

# Cellular Component of the Selected Renal Cell Platform Expresses a Podocyte-Parietal Epithelial Cell Transcriptome

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## INTRODUCTION | BACKGROUND

- Reciprocal inductive interactions between the ureteric bud (UB) and cap mesenchyme (CM) initiate nephron development<sup>1</sup>
- Podocyte-parietal epithelial cell (PEC) cross-talk maintains glomerular microarchitecture and glomerular barrier function<sup>2</sup>
- Biopsy-derived selected renal cells (SRCs) express developing kidney (UB+CM) and podocyte markers<sup>3</sup>
- In models of chronic kidney disease (CKD), administration of SRCs is associated with improvements in glomerular microarchitecture, glomerular barrier function and renal metabolic function<sup>4-7</sup>

## GOAL

Test the hypothesis that SRCs (REACT®/rilparencel) admixture of cellular components share a podocyte-PEC transcriptome associated with renal repair and restoration

## METHODS

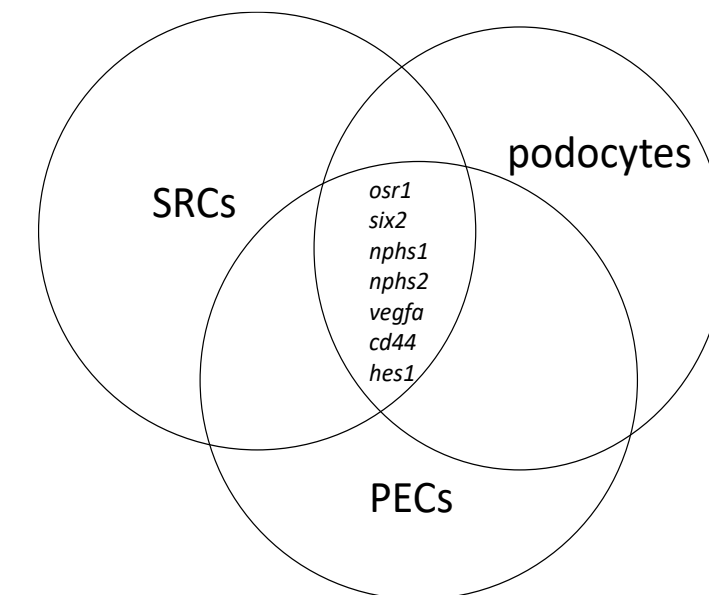
- The SRC admixture is composed of 3 components of the nephron characterized by ureteric bud, cap mesenchyme and podocyte markers<sup>3</sup>
- SRCs were isolated<sup>8</sup> from human kidneys obtained from the National Disease Research Interchange (Philadelphia, PA; protocol# RRON2 01 001A)
- scRNA seq was undertaken at UNC-Chapel Hill, NC to evaluate vascular endothelial growth factor A (VEGFA) gene expression by SRCs
- The intersection set of directionally aligned podocyte AND PEC AND SRC genes was seeded into knowledgebases<sup>9,10</sup> for identification of gene function and visualization of a hub node
- SRCs were placed in culture and the supernatant (24 hr) queried for secreted vascular endothelial growth factor A (VEGFA) using ELISA (#DVE00, R&D, MN)
- Angiogenic activity of VEGFA secreted by SRCs was evaluated using a human umbilical vein endothelial cell (HUVEC) tube formation assay

