

Gene Ontology Reveals Potentially Unique Mechanism of Action Underlying Selected Renal Cells Bioactivity

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1. Introduction/Background

- Selected renal cells (SRC), a renal epithelial cell-enriched platform, are being advanced in a Phase 3 Global Registrational trial for treatment of chronic kidney disease (CKD)¹.
- In CKD models, administration of SRC is associated with improved survival, preservation of renal microarchitecture, and reduced renal dysfunction^{2,3}.
- Preliminary data from a subset of diabetic kidney disease patients suggest randomization to SRC is associated with improvement in glomerular filtration rate¹.

2. Goal

To test the hypothesis that the renal restorative and reparative effects of SRC are mediated, at least in part, via its nephrogenic potential.

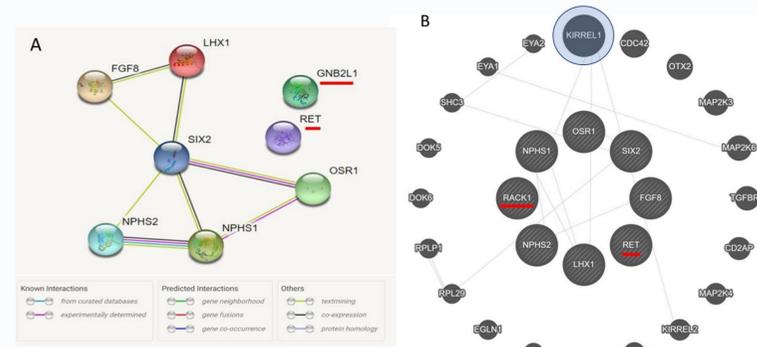
3. Methods

- Bioinformatics with empirical data were coupled to evaluate genes coding for Rack1 (Gnb2l1), Six2, Osr1, Ret, Fgf8, Lhx1, Nphs1 and Nphs2, proteins that are co-expressed by human SRC¹.
- Genes were seeded into a variety of knowledgebases and queried for co-expression, renal localization of their gene products and signaling interactomes, and function.
- Human SRC was placed in culture to evaluate formation of organoids and tubules.

4. Results

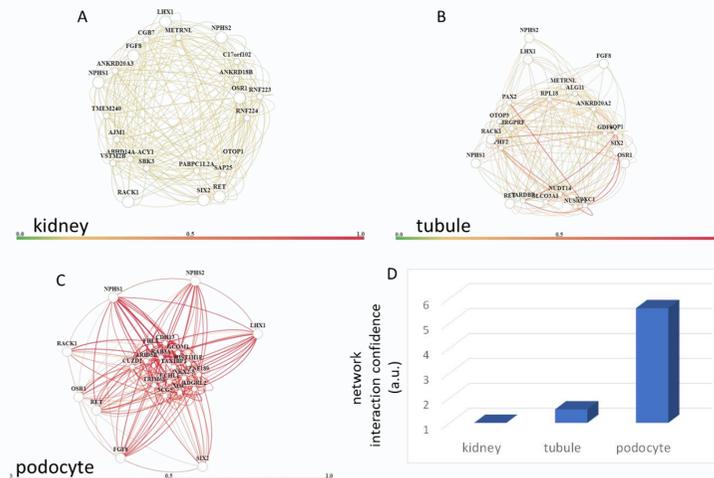
- Genes coding for SRC proteins typically do not appear to be co-expressed by human tissue; *kirrel1*, which codes for Neph1, and maintains glomerular barrier integrity, is potentially expressed by SRC.
- Both the SRC gene interactome and SRC gene products are compartmentalized within the tubules and/or glomeruli (podocyte) and participate in kidney development/nephrogenesis.
- SRC cultures form organoids which self-assemble into tubules in the presence of a scaffold.

Figure 1: Gene/Protein Co-expression Profile Unique to SRC



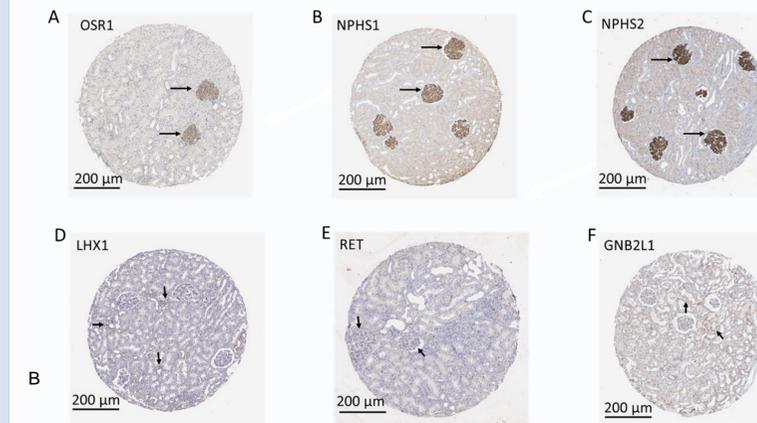
STRING (A) and GeneMANIA (B) queries suggest that genes coding for SRC proteins are not co-expressed by human tissue. The SRC interactome potentially comprises *kirrel1* (B) whose product (Neph1) participates in glomerular barrier function.

