ANDROGEN ACTION IN CELL FATE AND COMMUNICATION DURING PROSTATE DEVELOPMENT AT SINGLE-CELL RESOLUTION

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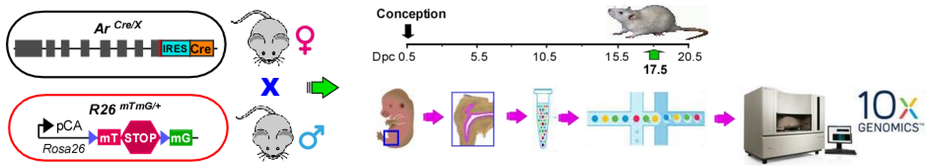
Introduction

Androgens/androgen receptor (AR) mediated signaling pathways are essential for prostate development, morphogenesis, and regeneration. Tissue recombination studies further demonstrated that mesenchymal, rather than epithelial, AR signaling plays a decisive role in inducing development of the prostatic epithelium through paracrine regulation. The cellular properties of AR-expressing cells and the mechanisms by which stromal androgen signaling initiates and regulates other pathways and regulators through mesenchymal-epithelial interactions during early prostatic development and morphogenesis remain unclear.

Experimental Design

To trace AR-expressing cells and assess their functions during prostate development, we used gene-targeting approaches to generate a mouse *Ar*$^{IRES\text{-}Cre}$ allele, which enables us to genetically mark AR-expressing cells and trace their fate and function in early prostate development. Utilizing single-cell mRNA sequencing (scRNAseq) and other experimental approaches, we evaluated the cellular properties of AR-expressing cells at single cell resolution.



Objectives

Determine the decisive role of mesenchymal AR action in prostate initiation

Conclusion & Discussion

- Androgen signaling-initiated signaling pathways were identified in mesenchymal niche populations at single cell transcriptomic resolution.

- We provide fresh, high resolution insight into AR signaling and its roles in initiating dynamic interactions with distinct signaling pathways between prostatic mesenchymal-epithelial cells.

Future Directions

Defining the cellular properties of *Gli1* and *Zeb1* expressing cells as prostatic stem cells and/or their niches cells in prostate development.

Acknowledgements

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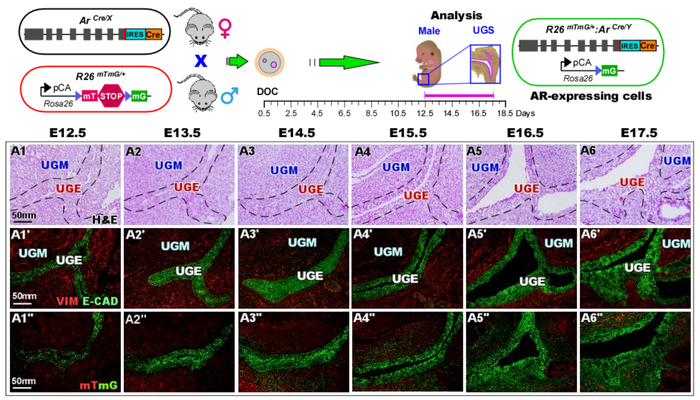
References

1. Cunha GR, Chung LW. Stromal-epithelial interactions--I. Induction of prostatic phenotype in urothelium of testicular feminized (Tfm/y) mice. J Steroid Biochem. 1981 Dec;14(12):1317-24. PubMed PMID: 6460136.
2. Cunha GR, Lung B. The possible influence of temporal factors in androgenic responsiveness of urogenital tissue recombinants from wild-type and androgen-insensitive (Tfm) mice. J Exp Zool. 1978 Aug;205(2):181-93. PubMed PMID: 681909. Epub 1978/08/01. eng.

