correlated with an FR-PT gene signature score from kidney biopsies were identified in patient-matched biofluid samples.
- A subset of serum and urine proteins were also correlated with FR-PT biopsy mRNA expression, suggesting a kidney origin for these proteins.
- Cell type-specific gene set enrichment analysis further identified 13 serum and 1 urine protein as potentially enriched, suggesting a kidney origin for these proteins.
- The relationship between candidate biomarkers and the FR-PT biopsy score will be assessed in a larger subset of the NURTuRE cohort.
- Independent datasets for the validation of FR-PT biomarker candidates will be identified.

**Next steps**

- Independent datasets for the validation of FR-PT biomarker candidates will be identified.

**Key observations**

- Matched serum & urine samples (122/297)
- High-quality biopsy transcriptome (297 total)
- Additional urine (100) and serum (71) samples identified to complement multiomics dataset

**References**

2. Ooi et al. (2020). Accumulation of multipotent tubule epithelial cells is ubiquitous in CKD and suggests a common initiating mechanism of disease progression. Neuroradiology 58, 253–263.