Figure 2: Tubular and/or Glomerular Compartmentalization of the SRC Gene Interactome



The SRC gene interactome (Humanbase) in the kidney (A), tubules (B), and podocytes (C) exhibits increasing interaction strength confidence kidney<tubule<glomerulus<podocyte (D) suggesting compartmentalization.

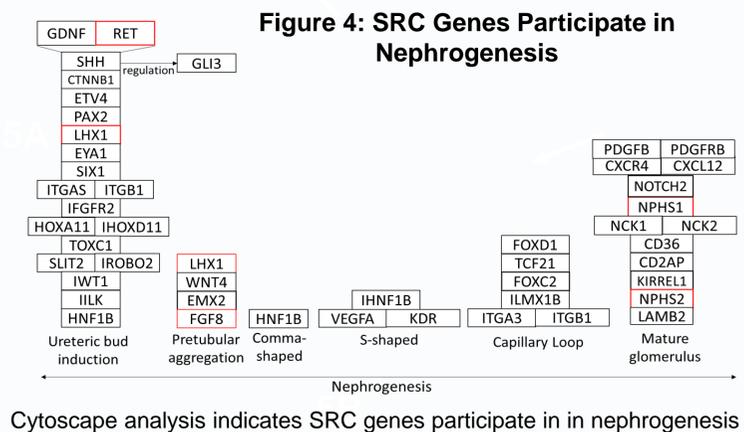
Figure 3: Tubular and/or Glomerular Compartmentalization of SRC Proteins



Antibody imaging data from The Human Protein Atlas indicate compartmentalization of SRC gene products within the tubules and/or glomeruli (arrows, A-F).

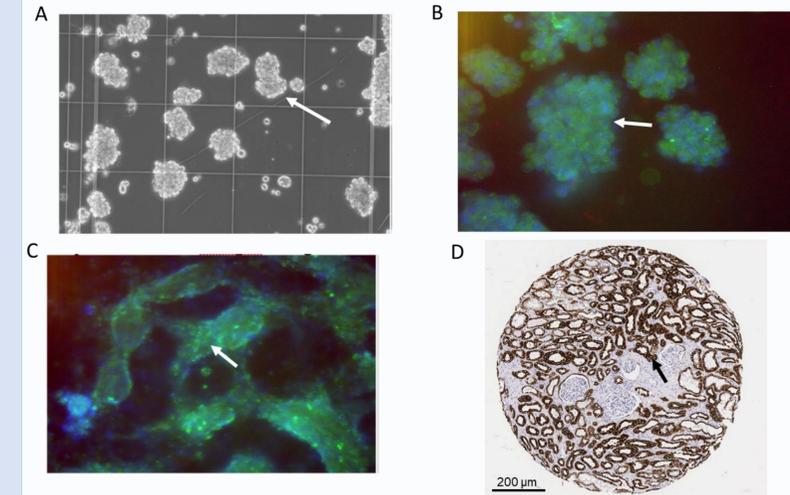
Table 1: SRC Genes Participate in Kidney Development

Gene Ontology Biological Process (GO BP)	
metanephric glomerulus development	mesonephric tubule morphogenesis
metanephric nephron development	mesonephric tubule development
nephron development	mesonephric epithelium development
kidney development	mesonephros development
renal system development	nephron tubule morphogenesis
metanephros development	renal tubule morphogenesis
glomerulus development	epithelial tube morphogenesis
renal vesicle development	morphogenesis of an epithelium
kidney epithelium development	tube morphogenesis
epithelium development	renal tubule development
tissue development	nephron tubule development
nephron epithelium development	nephron epithelium morphogenesis
cell differentiation involved in metanephros development	nephron morphogenesis
cell differentiation involved in kidney development	kidney morphogenesis
regulation of kidney development	ureteric bud development
epithelial cell differentiation involved in kidney development	anterior/posterior pattern specification



Cytoscape analysis indicates SRC genes participate in nephrogenesis

Figure 5: SRC Organoids Self-assemble into Tubules; Cytokeratin-18 Expression by SRC Tubules Consistent w/ Observation in Human Healthy Kidney



In culture SRC forms organoids (A, arrow, 5X) which express the tubular marker cubilin⁴ (B, arrow, 50X). In the presence of a scaffold such as hydrogel, these organoids self-assemble into tubules expressing the marker cytokeratin 18⁵ (C, arrow, 5X). Staining for Keratin-18 antibody in the human healthy kidney is also localized to the tubules (D, The Human Protein Atlas).

5. Discussion

- SRC (REACTTM), a renal epithelial cell-enriched platform with a unique protein co-expression profile, is being evaluated in a Phase 3 clinical trial in subjects at increased risk (CKD 3b/4) for kidney failure.
- The SRC gene interactome and SRC proteins appear compartmentalized within tubules and glomeruli (podocytes) and participate in processes associated with kidney development.
- SRC forms organoids which self-assemble into tubules *in vitro*.

6. Conclusions

The nephrogenic potential of SRC may underlie, at least in part, its renal restorative and reparative activity observed in clinical trials¹.

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