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

Rajal B. Shah, M.D.,[1] Zach Jeffery[1] and Angelo DeMarzo, M.D., Ph.D.[2]
[1]Miraca Life Sciences Research Institute, Miraca Life Sciences, Irving, TX, United States; [2]Johns Hopkins Hospital, Baltimore, Maryland, United States

Background

- Prostate cancer (PCa) is typically a multifocal disease. Multifocal PCa 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-ETS* 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); ERG as a marker of clonality and number of tumor foci 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 evaluation sampling strategy

Methods

- A cohort of 194 consecutively selected prostate NBXs containing >1 cancer cores were immunostained for ERG (C-terminus, EPR 3864 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 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 carcinoma in separate biopsy cores and Intra-tumor core when carcinoma within the same biopsy core demonstrated variation in staining pattern/ expression.
- 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 ($p<0.05$)

ERG overexpression	PTEN	
	No PTEN loss	PTEN loss
Absence (%)	64 (33)	14 (7)
Presence (%)	57 (29)	54 (28)

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

Gleason score (n)	ERG overexpression (%)	PTEN loss (%)
3+3 (66)	35 (53)	6 (9)
3+4 (79)	43 (54)	30 (38)
4+3 (20)	12 (60)	9 (45)
4+4 (21)	13 (62)	13 (62)
4+5 (8)	5 (63)	6 (75)

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

Biomarkers staining heterogeneity pattern	ERG overexpression (%)	PTEN loss (%)
Inter-tumor core	48 (43)	47 (68)
Intra-tumor core	41 (37)	47 (68)

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

	Highest grade core (%)	Highest tumor volume core (%)	Highest grade and volume combined (%)
ERG overexpression	53 (84)	58 (92)	58 (92)
PTEN loss	44 (96)	37 (80)	45 (98)

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.

	Highest tumor volume core	Highest and second highest volume combined
ERG overexpression	43 (90)	45 (94)
PTEN loss	19 (76)	21 (84)

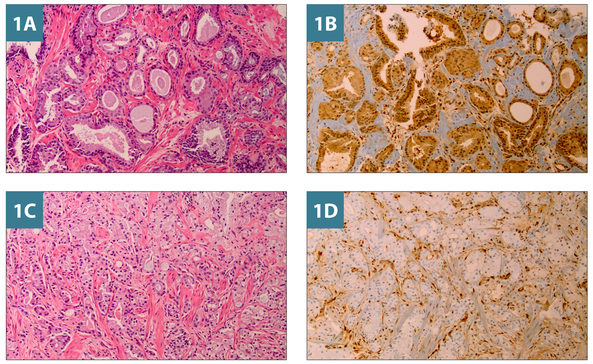

Figure 1, A-D. Representative examples of inter-tumor core PTEN heterogeneity. A-B. H&E section from the left apex NBX demonstrates Gleason score 3+3=6 PCa (A). There is uniform retention of PTEN protein expression in all malignant glands (B). C-D. H&E section from right mid biopsy demonstrated Gleason score 3+4=7 PCa. There is diffuse loss of PTEN in all cancer glands. Stromal cells and benign glands act as an internal control.

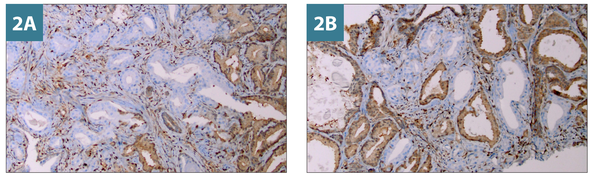

Figure 2, A-B. Representative examples of intra-tumor core PTEN heterogeneity. Malignant glands with PTEN loss and PTEN protein retention are intermingled.

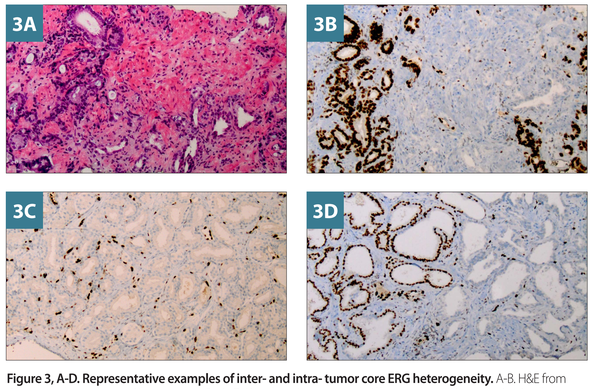

Figure 3, A-D. Representative examples of inter- and intra- tumor core ERG heterogeneity. A-B. H&E from right base demonstrates Gleason score 3+4=7 PCa. There is strong and diffuse nuclear ERG expression in all malignant glands. C. The cancer core from left apex biopsy for same patient demonstrates no ERG expression. Vascular endothelial cells staining serve as an internal control. D. Representative example of intra-tumor core ERG heterogeneity. Part of the tumor demonstrates strong ERG expression while part of the tumor lacks ERG expression.

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 needle 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

1. Mehra R et al. Heterogeneity of TMPRSS2 gene rearrangements in multifocal prostate adenocarcinoma: molecular evidence for an independent group of diseases. *Cancer Res* 2007; 67: 7991-95
2. Lotan TL 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