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Renin Cell Development: Insights from Chromatin Accessibility and Single Cell Transcriptomics

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Renin cells are essential for survival. They control the composition and volume of our extracellular fluid, arterial blood pressure, tissue perfusion, and oxygen delivery. *FoxD1*+ cells are precursors for renin cells which in turn differentiate into smooth muscle cells (SMCs), mesangial cells (MCs), and pericytes (PCs). When arteriole assembly completes, mature renin cells are confined to the tip of the renal arterioles near the glomeruli, thus their name juxtaglomerular (JG) cells. The chromatin states and transcription factor (TF)

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Authors' Contributions

RAG and MLSSL conceived and designed the project. AGM designed and performed animal experiments and library preparation. JPS and AGM discussed and performed computational data analysis. AGM analyzed single time points and JPS developed methods to identify JG cells and evaluate differentiation trajectories across all developmental timepoints. NCS provided bioinformatic support. SM made libraries and deleted the *Nfix* gene in As4.1 cells. JPS wrote the manuscript, and all authors discussed the results and contributed to the final manuscript.

Data and Code Availability

- The single cell omics data have been uploaded to the Gene Expression Omnibus (GEO) with the accession IDs GEO: GSE218570.
- Code to reproduce analyses is available at https://github.com/jpsmith5/renin_analysis
- Any additional information is available from the Lead Contact(s), R. Ariel Gomez, upon request.

Disclosures

None.

repertoires that determine the differentiation trajectory of renin cells from their early progenitors to mature JG cells is unknown. We isolated single cells from *FoxD1*cre;mTmG mice at key developmental times in embryonic (E12: 15 embryos, E18: 17 embryos, and post-natal (P5: 12 mice 6F|6M, P30: 10 mice; 4F|6M) life and subjected them to integrated scRNA-seq and scATAC-seq. This approach allowed us to i) construct a single-cell atlas of chromatin accessibility and gene expression profiles, ii) establish the developmental trajectory that leads to the mosaic of cells that compose the kidney arterioles, and iii) identify transcription factors (TFs) that control the elusive, myo-endocrine adult renin-secreting JG cell.

We uncovered how successive changes in the transcriptome, accessibilome, and enriched TFs instruct *FoxD1* progenitor' cells to adopt the different cell fates that encompass the kidney vasculature (Figure A). As *Ren1* expression is crucial to JG cell identity, we predicted which regulatory elements and TFs regulate *Ren1* and identified MEF2 family members (Figure B). We confirmed the presence of *Mef2c* using immunohistochemistry (IHC) on mouse kidney sections throughout development in JG cells and SMCs (Figure C).

We found the TF, nuclear factor I X (*Nfix*), was enriched in JG cells (Figure A, right), and confirmed co-localization with renin from E18 to P30 by RNAscope (not shown). We knocked out *Nfix* in renin-expressing As4.1 cells using CRISPR-Cas9 and showed a significant decrease (99.6% drop) in *Ren1* mRNA (Figure D), indicating the functional relevance of *Nfix*. Ongoing *in vivo* studies will define whether *Nfix* affects the development of JG cells, SMCs and/or PCs and their ability to switch on-off the renin phenotype in responses to homeostatic challenges.

We identified enriched TF binding sites at *Ren1* and *Akr1b7*, two independent markers of JG cells (Figure E). We observed significant motif occurrences for MEF2 members and *Nfix* in a region unique to JGs with co-accessibility to the *Ren1* core promoter. (Figure E). We also identify overrepresented MEF2 motif occurrences within open chromatin of JG populations at the *Akr1b7* locus (Figure E).

