A Multimodal Atlas of the Human Seminal Vesicle and Vas Deferens

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Abstract

The lower urinary tract includes the ureters, bladder, prostatic urethra, prostate, seminal vesicles and vas deferens in the male, bladder and urethra in the female (**Figure 1**). There are several benign and malignant urological diseases in need of personalized clinical treatment based on molecular data[1,2]. Our goal is to generate a reference atlas of the lower urinary tract in males and females of variable ancestry to understand cell type-specific alterations associated with aging, sex, ancestral background and disease, such as cancer. These foundational data are being used to design new mouse models to study novel hypotheses about dysfunction.

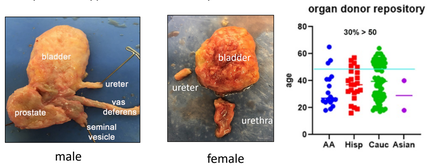

Figure 1. Human male and female lower urinary tract. Gross anatomy and spectrum of age and ethnicity represented in our human tissue biorepository.

We employ the latest multiomic single cell and spatial technologies on healthy human lower urinary tract specimens from a local organ transplant organization and diseased specimens from our university hospital. Single cell RNA sequencing and single nuclear RNA/ATAC sequencing has been performed on healthy distal ureter, bladder, urethra, seminal vesicle, vas deferens and prostate. New and previously established cell types were validated *in situ* with immunohistochemistry, RNAscope and Xenium spatial transcriptomics.

The vas deferens carries sperm from the testes and connects to the seminal vesicle before entering the dorsal side of the prostatic urethra. The seminal vesicle contributes to the seminal fluid to aid in fertility. Neither the vas deferens nor the seminal vesicle have been examined at the cellular level.

Single cell and spatial omics methods

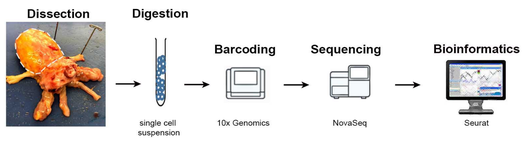

Figure 2. Single-cell RNA-sequencing workflow. Male LUT specimens were collected from organ donors aged 18-40 after consent at the Southwest Transplant Alliance according to approved IRB protocols. Vas deferens (n = 4) and seminal vesicle (n = 4) were dissected, digested with 2-4mg/ml collagenase for 4 hours for encapsulation and barcoding using the 10x Genomics Chromium X controller. Libraries were sequenced to ~50k reads/cell and analyzed using Seurat.

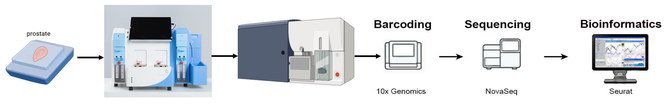

Figure 3. Single nuclear RNA/ATAC sequencing workflow. Male LUT specimens were collected from organ donors aged 18-40 after consent at the Southwest Transplant Alliance according to approved IRB protocols. Vas deferens (n = 4) and seminal vesicle (n = 4) were dissected, embedded in OCT, digested with the Singulator, flow sorted for viable nuclei, and barcoded using the 10x Genomics Chromium X controller. Libraries were sequenced to ~20k reads/nucleus and analyzed using Seurat.

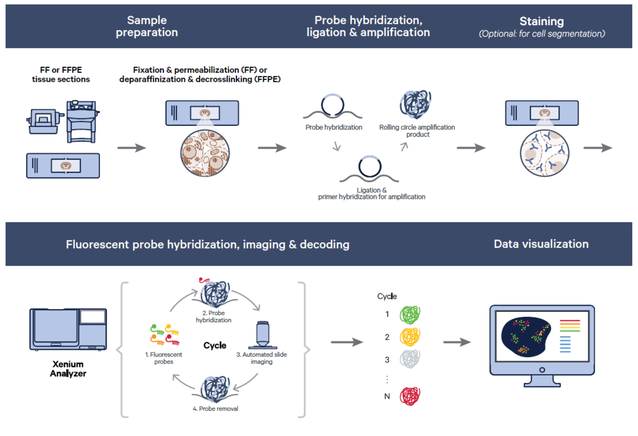

Figure 4. Xenium spatial transcriptomics workflow. Vas deferens and seminal vesicle were embedded in paraffin and sectioned into 5 um sections. Slides were incubated with 5,100 probes. Tissues were cycled with rounds of fluorescent probes and imaged for data visualization through the Xenium software.

