Macrophage-Derived Tumor Necrosis Factor Contributes to Benign Prostatic Hyperplasia Through Increased Fibroblast Proliferation

Renee E. Vickman[1], Nadia A. Lanman[2], Meaghan M. Broman[2], Omar E. Franco[1], Victoria Gil[1], Gregory M. Cresswell[2], Brittany Lapin[1], Max Greenberg[1], Brian T. Helfand[1], Alexander Glaser[1], Susan E. Crawford[1], Timothy L. Ratliff[2], and Simon W. Hayward[1]

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

Abstract

Background: Benign prostatic hyperplasia (BPH) is characterized by enlargement of the prostate, and commonly involves inflammation. High-grade inflammation has been demonstrated to limit the success of current therapies. However, the mechanisms by which immune cells promote expansion of the prostate and whether these can be controlled therapeutically are not well elucidated. Previous evaluation of patient medical records indicated that men with an autoimmune disease (AID) diagnosis were significantly more likely to also be diagnosed with BPH, while systemic therapy of AID using tumor necrosis factor (TNF)α-antagonists reduced BPH diagnoses back to a baseline incidence rate. Here, we investigated the inflammatory component of human BPH tissues and assess whether TNFα regulates proliferation of epithelial or stromal cells from BPH tissues.

Methods: These studies utilized histological evaluation of human BPH tissues from our prostate tissue repository as well as *in vitro* cell proliferation assays using benign prostate epithelial (BHPrE-1 and NHPrE-1) or stromal (BHPrS-1) cell lines, monocyte-like THP-1 cells, and primary fibroblast cultures isolated from BPH tissues following simple prostatectomy. We also isolated transition zone tissue from small (<40 grams) *versus* large (>90 grams) human prostates and sorted viable, CD45+EpCAM-CD200- immune cells for single-cell mRNA-sequencing (scRNA-seq) analysis using the 10x Genomics Chromium System. CellRanger and Seurat were used for data analysis, evaluation of cell clusters, and differential pathway analysis.

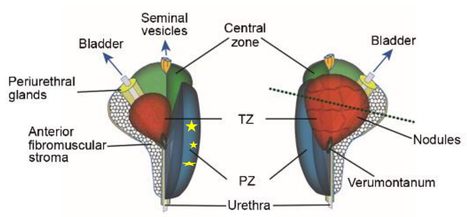
Results: Single-cell mRNA-sequencing (scRNA-seq) analysis of CD45+ immune cells from BPH tissues indicate significantly altered pathways related to autoimmune inflammatory (AI) conditions from large *versus* small tissues. TNFα and its receptors are expressed by macrophages and *in vitro* studies demonstrate that TNFα and macrophage conditioned medium stimulates proliferation in primary fibroblasts. Furthermore, macrophage-induced proliferation in primary fibroblast cultures can be abrogated by TNFα neutralization in some cultures but not others, indicating patient-specific responses to this pathway. Finally, histological evaluation of prostate specimens from seven patients taking TNFα-antagonists at the time of procedure indicate these tissues have reduced proliferation compared to matched controls.

Conclusions: These studies implicate macrophage-derived TNF as a driver of BPH. Studies to determine whether TNF targeting may yield therapeutic benefit for BPH patients are ongoing.

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:

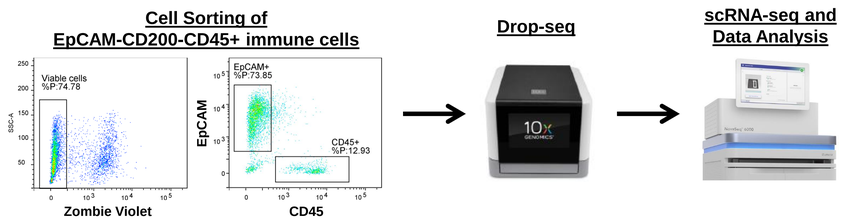
“Small” prostate representing early BPH
- prostate removed due to cancer
- used TZ tissue only
- size <40 grams
- N = 10



“Large” prostate representing advanced BPH
- prostate removed due to clinical BPH
- used TZ tissue only
- size >90 grams
- N = 4

CD45+ immune cells were isolated via fluorescence activated cell sorting on a BD FACSAria II. Cells staining positive for EpCAM (epithelial), CD200 (endothelial), or Zombie Violet (dead) were excluded from the analysis. Approximately 5000 CD45+ cells were loaded into the Chromium system (10X Genomics) and prepared for scRNA-seq analysis.