Past work highlighted the bivalent nature of renin-expressing JG cells between endocrine and contractile phenotypes[1]. *Mef2c* has an essential role in skeletal muscle growth and differentiation, and we observed significant increases in transcript abundance ($F_{Welch's}$ and Games-Howell post-hoc tests: early JGs versus: early SMCs ($p_{Holm\text{-}adj} = 3.27\times10^{-9}$) or PCs ($p_{Holm\text{-}adj} = 0.03$)) and accessibility (early JGs versus: early SMCs ($p_{Holm\text{-}adj} = 0.00$) or PCs ($p_{Holm\text{-}adj} = 8.99\times10^{-5}$)) for *Mef2c* by the formation of early JGs (Figure F). Furthermore, we observed increased expression of the *Mef2c* paralog, *Srf*, in P30 populations of JGs (late JGs versus: PCs ($p_{Holm\text{-}adj} = 0.00$), early JGs ($p_{Holm\text{-}adj} = 2.52\times10^{-10}$) or late SMCs ($p_{Holm\text{-}adj} = 1.87\times10^{-13}$)) which is involved in SMC development and maintenance and likely contributes to the plasticity of cells able to express renin[1] (Figure F).

While further experimental evidence of the importance of MEF2 members at the renin locus is ongoing, an accumulation of data supports their relevance. MEF2 family members are transcriptional effectors of the Notch pathway, and MEF2 activation has been linked to stimulation by p300[2], which is critical to remodeling chromatin at the renin locus[3].

Additionally, myocardin (*Myocd*) family factors are direct targets of MEF2 members[4], and *Myocd* expression is significantly increased (early JGs versus: early SMCs ($p_{Holm-adj} = 1.43\times10^{-9}$) or PCs ($p_{Holm-adj} = 9.95\times10^{-12}$); late JGs versus: late SMCs ($p_{Holm-adj} = 0.00$) or MCs ($p_{Holm-adj} = 1.39\times10^{-9}$)) in JG populations (Figure F). Clinical support for *Mef2c* relevancy is seen in Rett, Angelman, Pitt-Hopkins syndromes, CDKL5 deficiency disorder, and the observation of duplex kidneys in pediatric populations with *Mef2c* haploinsufficiency[5]. The evidence points to MEF2 members as providing a pioneering and sustaining role in guiding renin cells through specific developmental stages to form the classical JG cell.

We provide the first developmental trajectory of renin cells in a single-cell atlas of kidney vascular development enriched for the renin lineage (Figure A). Specifically, we identified the MEF2 and NFI TF families as enriched and likely to control renin cell fate (Figure A, F). It will be exciting to define whether other factors and chromatin domains identified in this trajectory atlas control the development and plasticity of renin cells. Ultimately, determining the epigenomic-transcriptomic landscape of the rare JG cell is necessary to understand and predict responses in cardiovascular and kidney pathologies[3] and in the design of pharmaceutical compounds to treat hypertension.

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Non-standard Abbreviations and Acronyms

E12	embryonic day 12
E18	embryonic day 18
FBs	fibroblasts
JGA	juxtaglomerular apparatus
JGs	juxtaglomerular cells
MCs	mesangial cells
MEF	myocyte enhancer factor
P5	five days old
P30	one month old
PCs	pericytes
SE	super-enhancer

