Decoding Stromal Heterogeneity across BPH Phenotypes

Background: Benign Prostatic Hyperplasia (BPH) is a non-malignant enlargement of the prostate that occurs with aging and is associated with Lower Urinary Tract Symptoms (LUTS). Therapeutic options often fail, necessitating surgical resection of the prostate. The phenotypic and cellular heterogeneity of BPH is thought to contribute to treatment resistance. BPH patients present with multiple nodules grouped around the prostatic urethra in the transition zone. The composition of these nodules vary with some being solely comprised of stromal cells and others containing a mixture of stromal and epithelial cells. In addition, some patients present with a band of fibrotic tissue around the prostatic urethra that we term as peri-urethral fibrosis. Here, we describe stromal cell heterogeneity in the normal human prostate and across the different BPH phenotypes.

Methods: We used unbiased single cell RNA-sequencing (scRNA-seq) to obtain transcriptomic identities of stromal cells from normal human prostates and prostates from BPH patients who underwent simple prostatectomy. Cell clusters identified from scRNA-seq were validated *in situ* using immunohistochemistry and RNA *in situ* hybridization.

Results: We found that the stromal composition of the normal prostate consists of two major fibroblast populations, a prostate smooth muscle cell type, a vascular smooth muscle cell type and pericytes. One fibroblast sub-type, marked by expression of MFAP4, is abundant around the prostatic urethra and in the interstitial space between prostate glands. The second fibroblast population, marked by expression of APOD, is found closely associated with the secretory epithelium of the prostate and is absent from the spaces between prostate glands. The MFAP4+ fibroblast subtype extends into the bladder and represents a lower urinary tract fibroblast whereas APOD+ fibroblasts are restricted to the prostate. MFAP4+ fibroblasts are present within stromal and glandular nodules from BPH patients and are increased in peri-urethral fibrosis. APOD+ fibroblasts are absent from stromal nodules and regions of peri-urethral fibrosis. Desmin expressing smooth muscle cells are largely absent from regions of peri-urethral fibrosis. Wisps of smooth muscle are present in stromal nodules while glandular nodules are packed with Desmin expressing cells.

Conclusions: Our results highlight the identity and anatomical location of stromal cell types in the normal and BPH prostate. We expect that a molecular understanding of stromal cell types in the prostate will aid in a better understanding of the etiology of BPH.