Characterization of Inflammatory Cells in Human Benign Prostatic Hyperplasia

Renee E. Vickman[1], Gregory M. Cresswell[2], Nadia A. Lanman[2], Meaghan M. Broman[2], Omar E. Franco[1], Brian T. Helfand[1], Alexander Glaser[1], Jacqueline Petkewicz[1], Pooja Talaty[1], Timothy L. Ratliff[2], and Simon W. Hayward[1]

[1]NorthShore University HealthSystem, Evanston, IL, USA
[2]Purdue University, West Lafayette, IN, USA

MP06-11

Abstract

Background: Benign prostatic hyperplasia (BPH) is a common disease in aging men characterized by enlargement of the prostate and often associated with lower urinary tract symptoms. Inflammatory cells are abundant within BPH tissues, and high-grade inflammation has been demonstrated to limit the success of current therapies. However, the mechanisms by which immune cells influence BPH progression and therapy resistance are not well understood. The purpose of these studies is to characterize the subpopulations of inflammatory cells within small (<60 grams) *versus* large (>70 grams) human prostate tissues, representing the progression of BPH.

Materials and Methods: To define immune cell types throughout BPH progression, the transition zone was isolated from fresh human prostate tissues after robotic-assisted laparoscopic prostatectomy or simple prostatectomy. Samples were minced and digested, followed by cell sorting of viable, CD45+EpCAM-CD200- immune cells. The 10x Chromium System was used to perform single-cell mRNA-sequencing (scRNA-seq) on the isolated CD45+ cells from small or large prostate tissues. CellRanger and Seurat were used for data analysis, evaluation of cell clusters, and differential pathway analysis.

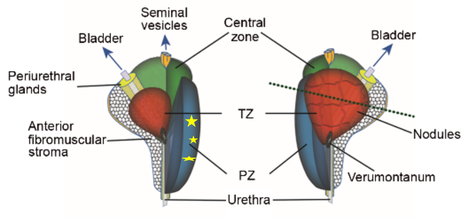
Results: CD45+ cells from ten small and four large tissues were evaluated from men ages 61-76. Patients with large prostates had a significantly increased International Prostate Symptom Score, but no significant differences in age or body mass index. CD45+ cells are more abundant in large versus small prostate tissues. Preliminary analysis using unsupervised clustering identified 14 immune cell clusters among all samples, with no significant changes in CD45+ subpopulations when comparing small and large samples. There may be a slight increase in B cell and plasma cell populations in patients with large prostates. Addition of cell surface protein markers aided in the classification of CD45+ cell types at the single-cell level and pathway analysis suggests altered cytokine signaling in samples from large versus small prostates.

Conclusions: CD45+ immune cells are abundant in human BPH and accumulate in large prostate tissues. scRNA-seq of CD45+ cells identified an array of immune cell subtypes present, with similar proportions of immune cell types within BPH samples as the disease progresses. Ongoing studies will investigate alterations in cytokine signaling between CD45+ cells in these two groups. These studies will functionally characterize inflammation during BPH progression and identify signaling pathways that may be utilized for therapeutic targeting.

Introduction and Methods

These data are a summary of an ongoing project in which complete analysis will include 10 small and 10 large prostate tissues. This will yield a dataset with comprehensive analysis of the immune cell compartment throughout BPH progression. An overview of patient selection criteria and tissue processing for scRNA-seq is summarized below:

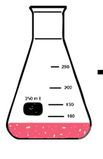
"Small" prostate representing early BPH
- prostate removed due to cancer
- used TZ tissue only
- size <60 grams
- N = 10



"Large" prostate representing advanced BPH
- prostate removed due to clinical BPH
- used TZ tissue only
- size >70 grams
- N = 4

Tissue Processing and CD45+ Cell Sorting:

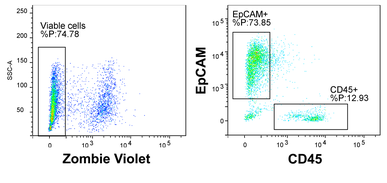
Tissue Digestion



Staining

Zombie Violet (viability)
CD45 (immune marker)
EpCAM (epithelial marker)
CD200 (endothelial marker)

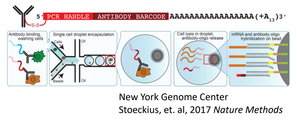
Cell Sorting of EpCAM-CD200-CD45+ immune cells



Single-cell Sequencing and Analysis:

TotalSeq™ Antibody Labeling

CD3
CD4
CD8
CD11b
CD19
CD279 (PD-1)



New York Genome Center
Stoecklius, et. al, 2017 *Nature Methods*

Drop-seq

scRNA-seq and Data Analysis

Results

Patient Characteristics:

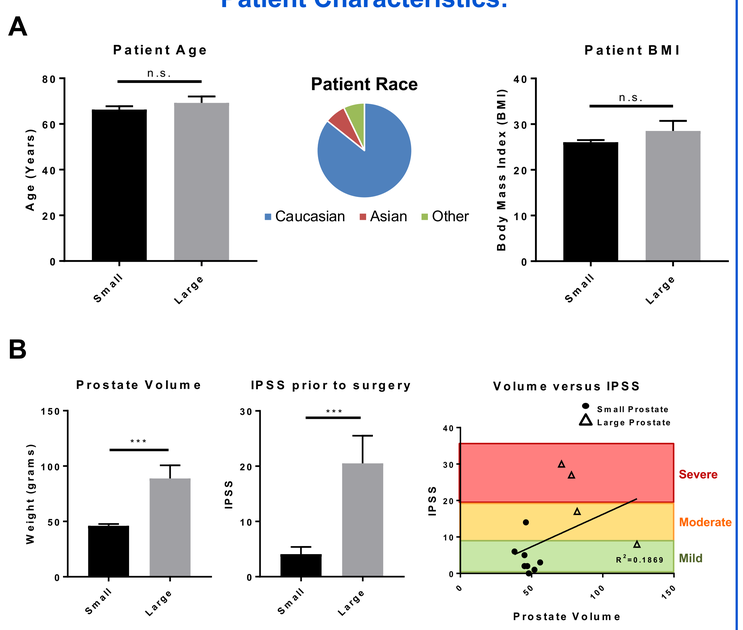


Figure 1. Patient characteristics and clinical parameters from patient samples used for scRNA-seq studies. Fresh transition zone from human prostate tissue was obtained after surgical removal of the prostate. **A)** Patient characteristics comparing the individuals with small (n=10) or large (n=4) including age, race, and body mass index (BMI). **B)** Post-surgical prostate volumes and International Prostate Symptom Score (IPSS) of patients with small (n=10) and large (n=4) prostates. A plot demonstrating the relationship between prostate volume and IPSS is also included.

CD45+ Immune Cells in Small and Large Prostates:

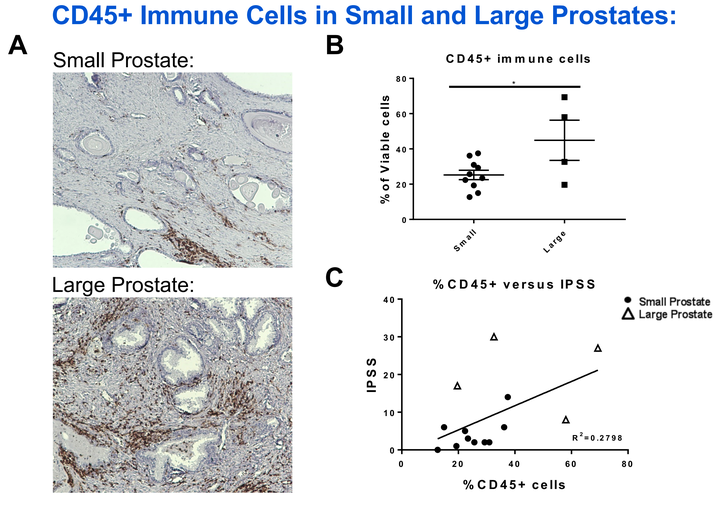


Figure 2. CD45+ immune cells accumulate in BPH. A) Immunohistochemistry indicating CD45+ cells within the transition zone of human prostate tissue. Samples are representative of the small (n=10) and large (n=4) prostate tissues used for scRNA-seq analysis. **B)** Comparison of the percentage of CD45+ cells (gated on Viable cells) from individual patients with small or large prostates by flow cytometry. **C)** Plot comparing %CD45+ cells *versus* IPSS for individual patients, with linear regression line shown.

Abbreviations

BMI = body mass index
BPH = benign prostatic hyperplasia
CITE-seq = cellular indexing of transcriptomes and epitopes by sequencing
IPSS = International Prostate Symptom Score
scRNA-seq = single-cell mRNA sequencing
UMAP = Uniform Manifold Approximation and Projection

Disclosures

The authors declare no conflicts of interest.

Funding

This work is funded by 1P20DK116185 from NIDDK. It has also been supported by the Rob Brooks Fund for Precision Prostate Cancer Care and Walther Cancer Foundation.