SMCs	smooth muscle cells

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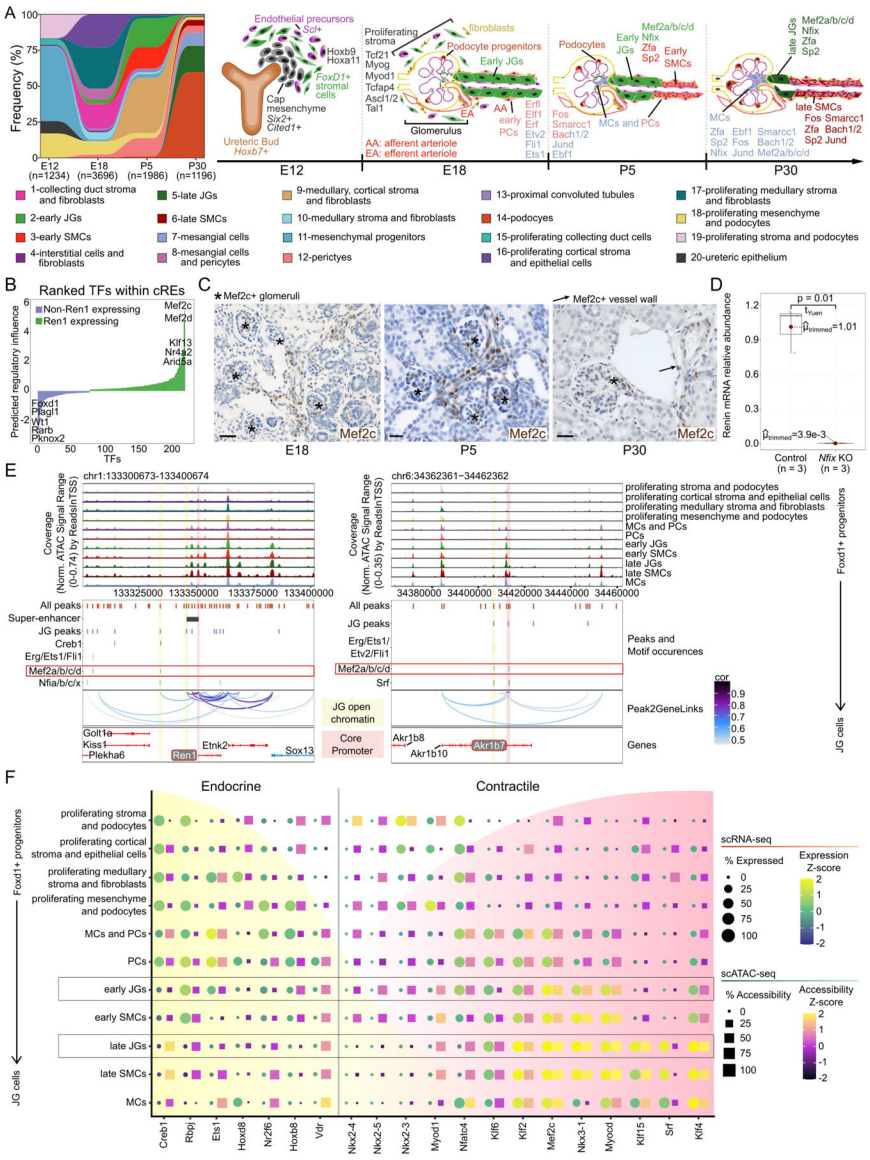

Figure: Chromatin and transcriptomic shifts during renin cell development.

(A), Alluvial plot of cell frequency distribution (left) and individually enriched TF motifs (right) across developmental time points. Hoxb9 and Hoxa11 are enriched at E12. At E18, Tcf21, Myog, Myod1, Tcfap4, Ascl1 and Ascl2, and Tal1 are enriched in proliferating stroma. Between E18 and P5, Erfl, Elf1, Erf, Etv2, Fli1, Fos, Smarcc1, Bach1 and Bach2, Jund, and Ebf1 are enriched in early PCs and mixed MCs and PCs. In early JGs and SMCs, Mef2a/b/c/d, Nfix, Zfa, and Sp2 motifs are enriched. By P30, MCs are enriched for Fos, Smarcc1, Bach1 and Bach2, Jund, Ebf1, and to a lesser extent Mef2a/b/c/d, Nfix, Zfa, and Sp2. Late JGs are enriched for Mef2a/b/c/d, Nfix, Zfa, and Sp2 motifs, and late SMCs for Fos, Smarcc1, Bach1 and Bach2, Jund, Ebf1, Zfa, and Sp2. (B), TFs ranked by predicted regulatory score. (C), Mef2c IHC in kidney sections at E18, P5, and P30. At E18, Mef2c is found along AAs, interlobular arterioles, and MCs while at P5, it is found inside glomeruli, MCs, AAs, interlobular arterioles, and at the JGA. Scale bars: 50μm. (D), Deletion of Nfix

in cultured As4.1 cells decreases Ren1 mRNA (n=3, p=0.01, Yuen-Welch's test). Three gRNAs were designed for Nfix knockout with 92% transfection efficiency. (E), Browser tracks identify enriched open chromatin and TF motif sites at Ren1 (left) and Akr1b7 (right). (F), Dot plot for markers of endocrine or contractile phenotypes throughout JG cell differentiation.