TH-OR40
Targeted Isolation of Urine-Derived Renal Tubular Cells for Person-
alised Medicine
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Background: The kidney achieves homeostasis through the complex actions of the nephron; a heterogeneous structure made up of 14+ segments. Rare monogenic tubular diseases are often characterised by impairment of specific tubular segments. Isolation and characterisation of patient-specific urine-derived renal tubular epithelial cells (uRTec) from disease-relevant segments can assist diagnosis and treatment planning.

Methods: Urine samples collected from healthy volunteers and patients with genetic tubular disease were pelleted by centrifugation at 400 RCF for 10min at RT, and the cell pellet was washed with a 50:50 mixture of DMEM:F12 (supplemented with 10% FBS, 100uM penicillin, 100mg/ml streptomycin, 1X insulin-transferrin-selenium, 2.5µg/ml nicoitamide, 500µg/ml hydrocortisone). Targeted isolation and enrichment of segment-specific cells was performed using a magnetic beads conjugated to target-specific antibodies/lectins. Primary urine-derived cells were either fixed using 4% w/v formaldehyde-PBS for 15min at RT at 60% confluency, or lysed with TRI reagent at 90% confluency for RNA isolation. Cell types were validated by staining with fluorescently-tagged marker antibodies/lectins and qPCR of segment specific mRNAs.

Results: Primary urinary cells successfully cultured from patients’ urine samples with different morphologies presented and can be maintained to the third passage. As can be seen in Figure 1, cells from several patients stained positively with Dolichos Biflorus Agglutinin indicating the presence of distal convoluted tubule cells. These was validated by confirmation of expression of NCC.

Conclusions: uRTec can be routinely isolated from patient’s urine, targeted for enrichment, and successfully subcultured for several passages. Future work, would aim to utilise these cells in 3D “Organ-on-a-Chip” systems, where we could potentially artificially reconstruct patient’s tubules from primary urinary cells and conduct individualised pharmacological experiments to optimize treatments, thereby bringing true personalised medicine to nephrology.

TH-OR41
CODEX Multiplex Imaging Uncovers Unique Cell Types, States, and Niches in Health and Disease
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Background: Co-Detection by indEXing (CODEX) multiplex imaging is a new and powerful tool for imaging many protein markers on one tissue specimen. A challenge in applying this technology on human kidney tissue is to establish a robust analytical pipeline to analyze the resultant multidimensional large-scale data and compare multiple datasets.

Methods: We imaged human cortical tissue from healthy reference and renal disease (AKI, Lupus, CKD, IgA) specimens with 38 different antibodies, including epithelial, immune, and injury markers. Segmentation of nuclei and unsupervised classification and visualization were performed using a customized open-source software tool: Volumetric Tissue Exploration and Analysis (VTEA). Additional analysis to combine datasets in a single analytical space was performed using R and visualized in VTEA. We also performed cell centric neighborhood analysis to define spatial relationships between cell types and immune cell subtypes. Neighborhood analysis showed unique cell niches that were altered in disease. Specifically, cell niches enriched in THY1+ PTs were markedly diminished, whereas immune-rich niches expanded with disease.

Conclusions: We established a unique analytical pipeline for CODEX multiplexed imaging data that can be utilized to define various cell types in the human kidney in health and disease and compare between patients. Our findings highlight unique cell niches and uncover alteration of specialized epithelial and immune cell types with disease, thereby identifying potential novel targets for therapy.

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TH-OR42
Super-Resolution Microscopy in Clinical Specimens Using Conventional Widefield Microscopes
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Background: Super-resolution microscopy (SRM) enables nanoscale molecular characterization of tissues, but the access to SRM systems is limited, hindering their applicability in the scientific and clinical pathology community. Expansion microscopy and computational image enhancement algorithms like “super-resolution radial fluctuations” (SRRF) are promising alternatives, but do not achieve sufficient resolution when combined with LED-based widefield microscopy (WFM). Here, we introduce expansion-enhanced super-resolution radial fluctuations (ExSRRF), enabling nanoscale molecular SRM in clinical pathology samples using WFM.

Methods: We performed expansion-enhanced fluorescence imaging of tissues, followed by hydrogel embedding, tissue expansion and time-stacked image acquisition with WFM. Subsequent computational processing using the SRRF algorithm yielded super-resolved images. To define the resolution range, nanorulers (synthetic molecules containing two fluorescent dyes at precisely predefined distances) were expanded and imaged in a similar fashion to tissues. Automated image analysis of the slit diaphragm (SD) was performed using a multi-step process including region of interest- and ridge-detection, followed by SD-density and dilatation measurements using both, custom and open-source tools.