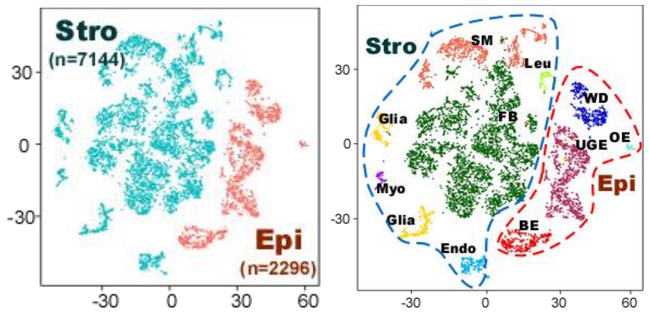
Results

Generation and characterization of *Ar*$^{IRES\text{-}Cre}$ mice



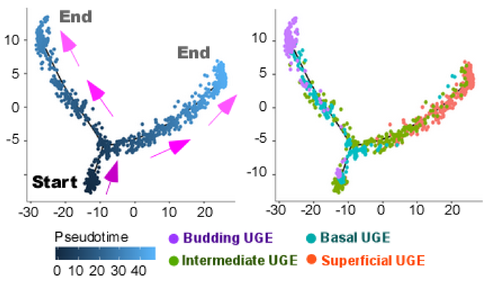
Genetic construct of the targeted Ar allele displaying the inserted IRES and Cre sequences. Schematics of the *Ar*$^{IRES\text{-}Cre}$ and *R26*$^{mTmG/+}$ alleles are shown in relation to the mating strategy for this experiment. Following the day of conception (DOC), a timeline is provided indicating the days of analysis as shown. A construct is displayed demonstrating the recombination event that will take place in *Ar* expressing cells resulting in a change from red to green fluorescence. A1-A6 Representative H&E images with dashed lines separating urogenital sinus epithelium and mesenchyme at the indicated time points. A1'-A6" Representative fluorescence imaging for the indicated proteins/antibodies

Single-cell RNA sequencing of E17.5 male mouse UGS



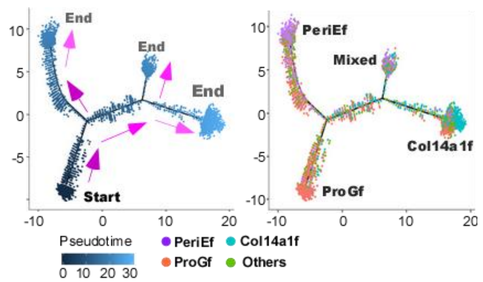
Gene expression tSNE plots for the indicated epithelial and stromal cell marker genes. Identification of cell types as indicated within the original clustering results

Trajectory analysis of the urogenital sinus epithelium



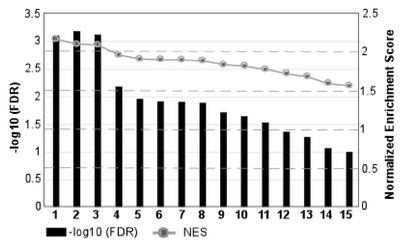
Pseudotime plot displays a predicted directional path of differentiation between cell types as indicated.

Trajectory analysis of fibroblast cells



Pseudotime indicates a predicted pathway of differentiation between the fibroblast subtypes as indicated

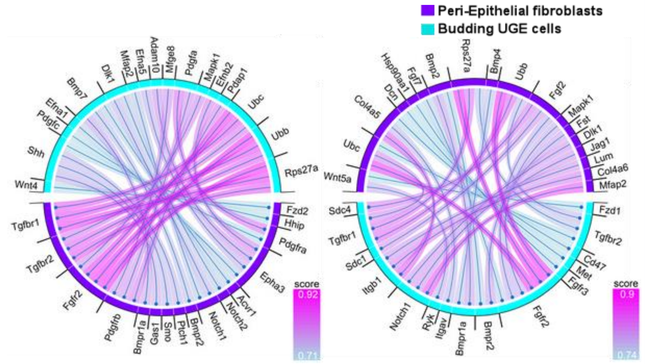
GSEA pathway analysis



1. GO_EMBRYONIC_ORGAN_MORPHOGENESIS
2. GO_EPITHELIAL_TO_MESENCHYMAL_TRANSITION
3. GO_EPITHELIAL_CELL_DIFFERENTIATION
4. GO_EPITHELIAL_TUBE_MORPHOGENESIS
5. GO_BRANCH_ELONGATION_OF_AN_EPITHELIUM
6. GO_RESPONSE_TO_RETINOIC_ACID
7. GO_REGIONALIZATION
8. GO_UROGENITAL_SYSTEM_DEVELOPMENT
9. GO_REGULATION_OF_STEM_CELL_DIFFERENTIATION
10. KEGG_PROSTATE_CANCER
11. HALLMARK_WNT_BETA_CATENIN_SIGNALING
12. GO_RESPONSE_TO_BMP
13. GO_PROSTATE_GLAND_DEVELOPMENT
14. HALLMARK_ANDROGEN_RESPONSE
15. KEGG_HEDGEHOG_SIGNALING_PATHWAY

GSEA pathway analysis results comparing the peri-epithelial fibro-blast clusters to the remaining fibroblasts.

Predicted ligand-receptor interactions



Predicted ligand-receptor interactions between budding UGE cells and peri-epithelial fibroblasts as indicated, generated using SingleCellSignalR. Color scale corresponds to interaction scores for each interaction.