CHARACTERIZATION OF FGF-5 EXPRESSION IN PROSTATIC TISSUE

Mary M. Stangis1,2,3, Emily A. Ricke1,3,4, Dalton T. McLean2,5, William A. Ricke1,3,4

Department of Urology, Cancer Biology Training Program, Carbone Comprehensive Cancer Center, George M. O’Brien Center for Research Excellence, Biotechnology Center: University of Wisconsin-Madison, Madison, WI

Introduction & Objectives

As other FGFs have been shown to play a role in cell-cell signaling between epithelial and stromal cells in the prostate, we predict that we will find FGF-5 present in both tissues. We also hypothesized that FGF-5 would be increased in diseased prostatic tissue when compared to benign tissue, as fibrosis and altered epithelial cell growth both contribute to diseases of the prostate.

Materials and Methods

Human Prostate Tissue Samples

For this study, we utilized two tissue microarrays (TMAs) composed of duplicate tissue biopsy cores from a total of 392 patients. Tissue states included benign, benign prostate hyperplasia (BPH), high grade intraepithelial prostate neoplasia (HGPIN), and various stages of prostate cancer. The breakdown of samples can be found below:

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>392</td>
</tr>
<tr>
<td>BPH</td>
<td>392</td>
</tr>
<tr>
<td>HGPIN</td>
<td>392</td>
</tr>
<tr>
<td>Stage 1 prostate</td>
<td>392</td>
</tr>
<tr>
<td>Stage 2 prostate</td>
<td>392</td>
</tr>
<tr>
<td>Stage 3 prostate</td>
<td>392</td>
</tr>
<tr>
<td>Stage 4 prostate</td>
<td>392</td>
</tr>
</tbody>
</table>

Immunohistochemistry, Imaging, and Analysis

TMA sections were multiplexed for FGF-5 (LS-B10340) using Bajoran Purple staining of FGF-5 in benign prostate tissue (false colored using Inform). When comparing whole cell FGF-5 staining optical density (OD), we found that FGF-5 OD is significantly higher in epithelial cells than adjacent stromal cells (p<0.0001). C. When comparing subcellular FGF-5 between stromal and epithelial cells in the same set of benign tissue cores, we found that FGF-5 OD is significantly increased in epithelial cells when compared to adjacent stromal cells (p<0.0001). Interestingly, there is no significant difference between nuclear and cytoplasmic FGF-5 in epithelial cells. When comparing nuclear and cytoplasmic FGF-5 between stromal and epithelial cells, we found that the OD is significantly increased in epithelial cells in both cases (p<0.0001). D. C4-2 cells were transfected with YFP tagged FGF-5 and observed over the course of 36 hours. Over the course of the first 10 hours, FGF-5 can be seen localizing to the nucleus and overlapping with DAPI.

Results (cont.)

Figure 1: Representative image of benign prostate tissue showing FGF-5 positive staining. Images have been false colored by Inform to better show Bajoran Purple staining. We observed FGF-5 positivity in both the prostatic epithelium, in A, putative basal cells and B, putative luminal cells, as well as in surrounding stroma in C, putative fibroblasts and smooth muscle cells.

Figure 2: FGF-5 staining was compared between stromal and epithelial cells within benign tissue cores, using Inform for tissue and cell segmentation. A. Representative image showing Bajoran Purple staining of FGF-5 in benign prostate tissue (false colored using Inform). B. When comparing whole cell FGF-5 staining optical density (OD), we found that FGF-5 OD is significantly higher in epithelial cells than adjacent stromal cells (p<0.0001). C. When comparing subcellular FGF-5 between stromal and epithelial cells in the same set of benign tissue cores, we found that the OD is significantly higher in epithelial cells than adjacent stromal cells (p<0.0001). Interestingly, there is no significant difference between nuclear and cytoplasmic FGF-5 in epithelial cells. When comparing nuclear and cytoplasmic FGF-5 between stromal and epithelial cells, we found that the OD is significantly increased in epithelial cells in both cases (p<0.0001, p<0.0001, respectively). D. C4-2 cells were transfected with YFP tagged FGF-5 and observed over the course of 36 hours. Over the course of the first 10 hours, FGF-5 can be seen localizing to the nucleus and overlapping with DAPI.

Figure 3: A. When repeating the comparison from Figure 2 across all prostatic disease subtypes, we found that the phenotype of increased epithelial vs stromal FGF-5 was consistent across all tissue types (p<0.0001 for all tissue types). B. When comparing stromal FGF-5 across disease subtypes, we found that the OD is significantly decreased between stage 2-3 prostate cancer and benign prostate tissue (p<0.0001). C. When comparing epithelial FGF-5 in the same manner, we found that OD is significantly increased between BPH and benign (p<0.0001), and between HGPIN and benign (p<0.0001). Interestingly, as with stromal FGF-5, epithelial FGF-5 is significantly decreased between stage 2-3 prostate cancer and benign tissue (p<0.0001).

Figure 4: Nuclear localization of FGF-5 in the absence of nuclear localization of AR, epithelial cells. A. Mean number of cells in which FGF-5 was found in the epithelial nucleus, but AR was not. The mean number of cells is significantly decreased in HGPIN (p<0.0021) and increased in stage 4 prostate cancer (p=0.0225). B. ROC analysis shows that number of epithelial cells with FGF-5 in the nucleus in the absence of AR can be used to predict whether a patient tissue is benign or HGPIN with an accuracy of 68% (p<0.0024). C. ROC analysis also shows that number of epithelial cells with FGF-5 in the nucleus in the absence of AR can be used to predict whether a patient tissue is benign or stage 4 prostate cancer with an accuracy of 67% (p=0.032).

Figure 5: Nuclear localization of both FGF-5 and AR, epithelial cells. A. Number of epithelial cells with nuclear localization of FGF-5 and AR is significantly increased in stage 4 prostate cancer compared to benign tissue (p=0.0141). B. ROC analysis shows that number of epithelial cells with nuclear localization of FGF-5 and AR can be used to predict whether a patient tissue is benign or stage 4 prostate cancer with an accuracy of 68% (p=0.0199).

Figure 6: The number of stromal cells with nuclear FGF-5 in the absence of AR is significantly decreased between benign tissue and stage 2-3 prostate cancer (p=0.0119).

Conclusions

Over the course of this study, we have shown that FGF-5 is expressed in the human prostate in both epithelial and stromal cells. We have also found that while FGF-5 is a secreted protein, it also localizes to the nucleus – though the function this serves is still unknown, interestingly, though FGF-5 is a growth factor, we found overall that expression is decreased in prostate cancer; though it is increased in other prostate diseases involving cell overgrowth such as BPH and HGPIN. The data we have gathered so far suggests that nuclear FGF-5, particularly in relation to the presence or absence of AR in the same nucleus, may have predictive value for both benign prostate disease as well as prostate cancer.

Future directions of this project may take include performing similar classification of FGF-5 expression on tissue samples from patients both treated with and resistant to Sex-steroid receptor inhibitors, as well as additional naïve benign tissues including stromal nodules and interstitial tissue. We also hope to include work utilizing mouse models of prostatic disease in the future.

Acknowledgements

U54DK104310