Unsupervised Characterization of the NURTuRE Cohort Reveals Gene Expression and Tissue Remodeling Dynamics along a Synthetic CKD Progression Axis

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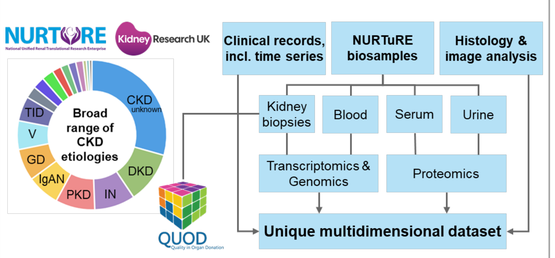
Background

- Conventional stratification by clinical and histopathological phenotypes is insufficient to describe the heterogeneity of chronic kidney diseases (CKD). Recent advances in CKD classification[1] integrate real-world molecular, morphological and clinical data from large patient cohorts to improve mechanistic disease understanding.
- Here, we combined molecular groups identified by unsupervised characterization of the NURTuRE[2] and QUOD[3] patient cohorts into a synthetic disease progression axis (sDPA) ranging from healthy to severe CKD, with the aim to explore gene expression and tissue remodeling dynamics along this pseudotime trajectory.

We will use this framework for a human data-driven, patient-centric and omics-enabled target identification focused on common cellular and molecular mechanisms of disease

Patient Cohorts & Data Sets

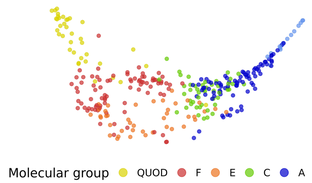


- NURTuRE[2] is a unique prospective cohort study involving > 3500 CKD patients that is linked to a biobank of matched patient samples covering a broad range of diagnoses and kidney functional states.
- A rich multidimensional dataset was generated by combining clinical and histopathological records with multiomics analyses of kidney and liquid biopsies.
- A data-driven (clinical reports, serum chemistry) selection of biopsies from the QUOD[3] initiative, representing kidney-healthy donors after brain death, was processed simultaneously to provide reference kidney transcriptomes.

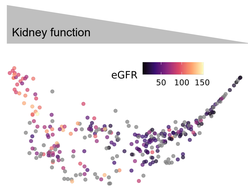
Methods

- Unsupervised classification of NURTuRE kidney transcriptomes via self-organizing maps[4] and characterization via PHATE[5] dimensionality reduction inferred 5 groups with distinct molecular profiles that aligned with clinical and histopathological disease progression.
- A data-driven selection of QUOD (healthy, n = 36) and NURTuRE (CKD, n = 139) kidney biopsy transcriptomes (FFPE, RNA-seq) representative of molecular groups was combined into a synthetic disease progression axis (sDPA) via PHATE[5] embedding.
- Groups of genes with similar expression dynamics were derived via local regression and hierarchical clustering enabling a pseudotemporal interpretation of gene expression dynamics along the sDPA.
- Gene set overrepresentation analysis based on > 600 manually curated kidney-focused scRNA-seq signatures and additional public sources (evoGO[6], Reactome, Hallmark) was employed to support a mechanistic interpretation of molecular disease progression.

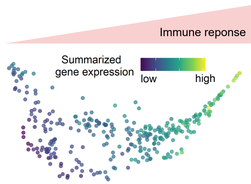
A human data-driven, patient-centric and omics-enabled target identification framework focused on common mechanisms of CKD



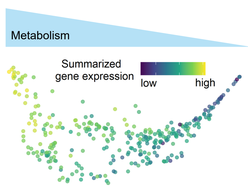
Unsupervised molecular stratification and multimodal characterization of kidney biopsy transcriptomes



Disease progression aligns with molecular stratification largely independent of clinical diagnosis.

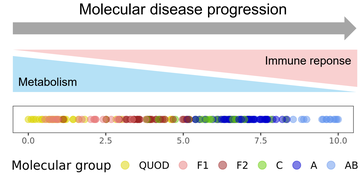


Immune reponse signature expression characterizes the polarized global data structure. (singscore of 200 genes, Hallmark M5932)

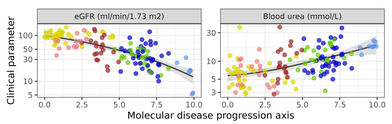


Fatty acid metabolism signature expression characterizes the polarized global data structure. (singscore of 158 genes, Hallmark M5935)

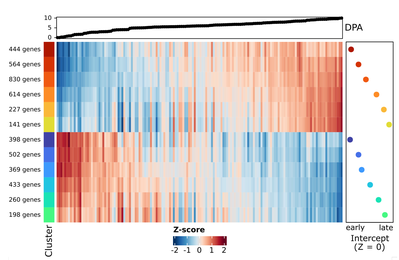
Molecular groups represent distinct transcriptomic disease states aligned with disease progression



Construction of a molecular disease progression axis and pseudotemporal stratification of gene expression

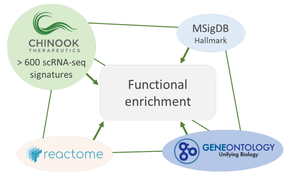


A data-driven selection (e.g. exclusion of biopsies affected by sampling bias) of **molecular subgroups** was **combined into a synthetic disease progression axis** (sDPA) ranging from healthy to severe CKD.