## RESULTS

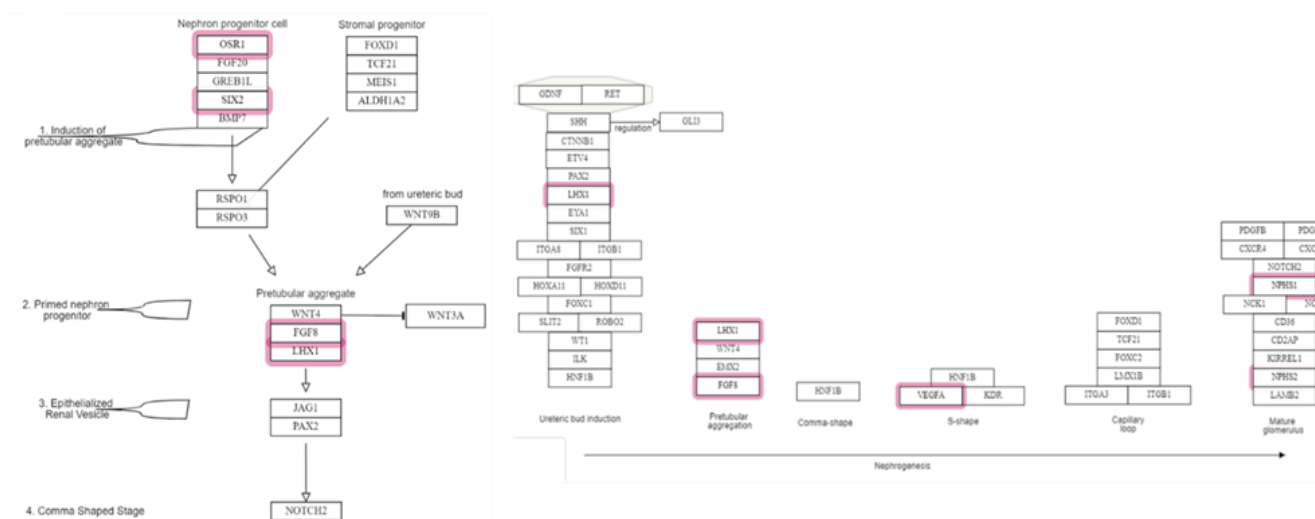
- osr1*, *six2*, *lhx1*, *fgf8*, *lfng*, *nphs1*, *nphs2*, *vegfa*, *cd44* and *hes1* are expressed by SRCs, podocytes and PECs
- These genes are associated with maintenance of undifferentiated nephron precursors, maturation of the glomerular barrier, formation of the glomerular capillary bed and nephron development
- vegfa* is a hub node within this network
- SRCs express *vegfa* and secrete VEGFA whose angiogenic activity is evidenced by HUVEC tube formation

