Selected Renal Cells Exhibit Renal Tubule Formation Associated with Transforming Growth Factor ß2 Expression

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INTRODUCTION | BACKGROUND

- Reciprocal inductive interactions between the ureteric bud (UB) and cap mesenchyme (CM) initiate kidney development[1]
- Transforming growth factorß2 (TGFß2) initiates mesenchyme-to-epithelial transition of the CM resulting in formation of an epithelialized nephron[1]
- Cortical administration[2-5] of biopsy-derived selected renal cells (SRCs) in animal models of chronic kidney disease (CKD) is associated with appearance of renal reparative and restorative markers, preservation of renal microarchitecture, improvement in survival, and preservation of both glomerular and tubular functions

GOAL

Test the hypothesis that SRCs (REACT®/rilparencel) recapitulate events associated with the developing kidney

METHODS

- SRCs were isolated[6] from human kidneys obtained from the National Disease Research Interchange (Philadelphia, PA; protocol# RRON2 01 001A)
- Canonical developing kidney markers (Table 1) were identified by verifying both locoregional expression of the marker from fetal kidney atlases[7-10] AND minimal marker expression by mature kidneys[11]. Podocyte markers (Table1) were identified using fetal and mature kidney atlases
- Single and bulk transcriptomic studies with SRCs were undertaken at UNC-Chapel Hill, NC. Differentially expressed genes were identified by comparing the SRCs vs. unselected biopsy digest transcriptomics (p_{adj} <0.05)
- Kidney-relevant ancestor-child pathways associated with SRC markers were generated by seeding SRC markers into a knowledgebase[12]. miR and mRNA interactomes were visualized by seeding SRC genes into a knowledgebase[13]
- SRCs were placed in culture, and secreted TGFß2 measured using ELISA. SRC organoids were stained for the CM marker Meis homeobox 2 (Meis2; ab73164, Abcam, United Kingdom), podocyte marker MPP5 (ab231560), epithelial marker CD13 (EPR4058, Abcam) and the nuclear stain Hoechst 33342 (1433, Sigma, St. Louis, MO). SRCs or unselected biopsy digest cells were cultured in Matrigel for visualization of epithelial networks. These networks were stained for the podocyte marker MPP5 (ab217068), the epithelial marker CK8/18/19 (aB 41825) and the nuclear stain Hoechst 33342

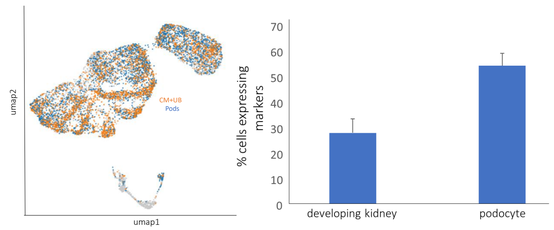
Table 1. Developing Kidney (UB+CM) and Podocyte Markers

Compartment	Gene Symbol
CM+UB	*ret*
CM+UB	*wnt11*
CM+UB	*fgf8*
CM+UB	*fgf10*
CM+UB	*grem1*
CM+UB	*etv5*
CM+UB	*cited1*
CM+UB	*eya1*
CM+UB	*hoxa11*
CM+UB	*ncam1*
CM+UB	*six2*
CM+UB	*osr1*
CM+UB	*gdnf*
Podocytes	*thsd7a*
Podocytes	*ptpro*
Podocytes	*mpp5 (pals1)*
Podocytes	*tppp3*
Podocytes	*lmx1b*
Podocytes	*nphs1*
Podocytes	*nphs2*

RESULTS

- SRCs express developing kidney (UB+CM) and podocyte markers associated with nephron development
- These developing kidney markers interact with *tgfß2*
- T*gfß2* is regulated by hsa-miRs 145-5p, 500b, 193a, 664a and 199a-5p; expression of these miRs is downregulated in SRCs
- SRCs overexpress tgfß2 and secrete TGFß2
- In 3-dimensional culture, SRCs form organoids expressing CM, tubular epithelial and podocyte markers
- Cultured in Matrigel, SRCs, but not unselected biopsy digest cells, form an epithelialized network expressing podocyte and tubular epithelial markers

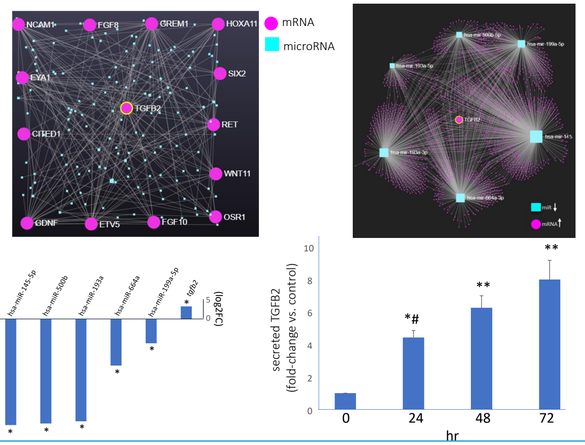
A **SRCs Express Developing Kidney & Podocyte Markers**



