Published in final edited form as:

Prostate. 2013 August ; 73(11): 1233–1240. doi:10.1002/pros.22675.

ERG Overexpression and PTEN Status Predict Capsular Penetration in Prostate Carcinoma

Ray B. Nagle¹, Amit M. Algotar², Connie C. Cortez³, Katherine Smith¹, Carol Jones¹, Ubaradka G. Sathyanarayana³, Steven Yun³, Janice Riley³, Dea Nagy³, Ryan Dittamore³, Bruce Dalkin⁴, Laura Brosh¹, and Gary Pestano³

¹University of Arizona Department of Pathology

²The University of Arizona Cancer Center

³Ventana Medical Systems, Inc

⁴University of Washington Department of Urology

Abstract

Background—This study examines the combined effect of two common genetic alterations, ERG and PTEN, in prostate carcinoma progression.

Methods—Prostate tissue from ninety patients having unilateral capsular penetrating lesions (CPL), and a contra-lateral organ confined (COC) second lesion, were examined by immunohistochemistry for the expression of the TMPRSS2:ERG transformation product ERG and the loss of expression of PTEN, a powerful phosphatase inhibiting the PI3 Kinase pathway. Multivariate logistic regression was carried out to analyze the data.

Results—After adjusting for Gleason score, the odds of having capsular penetration were 5.19 times higher ($p=0.015$) for ERG+/PTEN- group as compared to the wild type (ERG-/PTEN+).

Conclusions—This study presents the first evidence that ERG over expression and PTEN deletion is associated with greater risk of capsular penetration. Although further studies are needed, these results have the potential to change clinical assessment for prostate cancer.

Keywords

Prostate Carcinoma; ERG; PTEN; Capsular Penetration

INTRODUCTION

Previous studies have proven the common existence of ETS gene rearrangement in prostate cancers, and have indicated that the TMPRSS2:ERG gene fusion on chromosome 21 is the most common.¹ Studies that have examined the prognostic significance of this gene fusion have shown conflicting results to-date. Demichelis et al. described a statistically significant correlation between the TMRSS2:ERG gene fusion and metastases and disease-specific deaths.² They concluded that the TMPRSS:ERG gene fusion is associated with a more aggressive type of prostate cancer. Similar results were found in other studies.^{3,4} Attard et al. found that the gene fusion was associated with worse overall survival in a watchful waiting cohort, and Nam et al. found that TMPRSS2:ERG gene fusion was a strong

predictor for disease recurrence.^{4,5} Other studies examined the presence of this gene fusion in correlation with pathologic stage and found a significant association for rearrangement and high pathologic stage.^{2,6}

Additional studies have found opposing results. Two small studies found no association between gene fusion and tumor stage.^{7,8} In a larger cohort study by Darnel et al., the authors found no association between TMPRSS2: ERG fusion and pathologic stage, regardless of whether the gene fusion occurred by insertion or deletion.⁹ Finally, Petrovics et al. found that ERG expression in prostate cancer was associated with a lower pathologic stage and negative surgical margins, while Saramaki et al. found that the TMPRSS2: ERG fusion gene was actually associated with a longer progression free survival.^{10,11}

PTEN (phosphatase and tensin homolog) is located on chromosome 10 and reveals reduced expression in 70-80% of prostate cancers.^{12,13} It is an important negative regulator of the PI3K/AKT signal transduction pathway. This pathway is normally stimulated when a ligand binds to tyrosine kinase receptor, which initiates a phosphorylation cascade that ultimately activates the serine/threonine kinase AKT. AKT phosphorylates a number of substrates (BAD, MDM2) that enhance cell survival. AKT activation is also involved with inactivation of the TSC1/TSC2 complex (tumor suppressor 6 genes). Loss of PTEN function is one of the most common ways that PI3K/AKT signaling is upregulated. The regulation of PTEN gene expression is complex and includes transcription factors, microRNAs, competitive endogenous RNAs, and DNA methylation. In addition, PTEN protein levels can be altered through aberrant phosphorylation, ubiquitination, and acetylation.¹⁴

