TNF-alpha antagonism as a potential approach to modify BPH pathogenesis

Simon W. Hayward, Ph.D.
Inflammation in BPH

• Inflammation is evident in histological sections of BPH
• Could contribute to fibrotic changes in the prostate (“stiff” prostates)
• Could contribute to prostate growth
• Goals
  – Define the profile of leukocytes and their changes during prostate enlargement based upon single-cell RNA-seq analysis
  – Identify cellular clusters that possess distinct pathway topologies in BPH progression
  – Identify potential interventional strategies
Inflammatory cells accumulate as prostates enlarge.
Rationale for scRNA-seq Studies

• The currently defined subpopulations of immune cells in BPH are limited to known markers which may not include all intercellular signaling profiles

• Based on traditional M1/M2 polarization markers we know that as BPH progresses to surgery the ratio between these phenotypes become more pro-inflammatory. However, the M1/M2 continuum is not well-defined

• We want to understanding extracellular signaling pathways altered throughout BPH progression to identify potential therapeutic targets
Tissue processing and CD45+ cell sorting

~1 gram BPH tissue

Prep and Staining for Cell Sorting

Selection for non-epithelial (EpCam-) non-endothelial (CD200-) CD45+ immune cells
Clustering based on scRNA-seq
Cellular Indexing of Transcriptomes and Epitopes by sequencing (CITE-seq)

- Simultaneous gene expression and surface protein expression at the single cell level
- Validation of immune cell categorization after scRNA-seq

Marketed as TotalSeq by Biolegend

New York Genome Center
Stoeckius, et. al, 2017 Nature Methods
Correlation of transcriptomes and cell surface proteins (scRNA-seq plus CITE-seq)
Provisional assignment of mRNA clusters
Early hints about macrophage populations

General dogma regarding macrophage action in injury:

1) Inflammation (*IL1B*, *TNF*, *SPP1*, *CD36*, *FABP4/5*, *CCL2*, *CXCL3*, and *PTGS2*)

2) Anti-inflammation (wound repair)(*IL10*, *IL1RN*, *C1QA/B/C*, *MRC1*, *CD163*)

3) Resolution (*C1QA/B/C*, *MRC1*, *CD74*, *CD163*, *TGFB*, *IL10*, *VEGFA*, and *IL4*)
Greg Cresswell
Men with autoimmune inflammatory (AI) diseases have more BPH diagnoses. Men treated for AI diseases have less BPH diagnoses.

<table>
<thead>
<tr>
<th></th>
<th>Autoimmune, N (%)</th>
<th>No Autoimmune, N (%)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All Men, N (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Autoimmune</strong></td>
<td>10,796 (9.6)</td>
<td>101,383 (90.4)</td>
<td></td>
</tr>
<tr>
<td>BPH</td>
<td>3,294 (30.6)</td>
<td>20,586 (20.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No BPH</td>
<td>7,475 (69.4)</td>
<td>80,797 (79.7)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Autoimmune, N (%)</th>
<th>No Autoimmune, N (%)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men where Autoimmune occurred PRIOR TO BPH Diagnosis, N (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Autoimmune</strong></td>
<td>9,274 (8.4)</td>
<td>101,383 (91.6)</td>
<td></td>
</tr>
<tr>
<td>BPH</td>
<td>1,799 (19.4)</td>
<td>20,586 (20.3)</td>
<td>0.037</td>
</tr>
<tr>
<td>No BPH</td>
<td>7,475 (80.6)</td>
<td>80,797 (79.7)</td>
<td></td>
</tr>
</tbody>
</table>
### Effects of TNF-antagonists on human BPH

**Multivariable Regression Model Predicting BPH in Men with an Autoimmune Condition Prior to BPH Diagnosis**

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>1.05</td>
<td>0.79-1.40</td>
<td>0.745</td>
</tr>
<tr>
<td><strong>TNF-α antagonists</strong></td>
<td><strong>0.67</strong></td>
<td><strong>0.50-0.91</strong></td>
<td><strong>0.010</strong></td>
</tr>
<tr>
<td>Age 60+</td>
<td>4.92</td>
<td>1.30-5.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>1.16</td>
<td>0.81-1.65</td>
<td>0.427</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0.72</td>
<td>0.45-1.17</td>
<td>0.186</td>
</tr>
</tbody>
</table>

**Effects of TNF antagonist therapy on epithelial Ki67 labeling index in human BPH.**  
\( p < 0.01 \)
Ongoing and Planned Studies

- BPH is a T-cell dominant environment. We want to produce a clear picture of the B and T cell populations present in BPH.
- Develop the pathway analysis to determine how intercellular communication changes with progression, including communication with stromal and epithelial cells.
- Androgens are generally somewhat immunosuppressive. Therapies such as 5ARIs reduce androgen availability. We wish to quantify these effects on leukocyte populations in patient tissue.
- Explore the potential of immune signaling modifiers (such as TNF antagonists) in clinical BPH.
Acknowledgements

NorthShore
- Omar Franco
- Yana Filipovich
- Alejandro Morales
- Renee Vickman

• Susan Crawford
  - Max Greenberg
  - Philip Fitchev
  - Victoria Gil

• Brian Helfand
• Alexander Glaser
• Michael Paterakos
  - Lori Katz

Purdue University
- Timothy Ratliff
  - Greg Cresswell
  - Meaghan Broman
  - Paula Cooper

- Nadia A. Lanman
  - Sebastian Paez Paez

• FUNDING: 1P20DK116185
  1R01DK117906