Results: In a set of nanorulers, ExSRRF displayed non-overlapping nanoruler spread functions at distances between 120nm and 25nm, thus providing a resolution of at least 25nm. ExSRRF was applied across a broad range of formalin-fixed paraffin-embedded clinical and experimental tissues. In an experimental model of renal ischemia-reperfusion injury (iRIR), ExSRRF resolved endoplasmic reticulumic dilatation. In human kidney biopsies, ExSRRF resolved normal foot processes (FP) and detected EF effacement as a diagnostic feature of minimal change disease (MCD). In a small case series, ExSRRF resolved the SD and provided quantitative changes and a morphological disease signature of MCD.

Conclusions: ExSRRF is a flexible, scalable, inexpensive, and robust method for the molecular characterization of experimental and clinical specimens and thus has the potential to bridge SRM and both clinical and experimental pathology, enabling universal access to molecular nanopathology.

TH-OR43
Analyzing Cell Type-Specific Dynamics of Metabolism in Kidney Repair
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Background: Conventional single-cell metabolomics approaches such as MALDI mass spectrometry imaging (MALDI-MSI) generate biochemical snapshots, neglecting the inherent dynamic nature of metabolism. Here we describe a platform based on isotopic tracing and MALDI-MSI that allows in-situ dynamic measurements of cell type-specific metabolism at single-cell resolution, and thus unravels cellular metabolism within tissue architecture.

Methods: We applied different [13C]-isotopically labeled nutrients on vibratome slices of fresh mouse kidney. MALDI-MSI at single-cell resolution (i.e. pixel size of 5 × 5 µm) was applied to detect metabolite signals from the harvested tissues. Following MALDI-MSI analysis, post-MSI-processed sections were stained and subsequently imaged using multiplexed immunofluorescence (IF) microscopy for cell-type identification.

Results: We show that this method can map cell type-specific dynamic changes in the central carbon metabolism, as well as the contribution of different nutrients to energy metabolism in a complex heterogenous tissue architecture such as the kidney. Combined with multiplexed immunofluorescence staining, we can detect metabolic changes and nutrient partitioning in targeted cell types as demonstrated in a bilateral renal ischemia/reperfusion injury (iRIR) model. At baseline, we identified a marked heterogeneity in response to TCA metabolic consumption and glycolysis in the stripe spared outer medullary proximal tubular segments (PT-S3) when compared to the cortical PT-S1/S2 segments. After bIRI, PT cells that failed to repair remained in a hyperglycolytic state. Meanwhile, PT cells with an apparent normal phenotype in the recovery phase still display a striking difference in tricarboxylic acid (TCA) cycle substrate use when compared to those in sham kidneys. As TCA metabolites serve biosynthesis as well as gene regulation this may be of relevance to the homeostatic capacity of the kidney microenvironment.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral; PO - Poster; PUB - Publication Only
Underline represents presenting author.
Conclusions: In sum, this method allows to achieve single-cell resolution in situ and hence interpret cell type-specific metabolic dynamics in the context of kidney structure and metabolism of neighboring cells.

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TH-OR44
High Resolution Spatially Resolved Transcriptomic Atlas of Kidney Injury and Repair With Direct RNA Hybridization-Based In Situ Sequencing

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Background: Understanding how different kidney cell types contribute to acute kidney injury (AKI) requires knowledge of their spatial organization and connectivity, information that is lost in single cell techniques that rely on cell dissociation. Recent advances in spatial transcriptomic technologies enable visualization of multiplexed transcripts at cellular resolution.

Methods: We performed highly multiplexed direct RNA hybridization based in situ sequencing (dRNA-HybISS) (CARTANA, part of 10X Genomics) on the mouse kidneys from sham, 4h, 12h, 2d, and 6w after bilateral ischemia-reperfusion injury (IRI).

Results: We achieved sub-cellular transcript resolution and were able to map cell type specific markers precisely to their respective cell types (Fig 1). As expected, spatial expression of the injury marker Haver1 was confined to the PT-S3 segment at 4h, 12h and 2d, and was absent in sham and 6w. We segmented ~400,000 cells from all time points of IRI. Unsupervised clustering revealed 10 major kidney cell types including rare cell types such as podocytes and JGA cells. We were able to reconstruct glomerular cell type organization with podocyte, EC and JGA. We revealed dynamic changes in spatial distribution of immune cell subsets across AKI time course. For example, Cd14+ monocytes were increased in early time points of IRI whereas the Pircp+ macrophages accumulated only in later time point. Integration with scRNA-seq data increased resolution of our spatial map to 26 kidney cell types, including different PT injury states. We also compare these results to Viscum analysis of the same time course, revealing that dRNA-HybISS provided much higher cell resolution including classification of individual rare cell types.

