Identification of surrogate biomarkers reflecting tubular failed repair in CKD

Tobias Bohnenpoll[1], I-Ju Lo[1], Mark McConnell[2], Shawn S. Badal[2], Olivier Radresa[1], Jay Kuo[2], Jennifer Cox[2], Uwe Andag[1], Andrew King[2], N. Eric Olson[2]

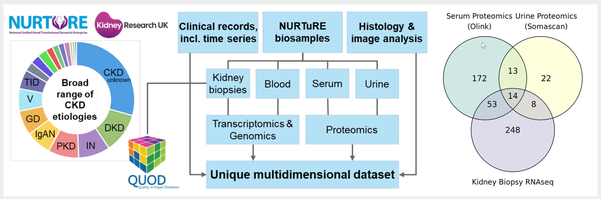
1. Evotec International GmbH, Gottingen, Germany; 2. Chinook Therapeutics Inc, Seattle, WA, United States,

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.[1] We have recently demonstrated that accumulation of FR-PTs in human disease predicts reduced renal event-free survival in multiple CKD etiologies.[2]

We used multiomics 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[3] 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 multiomics 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 Olink and Somascan proteomics platforms, respectively. Correlation analysis of biofluid 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).

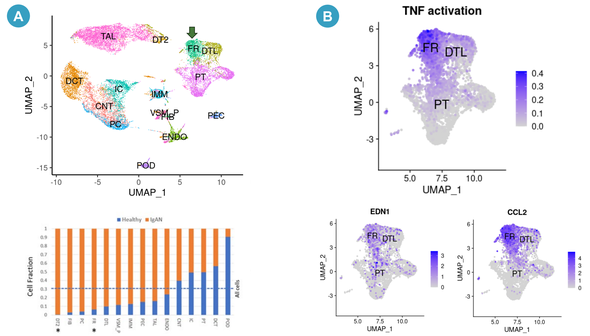
Kidney cell type-specific gene sets were derived from GSE171314[4] using FindAllMarkers in Seurat[5] (log2(fold change) ≥ 0.1 and p ≤ 0.05) and analyzed for enrichment of candidate biofluid protein encoding genes using hypeR[6]. A liver specific gene set was derived from Human Protein Atlas.

References

1. Kirita et al. (2020). Cell profiling of mouse acute kidney injury reveals conserved cellular responses to injury. *Proc Natl Acad Sci USA* 117, 15874–15883.
2. Olson et al. (2023). Accumulation of maladaptive tubular epithelial cells is ubiquitous in CKD and represents a common initiating mechanism of disease progression. *Nephrology Dialysis Transplantation* 38, gfad063b_4286.
3. https://www.nurturebiobank.org/
4. Tang et al. (2021). A partial picture of the single-cell transcriptomics of human IgA nephropathy. *Front Immunol* 12, 645988.
5. Butler et al. (2018). Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat Biotechnol* 36, 411–420.
6. Federico et al. (2020). hypeR: an R package for gene set enrichment workflows. *Bioinformatics* 36, 1307–1308.

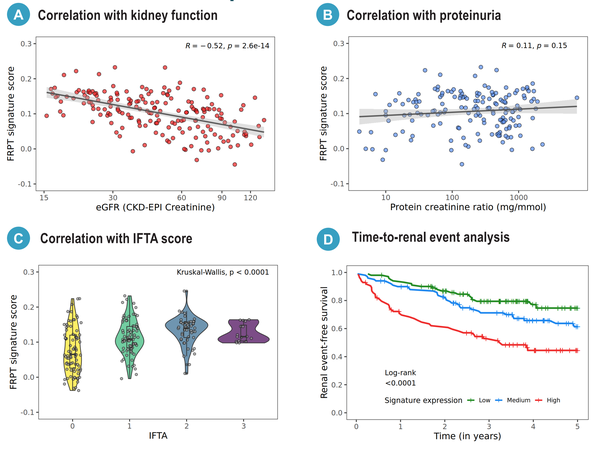
Results

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



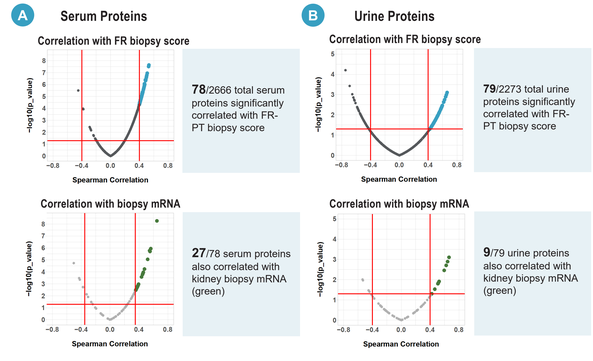
A Characterization of publicly available human CKD scRNA-Seq data (GSE171314) suggested that human FR-PT equivalents were detectable and increased with disease.
B Human FR-PTs were characterized by TNF pathway activation and increased expression of proinflammatory and pro-fibrotic factors.
A human failed repair gene signature was derived from marker genes for the FR-PT cluster.

