Heterogeneity of PTEN and ERG Biomarkers Expression in Prostate Cancer Needle Biopsies with More Than One Core Positive: Implications for Biomarkers Sampling Strategy

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Background

• Prostate cancer (PCA) is a multifocal disease. Multifocal PCAs is known to exhibit frequent clinical, histologic, and genomic heterogeneity. The dominant (index) tumor is considered to be biologically the most representative tumor that typically determines clinical behavior.

• Genomic rearrangements leading to the formation of TMPRSS2-ETV gene fusions and deletion of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) tumor suppressor gene are the two most frequent alterations observed in PCA.

• ERG and PTEN immunohistochemistry has been shown to be a sensitive and specific approach to detect underlying genetic abnormality and are increasingly analyzed in prostate needle biopsies (NBXs) as a marker of donality and number of tumor loss and PTEN due to its prognostic relevance.

Aims

• To evaluate heterogeneity of PTEN and ERG biomarkers expression in PCA NBXs setting.

• To assess biomarkers staining pattern in reference to cancer cores with highest Gleason score and/or tumor volume to evaluate optimal biomarkers sampling strategy.

Methods

• A cohort of 194 consecutively selected prostate NBXs containing ≥1 cancer cores were immunostained for ERG (C-terminus, E069 clone, Epitomics) and PTEN (rabbit monoclonal antibody, cell signaling) antibodies and analyzed for the presence of ERG overexpression and PTEN loss.

• ERG overexpression was defined as diffuse (entire tumor) or partial nuclear staining in a tumor cells. PTEN loss was defined as complete or partial (any amount of the tumor) loss of cytoplasmic staining.

• Biomarkers staining heterogeneity was defined as: Inter-tumor core when different Gleason scores or tumor volumes are observed in separate biopsy cores, Intra-tumor core heterogeneity when two cores representing highest Gleason score and tumor volume are present within the same core.

• Biomarkers staining pattern in each case was evaluated in reference to cancer cores with highest Gleason score and/or tumor volume to evaluate optimal biomarkers evaluation sampling strategy.

Results

Of 194 PCA cases, ERG overexpression and PTEN loss in at least one PCA core was present in 111 (57%) and 69 (36%) cases respectively.

Table 1: ERG overexpression is significantly associated with PTEN loss in prostate cancer (p<0.05)

<table>
<thead>
<tr>
<th>ERG overexpression</th>
<th>PTEN absence</th>
<th>PTEN presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>No PTEN loss</td>
<td>64 (35)</td>
<td>14 (7)</td>
</tr>
<tr>
<td>PTEN loss</td>
<td>57 (29)</td>
<td>54 (26)</td>
</tr>
</tbody>
</table>

Table 2: PTEN loss in prostate cancer is associated with Gleason score (p<0.05)

<table>
<thead>
<tr>
<th>Core heterogeneity pattern</th>
<th>ERG overexpression (%)</th>
<th>PTEN loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-tumor core</td>
<td>48 (31)</td>
<td>47 (68)</td>
</tr>
<tr>
<td>Intra-tumor core</td>
<td>41 (25)</td>
<td>47 (68)</td>
</tr>
</tbody>
</table>

Table 3: ERG and PTEN biomarkers demonstrate frequent staining heterogeneity in prostate cancer

Table 4: In the setting of different Gleason score across tumor sites, combined highest Gleason grade and highest tumor volume cores are representative of ERG overexpression and PTEN loss

<table>
<thead>
<tr>
<th>Highest grade combination</th>
<th>Highest tumor volume (50%)</th>
<th>Highest grade and volume combined (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERG overexpression</td>
<td>53 (94)</td>
<td>38 (93)</td>
</tr>
<tr>
<td>PTEN loss</td>
<td>44 (95)</td>
<td>37 (85)</td>
</tr>
</tbody>
</table>

Table 5: In the setting of uniform Gleason score across tumor sites, addition of second highest tumor volume improves representation of ERG overexpression and PTEN loss

<table>
<thead>
<tr>
<th>Highest tumor volume core</th>
<th>Highest and second highest volume combined (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERG overexpression</td>
<td>43 (96)</td>
</tr>
<tr>
<td>PTEN loss</td>
<td>19 (76)</td>
</tr>
</tbody>
</table>

Study Highlights

• Both PTEN and ERG biomarkers demonstrate significant inter- and intra-tumor core staining heterogeneity, suggesting that sampling may affect biomarkers interpretation.

• Inter-tumor core (carcinoma in separate biopsy cores) heterogeneity for ERG suggests the presence of at least two separate clones in the prostate. Intra-tumor core heterogeneity for ERG (carcinoma within the same biopsy core) indicates multiple clones can be present even within the same core biopsy/site.

• ERG overexpression shows strong relationship with PTEN loss.

• PTEN loss is significantly associated with Gleason score.

• When multiple cores are involved by PCA, selection of at least two cores representing highest Gleason score and tumor volume provide cost-effective yet optimal representation of underlying molecular abnormality. For cases with similar Gleason score across tumor sites, addition of second highest tumor volume improves representation of ERG overexpression and PTEN loss.

• Due to significant inter- and intra-tumor core expression heterogeneity, complete assessment of the status of these markers for all positive cores may be appropriate in cases where both selected cores lack molecular abnormality.

• Since immunohistochemistry (IHC) is much more cost effective, and much less laborious to evaluate than FISH, IHC analysis of these markers appears most efficient.

References

2. Lotan TI et al. PTEN Protein Loss by Immunostaining: Analytic Validation and Prognostic Indicator for a High-Risk Surgical Cohort of Prostate Cancer Patients. Clin Cancer Res 2011; 17(20); 6563-73
3. Yoshimoto et al. PTEN losses exhibit heterogeneity in multifocal prostatic adenocarcinoma and are associated with higher Gleason grade. Mod Pathol 2012; 1-13

Figure 1: A-D. Representative examples of inter-tumor core PTEN heterogeneity. A-B: H&E section from left apex biopsy demonstrates Gleason score 4+3 in both cores. There is diffuse loss of PTEN protein expression in all malignant glands. C-D: H&E section from right mid biopsy. Demonstrated Gleason score 3+4 in both cores. There is diffuse loss of PTEN in all malignant glands, all stromal cells and benign glands are an internal control.

Figure 2: A-B. Representative examples of intra-tumor core PTEN heterogeneity. Malignant glands with PTEN loss and PTEN protein retention are intermingled.

Figure 3, A-D. Representative examples of inter- and intra-tumor core ERG heterogeneity.

Figure 4: A-B. H&E section from left apex biopsy demonstrates Gleason score 4+3 in both cores. There is diffuse loss of ERG expression in all malignant glands. C. The cancer core from left apex biopsy for same patient demonstrates no ERG expression. Intra-tumor core heterogeneity as separate clones in the prostate. Intra-tumor core heterogeneity for ERG suggests the presence of at least two separate clones in the prostate. Intra-tumor core heterogeneity for ERG (carcinoma within the same biopsy core) indicates multiple clones can be present even within the same core biopsy/site.