Combined Clustering of CD45+ Cells From 10 Small and 4 Large Prostates:

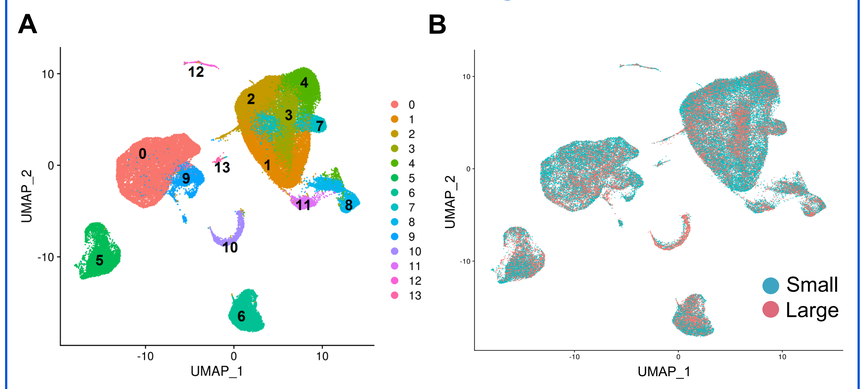


Figure 3. Combined clustering of CD45+ cells from 14 human prostate tissues. Fresh transition zone from small (n=10) or large (n=4) human prostate tissues were obtained. Tissue digestion and cell sorting allowed for scRNA-seq of ~5,000 CD45+ cells per sample. Unsupervised clustering of all 14 samples yielded 14 clusters with unique differential gene expression profiles. Uniform Manifold Approximation and Projection (UMAP) plots indicate the 14 unique clusters in the combined clustering (A) or contributions of the small *versus* large prostates to each cluster (B).

Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq) Technology Aids in Cluster Identification:

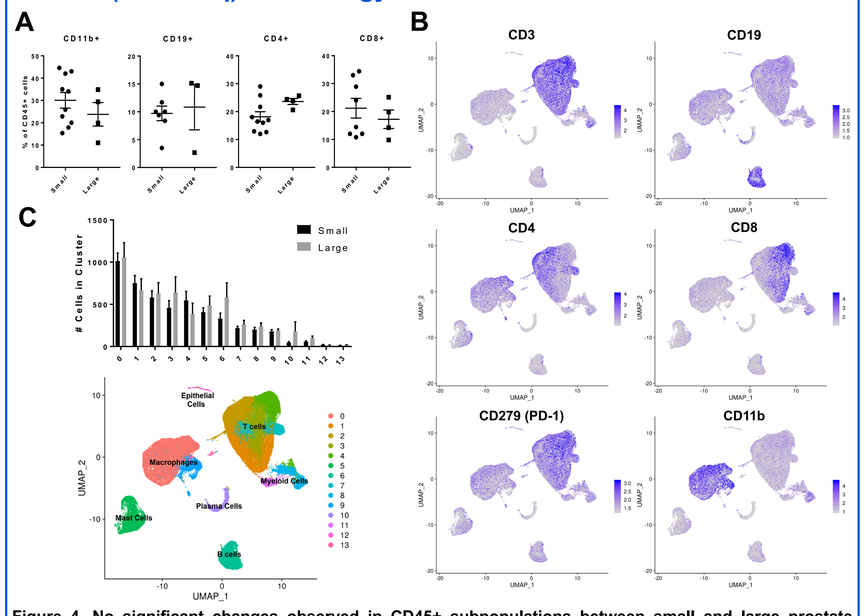


Figure 4. No significant changes observed in CD45+ subpopulations between small and large prostate samples. A) Evaluation of percent CD11b+ myeloid cells, CD19+ B cells, CD4+ T cells, and CD8+ T cells (gated on CD45+ cells) within patient samples by flow cytometry. **B)** Expression profile of indicated cell surface protein markers within individual cells in combined UMAP plot. This analysis was completed using CITE-seq technology. Blue indicates positive expression. **C)** Comparison of the number of cells within each cluster between small and large specimens. A UMAP plot indicating general cell types is included for reference.

Summary and Future Directions

- CD45+ cells accumulate in human BPH tissue as the disease progresses.
- A variety of immune cells are present in human prostate, including T cells, macrophages and other myeloid cells, B cells, plasma cells, and mast cells. However, the relative proportions of these cell types does not change significantly as prostates enlarge.
- CITE-seq, using TotalSeq™ antibodies, can be used to quickly identify cell populations in scRNA-seq analysis.
- Preliminary KEGG analysis for each cluster has identified many intracellular signaling pathway alterations between small and large samples.
- Functional characterization of the immune cell subpopulations in BPH is ongoing.
- Analysis of intracellular and extracellular signaling pathways will be evaluated for changes throughout BPH progression. Identification of targetable pathways that contribute to BPH pathogenesis will yield novel therapeutic strategies to limit progression of this disease.