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Integrated multi-omics in animal and observational human datasets provides insights into potential molecular mechanisms and biomarkers for atrasentan

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Identifying, characterizing and validating an atrasentan response signature in animal models

*Insights into atrasentan’s mechanism of action*

**Goal:** identify gene signature and non-invasive biomarkers to differentiate MOAs in IgAN pathogenesis

**Atrasentan Blocks Central Drivers of IgAN Pathogenesis**

**Approach:** Apply translational cellular & in vivo models to investigate potential impact of atrasentan on key mechanisms of IgAN pathogenesis

*ETA receptor activation drives proteinuria, mesangial cell activation & kidney inflammation & fibrosis, all hallmarks of IgAN*
An atrasentan response signature derived from preclinical models

- A cluster of cells corresponding to failed repair proximal tubules (FR-PTEC) was highly expanded in the gddy mice compared to naïve mice.
- FR-PTEC showed the largest response to atrasentan as measured by number of differentially expressed genes.
- A 31 gene signature (Atra_31) was derived from the genes that were increased in gddy and decreased by atrasentan in the FR-PTEC cells.
- The signature score was also found to be decreased by atrasentan in the anti-Thy1.1 model of Mesangio-proliferative Glomerulonephritis.

~150,000 nuclei were isolated and sequenced from naïve, gddy and gddy + atrasentan mice.

FR PTEC are a source of chemokines and cytokines for immune cells and fibroblasts:

Putative relationships for key FR PTEC ligands and corresponding receptors on potential target cell types:
- Pro-inflammatory and profibrotic effects on fibroblasts may be mediated through Tnf and Tgfb2 signaling from FR PTEC.
- FR PTEC may play a key role in recruitment of immune cells through their expression of Ccl2.
Conduct an analysis of patient-matched kidney biopsies and biofluids from the NURTuRE cohort with the aim to identify non-invasive biomarkers associated with the atrasentan response signature score.

**Project Aims:**

**NURTuRE Disease Cohort Data Availability**

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- There is substantial overlap for many of the data types available for the NURTuRE disease cohort.
- 67 subjects with serum proteomics and biopsy rnaseq and 22 subjects with both urine proteomics and biopsy RNAseq were used for analysis.

https://www.nurturebiobank.org
Biomarker discovery strategy in the NURTuRE cohort

1. Signature score is correlated with eGFR and IFTA

\[ r = -0.64, \ p < 2.2 \times 10^{-16} \]

Urine (Somascan)

Kruskal-Wallis \( p < 0.0001 \)

Serum (Olink)

\[ r = 0.001, \ p = 0.93 \]

2. Correlation of biofluid proteins with patient biopsy score

174/2273 total urine proteins significantly correlated with patient biopsy rank

12/174 urine proteins correlated with patient biopsy rank are also significantly correlated with biopsy mRNA

3. Correlation of biofluid proteins with patient biopsy mRNA expression

173/2666 total serum proteins significantly correlated with patient biopsy rank

39/173 serum proteins correlated with patient biopsy rank are also significantly correlated with biopsy mRNA
Serum proteins are enriched for proteins expressed by failed repair tubules

A) Urine Proteins – enrichment for kidney cell type markers

B) Serum Proteins – enrichment for kidney cell type markers

- Serum showed an enrichment for FR proteins compared to urine
- Including biopsy correlation reduced enrichment for liver associated proteins
- FR expressed protein identified in urine
  - 26 correlated with biopsy score
  - 1 also correlated with biopsy gene expression
- FR expressed proteins identified in serum
  - 42 correlated with biopsy score
  - 19 also correlated with biopsy gene expression

Cell type Enrichment Analysis

Cell type genesets for 17 cell types derived from scRNA-seq data (GSE171314)

Significant genes for each cell type from FindAllMarkers (padj < 0.05)

Liver specific gene set derived from Human Protein Atlas RNA data (enriched in only liver)
### Summary and future plans

#### Key observations
- A gene signature associated with atrasentan response in failed repair cells was identified in the gddY mouse model of IgAN
- Proteins associated with the gene signature score in patient biopsies were identified in urine and serum samples
- A subset of proteins that were also correlated with gene expression in the biopsies were identified and were enriched for proteins associated with the tubules
- Serum proteins had a stronger enrichment for tubular proteins than urine

#### Future steps
- Assess relationship between candidate proteins and signature score in a larger set of subjects for the NURTuRE cohort
- Assess effects of atrasentan treatment on proteins levels in urine and serum in subjects from the AFFINITY trial
  - Are signature-associated proteins reduced by atrasentan treatment?

#### Goals
- To identify non-invasive biomarkers associated with specific cellular responses that will enable precision treatment in CKD
Thank You!

To all members of the Chinook-Evotec Strategic Partnership...

... and all contributors of the NURTuRE Consortium!

Elaine Davies, Director at Kidney Research UK

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