Cell Sorting of EpCAM-CD200-CD45+ immune cells → **Drop-seq** → **scRNA-seq and Data Analysis**



An electronic data warehouse study including over 80,000 patient records of men over the age of 40 at NorthShore was completed to determine whether autoimmune inflammatory (AI) conditions were positively associated with BPH diagnosis. The baseline incidence rate for men without AI conditions is 23.6%, but men with AI conditions have a significantly increased BPH incidence rate of 33.8% (Table 1). However, if men with AI conditions were treated for that condition prior to BPH diagnosis, their incidence rate fell below the baseline level to 20.9% (Table 2). The clinical therapy that significantly attributed to this decrease was TNFα antagonist treatment (Table 3).

Disclosures

The authors declare no conflicts of interest.

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Results

Electronic Database Warehouse Study: Men with AI Conditions Have Increased BPH Incidence

Table 1. All Men, N (%)

Autoimmune 8203 (10.2)		No Autoimmune 72503 (89.8)		
BPH	No BPH	BPH	No BPH	P-Value
2774 (33.8)	5429 (66.2)	17113 (23.6)	55390 (76.4)	<0.001

Table 2. Men where Autoimmune occurred PRIOR TO BPH Diagnosis, N (%)

Autoimmune 6860 (8.6)		No Autoimmune 72503 (91.4)		
BPH	No BPH	BPH	No BPH	P-Value
1431 (20.9)	5429 (79.1)	17113 (23.6)	55390 (76.4)	<0.001

Multivariable Regression Model: Predicting BPH in Men with AI Conditions Based on Treatment Prior to BPH Diagnosis

Table 3. TNFα antagonist treatment reduces BPH incidence in men with autoimmune inflammatory conditions.

Predictors	Odds Ratio	95% CI	P-Value
Methotrexate	1.05	0.79-1.40	0.745
TNFα antagonists	**0.67**	**0.50-0.91**	**0.010**
Age 60+	4.92	1.30-5.63	<0.001
Race			
Caucasian	Reference		
African-American	1.16	0.81-1.65	0.427
Hispanic	0.72	0.45-1.17	0.186

Macrophages from Small and Large BPH Tissues Express TNF and TNF Receptors:

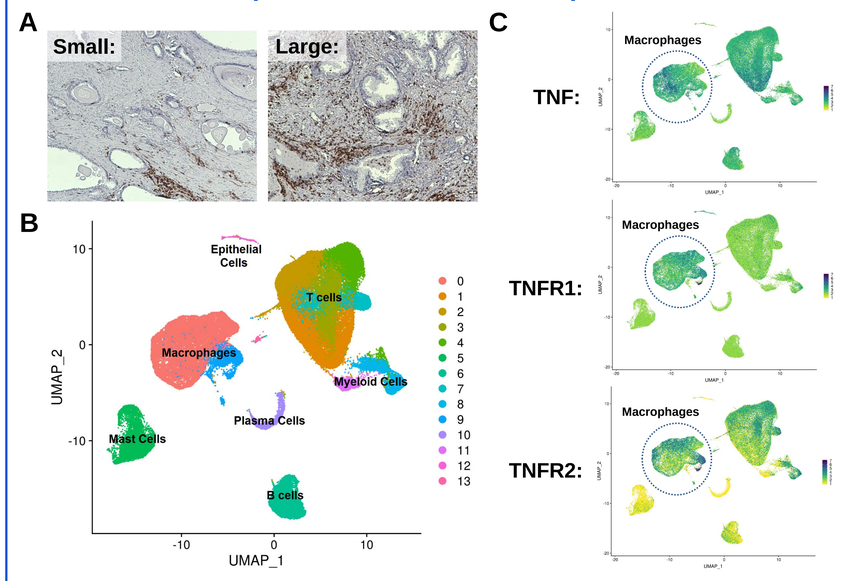

Figure 1. Combined clustering of CD45+ cells from 14 human prostate tissues. A) Immunohistochemistry indicating CD45+ cells within the transition zone of human prostate tissue. Samples are representative of the small (n=10) and large (n=4) prostate tissues used for scRNA-seq analysis. B-C) Fresh transition 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 (B) putative cell types from the 14 unique clusters in the combined clustering, or (C) relative expression of TNF, TNFRSF1 (TNFR1), or TNFRSF1B (TNFR2) genes in individual cells, where yellow indicates low expression and purple indicates high expression.