B **Biological Process: Developing Kidney & Podocyte Markers**

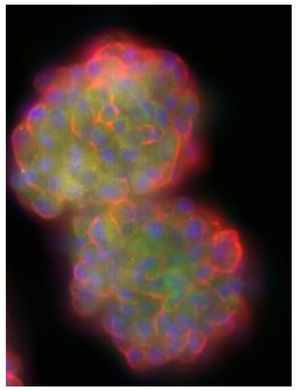
Biological Process	Fold-enrichment	False Discovery Rate
slit diaphragm assembly		
filtration diaphragm assembly	>100	<0.01
ureteric bud formation	>100	<0.01
mesenchymal to epithelial transition involved in metanephros morphogenesis	>100	<0.01
mesonephric tubule formation	>100	<0.01
renal vesicle development	>100	<0.01
mesenchymal to epithelial transition	>100	<0.01
mesenchymal cell differentiation involved in kidney development	>100	<0.01
mesenchymal cell differentiation involved in renal system development	>100	<0.01
renal vesicle morphogenesis	>100	<0.01
metanephric glomerulus development	>100	<0.01
podocyte development	>100	<0.01
glomerular epithelial cell development	>100	<0.01
epithelial cell differentiation involved in kidney development	>100	<0.01
nephron tubule formation	>100	<0.01
metanephric nephron development	>100	<0.01
renal filtration cell differentiation	>100	<0.01
regulation of kidney development	>100	<0.01
glomerular epithelial cell differentiation	>100	<0.01
nephron epithelium morphogenesis	>100	<0.01
glomerular epithelium development	>100	<0.01
renal tubule development	90	<0.01
nephron development	89	<0.01

C **SRCs & TGFß2**



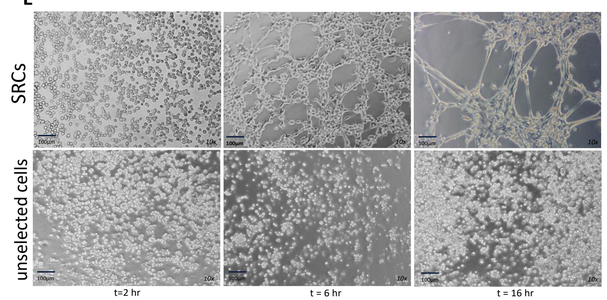
RESULTS CONTD.

D **SRC Organoid**

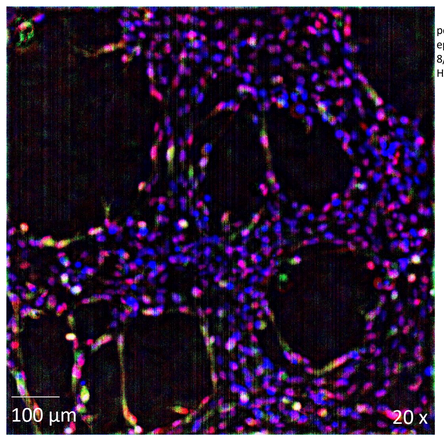


CM (MEIS2) - yellow
podocyte (PTPRO) - green
epithelial tubule (CD13) - red
HOECHST (nuclei) - blue

E **SRCs Assemble into Networks**



F **SRCs Express Nephron Markers**



podocyte (MPP5) – red
epithelial tubule (CK 8/18/19) - green
HOECHST (nuclei) - blue

A. SRCs comprise cells expressing both developing kidney (UB+CM) and podocyte markers. B. Gene Ontology Biological Process analysis indicates these markers are associated with nephron development. C. Developing kidney markers interact with *tgfß2*, expression of which is regulated by miRs. Expression of these miRs are reduced in SRCs (*, p_{adj} <0.05 vs. unselected cells) with concomitant increase in the *tgfß2* expression level (*, p_{adj} <0.05 vs. unselected cells). Cultured SRCs secrete TGFß2 (*, p<0.05 vs. 0 hr, #, p<0.05 vs. 72 hr, ** p<0.01 vs. 0 hr). D. In 3-dimensional culture, SRCs form organoids expressing CM, podocyte and tubular epithelial markers. E. Cultured in Matrigel, SRCs, but not unselected biopsy cells, form tubular networks expressing, F. nephron-like markers

DISCUSSION

- Biopsy-derived SRCs from the kidneys of adult cadavers express developing kidney (UB+CM) and podocyte markers
- These SRCs secrete TGFß2 associated with which is formation of epithelialized networks; these networks express nephron-associated markers
- Results from these *in vitro* studies suggest that SRCs recapitulate events associated with kidney development which may underlie their reparative and restorative effects observed in diabetic patients with CKD
- SRCs represent a standalone cell-based platform with renal reparative and restorative potential

CLINICAL STATUS

- Preliminary data[14,15] from patients with advanced CKD (Stage 3A-4) suggest that administration of SRCs (REACT®/rilparencel) is associated with preservation of kidney function
- REACT®/rilparencel is currently being evaluated[16] in a Phase 3 Global Registrational trial for treatment of CKD and has been awarded Regenerative Medicine Advanced Therapy Designation by Food and Drug Administration

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