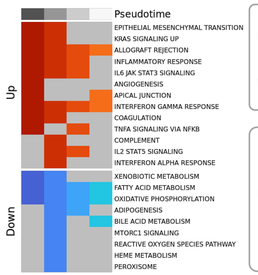


Genes highly correlated with the sDPA (~ 5000) were **clustered hierarchically by their expression dynamics** resulting in 12 clusters with different pseudotemporal change.

Integration of selected molecular subgroups into a disease progression axis reveals pseudotemporal gene expression dynamics

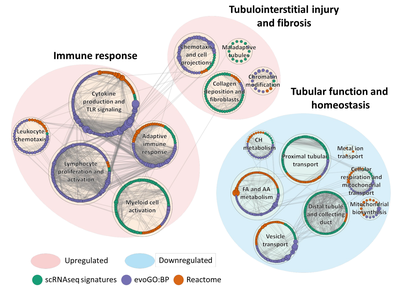


Functional enrichment analysis and mechanistic interpretation of molecular disease progression



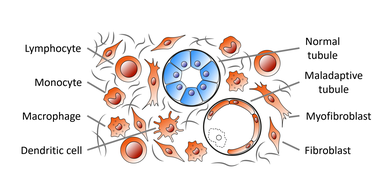
Overrepresentation analysis along the sDPA **enables a mechanistic interpretation** of pseudotemporal disease progression.

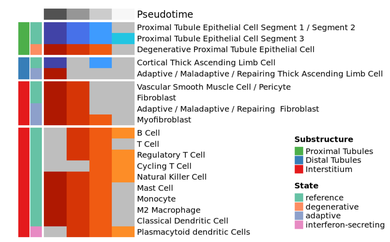
Combined **similarity network analysis** of enriched kidney scRNA-seq signatures and other mechanistic genes sets **unravels complex cellular and molecular mechanisms** of CKD.



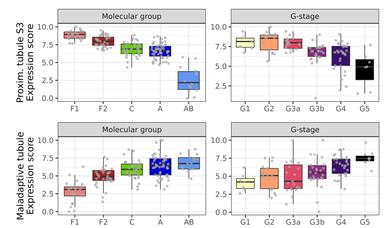
Tubular injury and atrophy, interstitial fibrosis and immune response are the main functional concepts associated with molecular disease progression

Characterization of molecular mechanisms of disease in normal and maladaptive cell states





Cell type-specific pseudotemporal expression profiles reflect **tubulointerstitial tissue remodeling dynamics** characterized by a gradual loss of healthy tubular gene expression and a complementary increase of maladaptive tubular, fibrotic and immune gene expression.



Our framework captures accumulating maladaptive tubules and associated molecular programs as common drivers of CKD progression

Conclusions and Outlook

- Unsupervised cohort characterization and multimodal data exploration enabled the careful selection and integration of disease-relevant biopsy transcriptomes into a molecular CKD progression axis. Pseudotemporal stratification of gene expression along this axis revealed groups of genes with shared expression dynamics corresponding to CKD tissue remodeling.
- Our framework captures major concepts of CKD progression, including but not limited to tubular injury and atrophy, interstitial fibrosis, inflammation and immune infiltration as reflected by the enrichment of curated scRNA-seq-derived cell type-specific signatures and other mechanistic gene sets.
- Importantly, our human data-driven and omics-enabled analysis provides translational evidence for an early accumulation of profibrotic and proinflammatory maladaptive cell tubular states reflecting failed repair as common drivers of CKD progression in this large patient cohort.

Chinook Therapeutics and Evotec joined forces for a patient-centric target identification supported by strong translational evidence to initiate drug discovery programs with a focus on tubular failed repair and cross-talk in the tubulointerstitial niche

References and Acknowledgement

1. Bohnenpoll et al. A Systems Nephrology Framework for the Molecular Classification of Chronic Kidney Disease. *Nephrology Dialysis Transplantation*. 2022; 37:gfac114.004.
Follow QR codes for additional information.
2. NURTuRE - The National Unified Renal Translational Research Enterprise; https://www.nurturebiobank.org
3. QUOD – The Quality in Organ Donation Initiative; https://quod.org.uk/
4. Löffler-Wirth et al. oposSOM: R-package for high-dimensional portraying of genome-wide expression landscapes on bioconductor. *Bioinformatics*. 2015;31(19):3225-3227.
5. Moon et al. Visualizing structure and transitions in high-dimensional biological data. *Nat Biotechnol.* 2019;37(12):1482-1492.
6. evoGO – An Evotec R package providing advanced functionality for performing a Gene Ontology (GO) enrichment analysis. https://github.com/Evotec-Bioinformatics/evoGO

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