Primary BPH Fibroblast Growth is Stimulated by TNF and Macrophage Conditioned Medium:

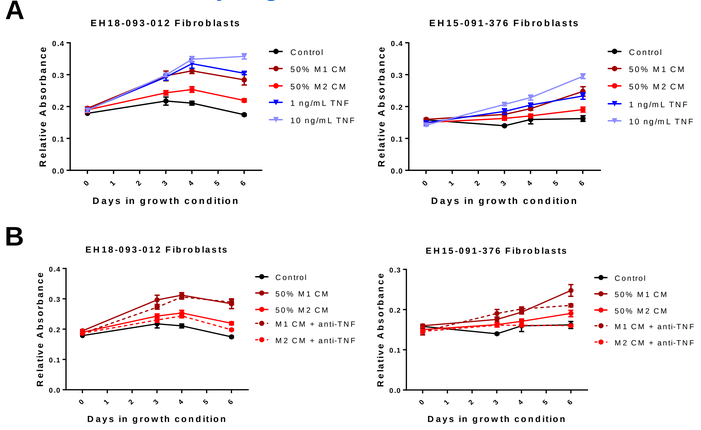

Figure 2. Primary BPH fibroblast proliferation is stimulated by TNF and macrophage conditioned medium. THP-1 cells were treated with PMA and polarized to M1 or M2 macrophages. Conditioned medium (CM) from polarized cells was harvested after 24 hours, then cultured with primary BPH fibroblasts isolated from two different BPH patients. Growth assays were performed with 0.5% final serum concentration and growth was monitored via crystal violet. A) Growth assays with 50% macrophage CM or treatment of recombinant human TNF. B) Macrophage CM was treated with or without 40μg/mL anti-TNF neutralization antibody (P300A) for 6 hours prior to cell treatment. Data indicate that neutralization of TNF reduces macrophage-stimulated cell growth in fibroblasts from one patient but not from another patient.

Epithelial Cell Proliferation is Reduced in BPH Tissues from Patients Taking TNFα Antagonists:

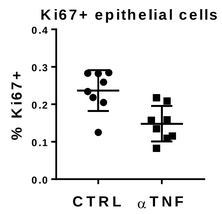

Figure 3. BPH patients treated with TNFα antagonists have decreased epithelial Ki67 staining. Immunohistochemistry of Ki67 in the epithelial cells of prostate transition zone tissues from patients treated with TNFα antagonists prior to prostatectomy versus untreated age-matched controls. Data is represented as % Ki67+ epithelial cells per field of view. Eight patients were included in each group for analysis.

Evaluation of Altered Pathways between Leukocytes from Large *versus* Small BPH Tissues Includes AI-Related Pathways:

Table 4. Top 18 pathways from Ingenuity Pathway Analysis (Qiagen) of differentially expressed genes between large versus small CD45+ cells.

Ingenuity Canonical Pathways	-Log(p-value)	Ratio
Communication between Innate and Adaptive Immune Cells	11.5	0.177
Hematopoiesis from Pluripotent Stem Cells	10	0.245
EIF2 Signaling	7.94	0.0893
NRF2-mediated Oxidative Stress Response	7.64	0.0952
Systemic Lupus Erythematosus In B Cell Signaling Pathway	7.13	0.0764
Atherosclerosis Signaling	6.91	0.111
B Cell Receptor Signaling	6.29	0.0865
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	6.21	0.0673
IL-10 Signaling	6.17	0.145
IL-6 Signaling	6.12	0.104
Dendritic Cell Maturation	5.64	0.082
Primary Immunodeficiency Signaling	5.38	0.16
Systemic Lupus Erythematosus Signaling	5.08	0.0699
Granulocyte Adhesion and Diapedesis	5.04	0.0778
Cholecystokinin/Gastrin-mediated Signaling	4.78	0.0924
Graft-versus-Host Disease Signaling	4.5	0.146
Autoimmune Thyroid Disease Signaling	4.44	0.143
Allograft Rejection Signaling	4.44	0.105

Summary and Future Directions

- Men with AI conditions are more likely to be diagnosed with BPH, but treatment with TNFα antagonists reduce BPH diagnoses in these patients back to a baseline level.
- CD45+ cells accumulate in human BPH tissue as the disease progresses, 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.
- The top most significantly altered canonical pathways between CD45+ cells from large *versus* small BPH tissues include pathways related to AI conditions.
- Macrophages from BPH tissues express TNF and high levels of both TNF receptors.
- Primary BPH fibroblast growth is stimulated by TNF and macrophage CM, but neutralizing TNF reduces macrophage-stimulated growth in only a subset of fibroblast cultures.
- Functional characterization of the immune cell subpopulations in BPH is ongoing.
- We will continue to investigate the precise mechanism(s) by which TNF aids in BPH pathogenesis, including its role in altering the inflammatory cell milieu in BPH. These studies aim to identify novel therapeutic strategies to limit progression of this disease.