## RESULTS CONTD.

### A SRCs Share Podocyte-PEC Genes



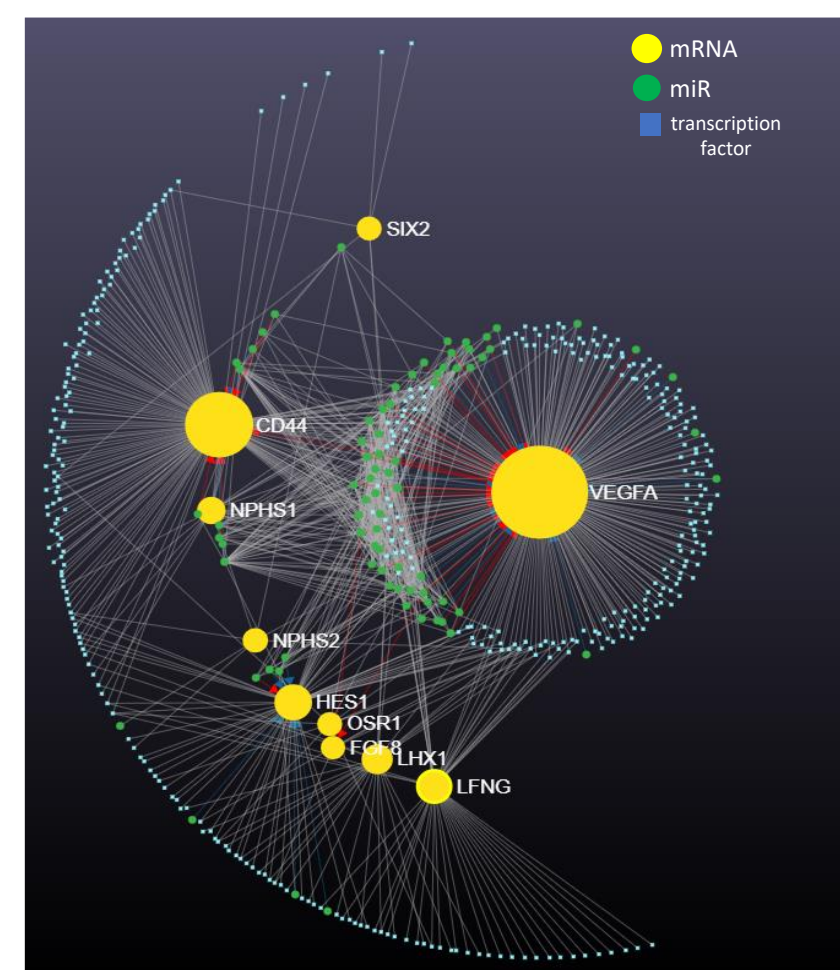
### B Shared Genes Pathways



### C Shared Genes Functions

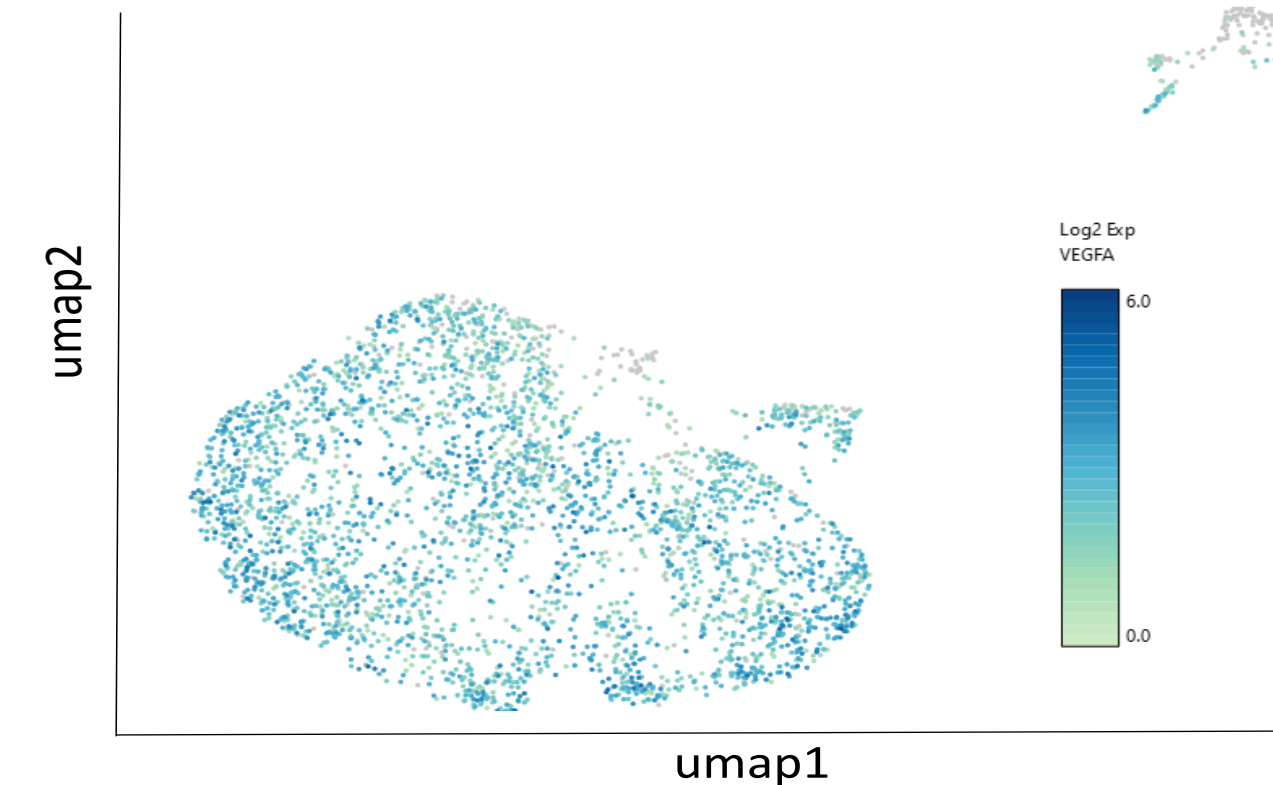
Gene	Function
<i>osr1</i>	maintains CM in undifferentiated state
<i>six2</i>	maintains CM in undifferentiated state
<i>lhx1</i>	drives intermediate mesoderm towards a renal progenitor cell population
<i>fgf8</i>	coordinates tissue elongation and cell epithelialization during kidney tubulogenesis
<i>lfng</i>	induction and patterning of the metanephric nephron
<i>nphs1</i>	maturation of podocytes and slit diaphragm structure
<i>nphs2</i>	maturation of podocytes and slit diaphragm structure
<i>vegfa</i>	formation of glomerular capillaries, guiding endothelial cells into the vascular cleft of the nephron
<i>cd44</i>	nephron formation and branching morphogenesis
<i>hes1</i>	nephron development