Harmonization of single nuclear sequencing and spatial transcriptomics

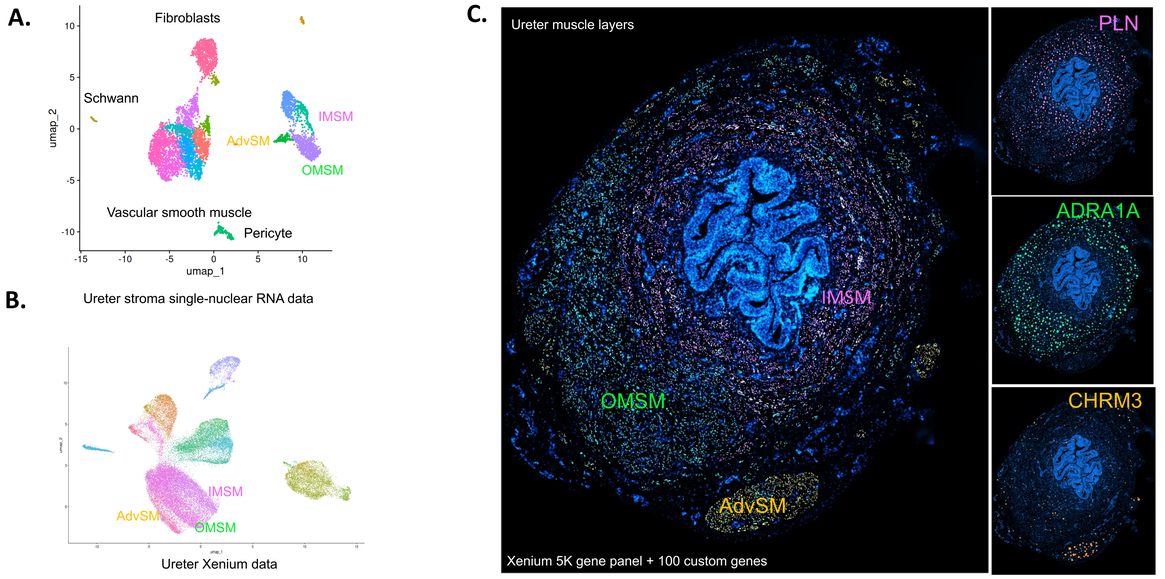

Figure 5. Validation of ureter muscle layers in the snRNA data using Xenium spatial transcriptomics. (A) snRNA sequencing data of male and female ureter. The smooth muscle clusters in into four clusters, three of which we have labeled outer-muscularis smooth muscle (OMSM), inner-muscularis smooth muscle (IMSM), and advential smooth muscle (AdvSM). **(B)** Xenium spatial transcriptomics was performed on one male and one female ureter using a 5,000 gene panel + 100 custom genes. Xenium software segments cells within tissue sections that can then be clustered based on shared transcripts. We have highlighted that the cell types verified in **(C)** do exist in the clustered data. **(C)** Xenium spatial transcriptomics of a male ureter (female not shown) showing cell annotation labeled segmented cells, showing OMSM (green), IMSM (pink), and AdvSM (orange). Cell types are labeled by anchor genes including ADRA1A (OMSM, green), PLN (IMSM, pink), and CHRM3 (AdvSM, orange).

Motif activity inference from snRNA/ATAC-seq of human prostate

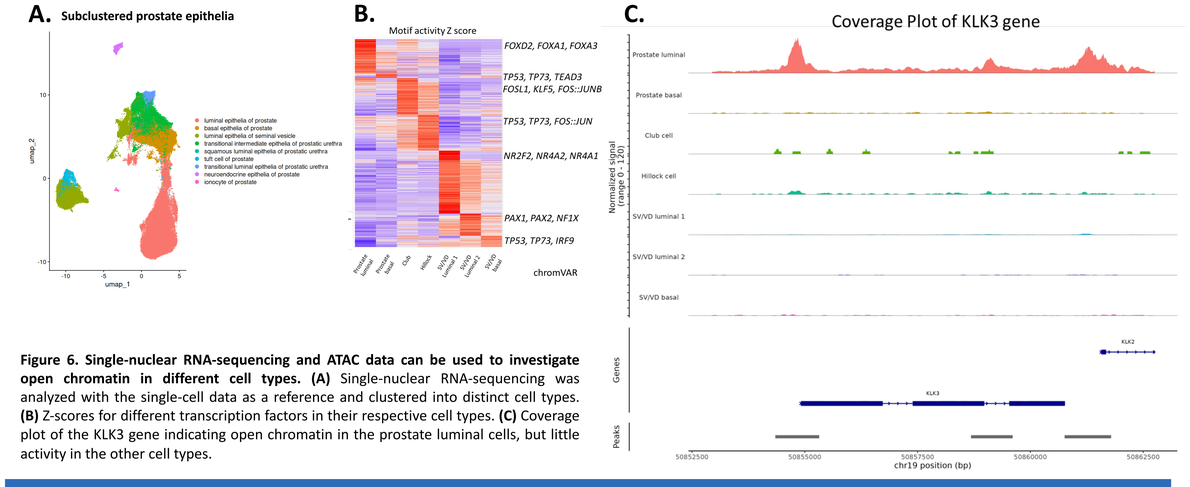

Figure 6. Single-nuclear RNA-sequencing and ATAC data can be used to investigate open chromatin in different cell types. (A) Single-nuclear RNA-sequencing was analyzed with the single-cell data as a reference and clustered into distinct cell types. **(B)** Z-scores for different transcription factors in their respective cell types. **(C)** Coverage plot of the KLK3 gene indicating open chromatin in the prostate luminal cells, but little activity in the other cell types.