Conclusions: dRNA-HybISS enables in situ identification and spatial mapping of cell types in the kidney. When applying this technique to IRI, we reveal the dynamics of immune cell migration both in time and region during kidney injury and repair.

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TH-OR45
Genome-Wide CRISPR Screen Reveals That Elamipretide (SS-31) Mediated PLSCR3 Activation Mitigates Mitochondrial Dysfunction During AKI

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Background: Mitochondrial dysfunction is a hallmark of several human disorders, including acute kidney injury (AKI). SS-31 is a cell-permeable mitochondrial-targeted peptide restores healthy mitochondrial function and is currently undergoing clinical trials. SS-31 interacts with cardiolipin in the inner mitochondrial membrane; however, the pharmacological basis of its protective effects remains obscure. Importantly, the role of phospholipid scramblase 3 (PLSCR3), a mitochondrial cardiolipin binding protein, in SS-31 and renal biology is unknown.

Methods: We performed a genome-wide CRISPR screen in a nephrotoxic model of tubular epithelial cell-death with SS-31. The primary and secondary screens in BUMPT and HK-2 cells showed that PLSCR3 is essential for the protective effects of SS-31. Seahorse based analysis of mitochondrial function was also carried out in WT and PLSCR3 KO HK-2 cells. Conditional KO mice were generated by crossing PLSCR3-flxed with Ggt1-Cre mice (Fig. 1). Next in vivo KO of PLSCR3 through hydrodynamic intravenous RNAi injections was done. The severity of renal injury (IRI and cisplatin) was monitored in control and KO littermates or mice injected with control and PLSCR3 siRNA through measurement of BUN, serum creatinine, histological analysis, and biomarker analysis.

Results: In cell culture models of cisplatin nephrotoxicity, cell survival and mitochondrial protection provided by SS-31 is abrogated by PLSCR3 knockdown or knockout. In vivo, PLSCR3 gene ablation or knockdown in RTECs suppresses the protective effects of SS-31 in cisplatin and ischemia-reperfusion associated models of AKI (Fig 1). Using liposome-based assays, we also found that SS-31 activates PLSCR3 scramblase activity.

Conclusions: Our studies have discovered phospholipid scramblase 3 as the crucial mediator of the cell protective effects of SS-31. We propose that SS-31 activates PLSCR3 phospholipid scramblase activity resulting in mitochondrial protection under stress conditions associated with AKI.

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TH-OR46
Downregulation of Syndecan-1 and Alternative Complement in Renal Proximal Tubular Epithelial Cells by Crotamine/SiRNA Complexes

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Background: In proteinuria, syndecan-1, an epithelial heparan sulfate proteoglycan, serves as a docking platform for filtrated urinary protein in the apical membranes of proximal tubular epithelial cells (PTEC) activating the complement system via alternative pathway. Targeting PTEC aiming to reduce syndecan-1 expression might be useful to slow down the alternative complement activation during proteinuria. Crotamine is a non-viral cell-penetrating peptide which after ip injection accumulates in PTEC via apical endocytosis. We now tested crotamine-siRNA complexes for in vitro and in vivo targeting of PTEC.

Methods: The complexes formed by crotamine and syndecan-1 siRNA were characterized by biophysical methods. After the in vitro transfection of HK2 cells with crotamine-siRNA complexes, the efficiency to downregulate the syndecan-1 expression, properdin binding, and subsequently, complement deposition was assessed by FACS and qRT-PCR. The targeted internalization into PTEC in vivo was evaluated by confocal microscopy of kidney sections from mice injected with fluorescein-labeled crotamine-siRNA complexes.

Results: We demonstrated that the efficient complex formation is time- and crotamine-siRNA ratio-dependent and that crotamine is able to protect siRNA against degradation by endolysosomes. After 48 h, the transfection with the complex reduced ~50% of syndecan-1 expression at both mRNA and protein levels (both p<0.01) in vitro. Subsequently, properdin binding was also comparably reduced (p<0.001) and the alternative pathway activation declined ~60% (p<0.001). Moreover, ip injection of the fluorescein-labeled crotamine-siRNA complexes in mice showed siRNA presence in the cell membranes of proximal tubular cells, followed by internalization into these tubular cells.

Conclusions: We show for the first time the use of crotamine as a non-viral nanocarrier for PTEC-specific delivery of siRNA both in vitro and in vivo. Successful reduction of the expression of syndecan-1 was accompanied by down modulation of alternative complement system by PTECs in vivo. The use of crotamine as a prototypic next generation kidney-specific non-viral vector to modulate aberrant gene expression in kidney PTECs, for instance, in proteinuric renal diseases.

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