### D Vegfa is a Shared Genes Hub Node

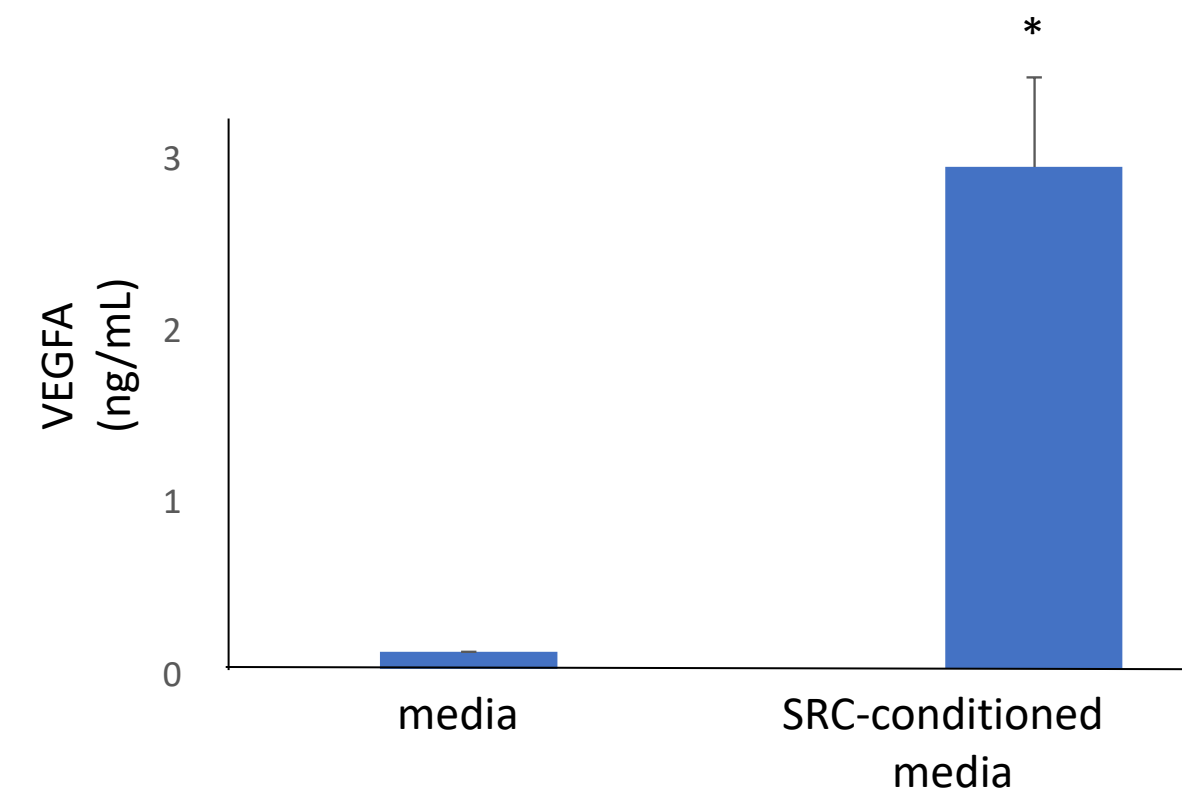


## RESULTS CONTD.

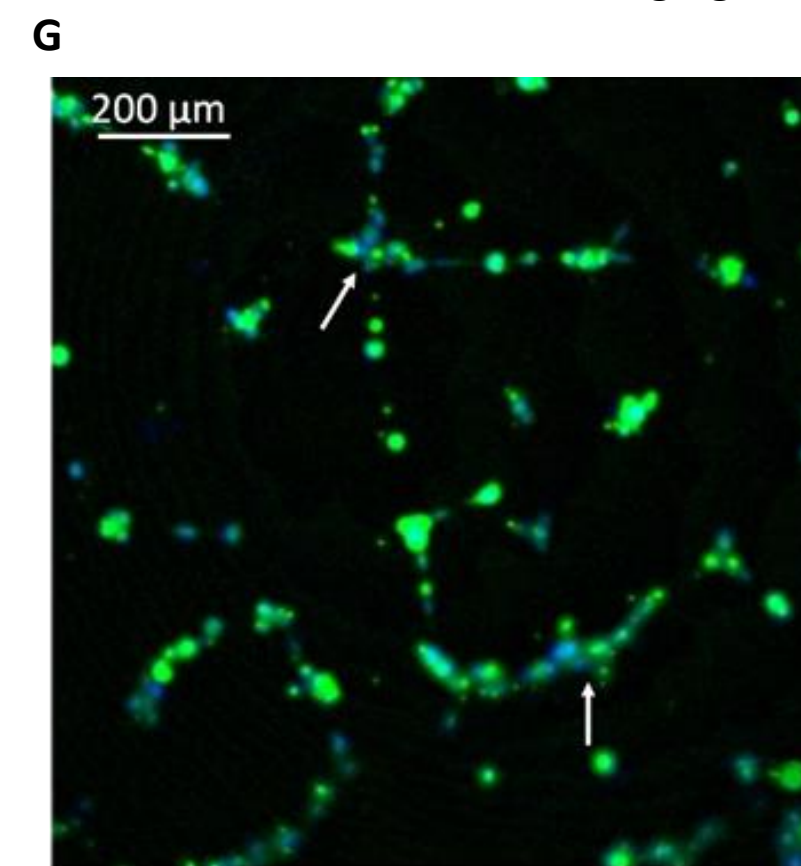
### E SRC Cellular Components Express vegfa



### F SRCs Secrete VEGFA



### G Secreted VEGFA Stimulates Angiogenesis



A. Genes expressed by SRCs, podocytes and PECs. B and C. Biological pathways and functions associated with these shared genes. D. Shared genes form an interactome with *vegfa* as the hub node. E. scRNA-seq analysis indicates that human SRCs express *vegfa*. F. SRC-conditioned (24 hr) media contains VEGFA (\*, p<0.05 vs. control media). G. Assembly of tubular networks from HUVEC in culture in the presence of SRC-derived conditioned media.

## DISCUSSION

- A *vegfa* anchored podocyte-PEC transcriptome expressed by cellular components of SRCs may participate in recapitulating events associated with the developing nephron and mediate the improved glomerular microarchitecture and glomerular barrier function associated with its administration in CKD models<sup>4-7</sup>
- SRCs represent a standalone cell-based platform with renal reparative and restorative potential
- SRC-secreted VEGFA may act as a potency marker for product (REACT®/rilparencel) release

## CLINICAL STATUS

- Preliminary data<sup>3, 11</sup> 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<sup>12</sup> 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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