2 An elevated FR-PT signature score is associated with reduced renal event-free survival in NURTuRE CKD patients



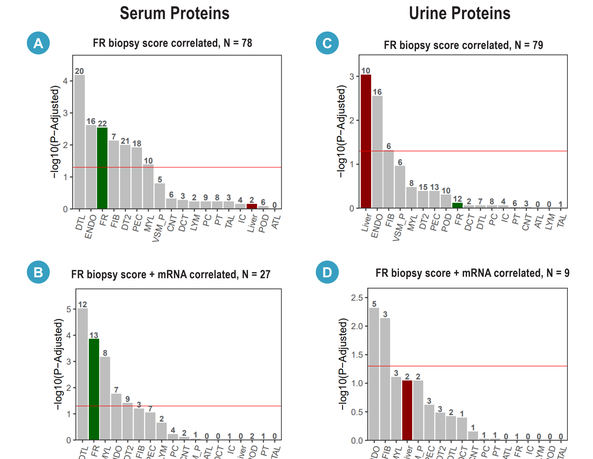
A FR-PT signature scores were inversely correlated with kidney function.
Pearson correlation of log-transformed eGFR and FR-PT signature in 198 kidney biopsies.
B FR-PT signature scores did not correlate with proteinuria.
Pearson correlation of log-transformed urinary protein creatinine ratio and FR-PT signature score in 169 kidney biopsies.
C FR-PT signature scores were correlated with IFTA severity.
Kruskal-Wallis test of IFTA and FR-PT signature score correlation in 227 kidney biopsies.
D FR-PT signature scores at time of biopsy were predictive for future disease progression.
Kaplan-Meier and log-rank analysis comparing low, medium and high FR-PT signature expression in 285 kidney biopsies over 5 years. Renal events were defined as 40% eGFR decrease or incident ESRD.

3 Multiomics biomarker discovery from patient biopsy-matched NURTuRE biofluids



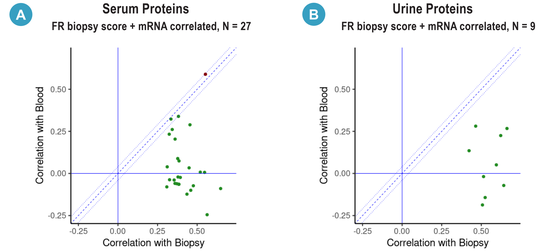
A Discovery of serum biomarker candidates by sequential filtering for correlation with FR-PT biopsy score and kidney mRNA expression.
B Discovery of urine biomarker candidates by sequential filtering for correlation with FR-PT biopsy score and kidney mRNA expression.

4 Serum but not urine proteins are enriched for FR-PT specific kidney expression



A Serum protein correlated with the FR-PT biopsy score (n = 78) were enriched for FR-PT and kidney cell specific expression.
B Serum protein correlated with the FR-PT biopsy score and kidney mRNA (n = 27) were enriched for FR-PT specific expression.
C Urine protein correlated with the FR-PT biopsy score (n = 79) were enriched for liver and endothelial but not FR-PT or kidney cell specific expression.
D In contrast to serum proteins, FR-PT biopsy score and mRNA correlated urine proteins (n = 9) were not enriched for FR-PT specific expression.

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



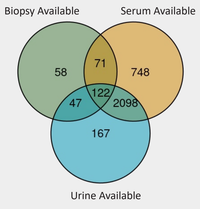
A FR-associated serum proteins were strongly correlated with kidney biopsy (left) but not with whole blood mRNA (right).
B FR-associated urine proteins were strongly correlated with kidney biopsy (left) but not with whole blood mRNA (right).

Additional patient-matched samples available for validation of biomarker candidates

Cohort of 100 new NURTuRE subjects with:

- Rich clinical metadata
- High-quality biopsy transcriptome (297 total)
- Matched serum & urine samples (122/297)
- Acceptable time between biopsy and biofluid sample collection

Additional urine (100) and serum (71) samples identified to complement multiomics dataset



Summary & Next Steps

Key observations

- 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 27 serum and 9 urine proteins were also correlated with matched 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 derived from kidney FR-PTs

Next steps

- 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

Goal

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