Single nuclear RNA/ATAC sequencing of seminal vesicle and vas deferens

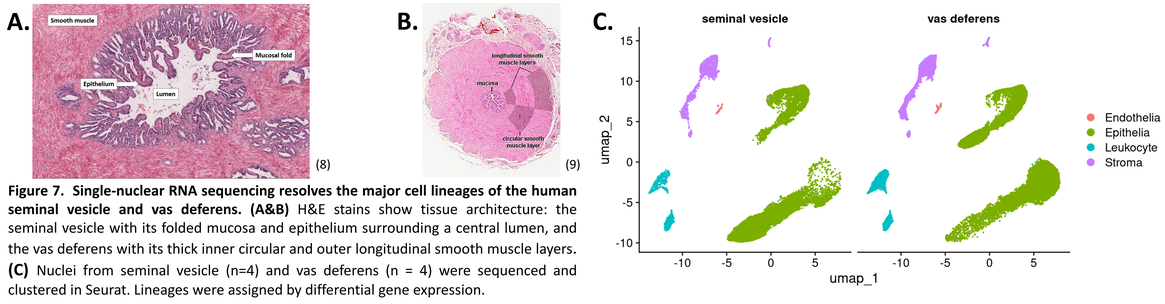

Figure 7. Single-nuclear RNA sequencing resolves the major cell lineages of the human seminal vesicle and vas deferens. (A&B) H&E stains show tissue architecture: the seminal vesicle with its folded mucosa and epithelium surrounding a central lumen, and the vas deferens with its thick inner circular and outer longitudinal smooth muscle layers. **(C)** Nuclei from seminal vesicle (n=4) and vas deferens (n = 4) were sequenced and clustered in Seurat. Lineages were assigned by differential gene expression.

Identification of anchor genes in ejaculatory duct lineages

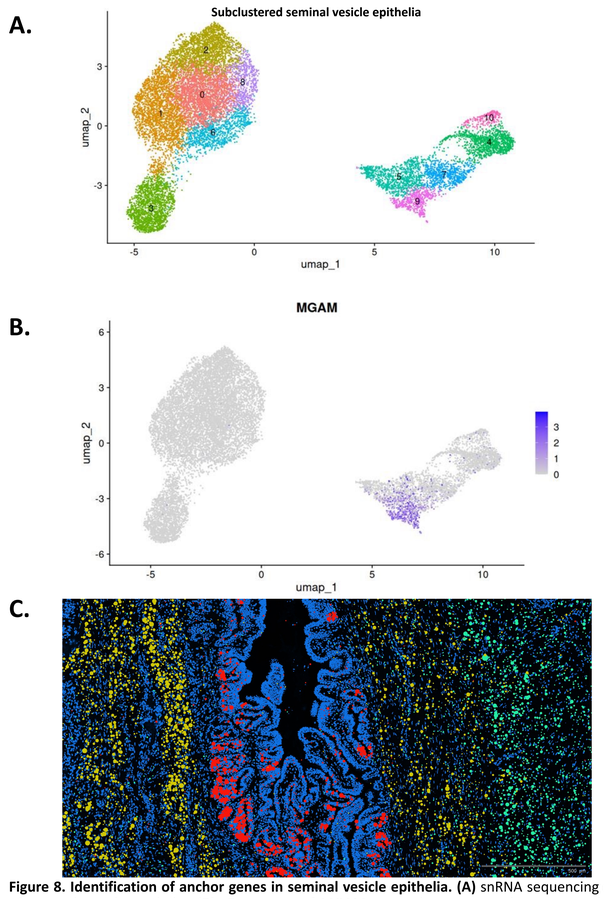

Figure 8. Identification of anchor genes in seminal vesicle epithelia. (A) snRNA sequencing data of seminal vesicle epithelia. **(B)** Expression of MGAM, an anchor gene marking one epithelial subtype. **(C)** Xenium spatial transcriptomics of ejaculatory duct confirms markers *in situ:* PI16 fibroblast (yellow), MGAM epithelia (red), and FLNC muscle (green).

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Acknowledgements and Funding

We want to thank the staff and transplant surgeons at the Southwest Transplant Alliance as well as the families of organ donors for their commitment to basic science research.

RC2DK129994 (2022-2027) PI
R01DK135535 (2022-2027) PI
R01CA245294 (2021-2025) Co-I
CZI 13455SC (2022-2024) Co-I
R01DK107436 (2021-2025) Co-I
R01DK124067 (2021-2024) Co-I
U54DK104310 (2020-2024) Co-I