Yoshimoto et al showed that deletions of PTEN, as demonstrated by FISH (fluorescence *in situ* hybridization) were associated with earlier biochemical disease recurrence in prostate cancer.¹⁵ Additionally, the authors found that at the time of prostatectomy, loss of PTEN (either hemizygous or homozygous deletion) was significantly associated with extraprostatic extension and seminal vesicle invasion, parameters indicative of more advanced disease. In a second study by Yoshimoto et al, they examined PTEN deletions and TMPRSS2: ERG rearrangements in prostatic adenocarcinoma prognosis.¹⁶ Using Kaplan-Meier and multivariate analysis, duplication of TMPRSS2/ERG showed a shorter survival than TMPRSS2: ERG rearrangement alone. PTEN deletion, regardless of TMPRSS2/ERG status, resulted in the shortest survival duration. Similar results were found by Reid et al.¹⁷ In a study examining the prevalence of ERG rearrangement and PTEN deletion in both HGPIN and metastatic cancer, Han et al. found that ERG aberration, but not PTEN deletion, was found in both HGPIN (High Grade Prostatic Intraepithelial Neoplasia) and cancer, and that the ERG status was similar from localized cancer to metastasis.¹⁸ The authors found that PTEN deletion was observed more frequently in metastatic cancers.

Recently, a rabbit monoclonal anti-ERG antibody (clone EPR3864, Ventana # 790-4576) was characterized by Park et al.¹⁹ The antibody binds to the 3' end of the ERG protein and has a high correlation with gene arrangement, as determined by ERG break-apart assays. Given the ongoing debate about the prognostic significance of the TMPRSS2:ERG fusion gene, we investigated the relationship of this fusion gene combined with PTEN status to

capsular penetration using immunohistochemical methods (IHC) and four-color Quantum dot FISH.

MATERIALS AND METHODS

Clinical Material

Four hundred and twenty six men underwent open prostatectomy by a single urologist between 1997 and 2008. The prostates were put on ice and delivered to the pathologist. The prostates were inked, the apex removed, and two or three coronal incisions were made to allow better fixation. After overnight fixation in 10% neutral buffered formalin, the prostates were sectioned at 5mm coronal intervals which were then cut into quadrants and totally embedded in paraffin blocks. H &E stained sections were examined by a single pathologist. All lesions were mapped and Gleason sum scores, volume of largest tumor, and pathologic stage were determined. Review of the tumor distribution on the maps revealed 108 cases with capsular penetration (pT3) and a second, organ confined contra-lateral lesion. Formalin fixed, paraffin block (FFPE) from the capsular penetration lesion and from the contra-lateral lesion were used in the current analysis. Following re-sectioning of the blocks, 90 cases were selected for the final study (Fig. 1). Clinical and patient follow up information was obtained after patient consent using protocols approved by the Institutional Review Board of the University of Arizona and Tucson Medical Center, Tucson, Arizona. The descriptive data of the final cohort is shown in Table 1.

Immunohistochemistry

PTEN and ERG status were determined by immunohistochemistry on 4µm FFPE prostatectomy sections. Immunostaining was performed by employing the automated DISCOVERY®XT staining platform from Ventana Medical Systems, Inc., (Tucson, AZ). A 1:25 dilution of the PTEN antibody (catalog #.9559, Cell Signaling Technology, Danvers, MA) was incubated for 1 hour at room temperature. This PTEN antibody has been well characterized by using a blocking peptide characterized by reverse phase HPLC and mass spectroscopy. This peptide blocks PTEN (138G8) Rabbit mAb #9559 in immunohistochemistry. The PTEN antibody was then further detected using the UltraMap anti-Rb HRP (VMSI, # 760-4315) and the ChromoMap DAB detection kit (VMSI, # 760-159). Slides were counterstained with Hematoxylin II (VMSI, # 790-2208) for 8 minutes followed by Bluing Reagent (VMSI, # 760-2037) for 4 minutes. Normal prostate cells were used as an internal positive control for PTEN.

The primary rabbit monoclonal ERG antibody (clone EPR3864) was obtained from Epitomics (Burlingame, CA), or from Ventana Medical Systems (# 790-4576). The EPR clone 3864, used in this study has been shown to have a 98% correlation with positive ERG protein expression and gene rearrangement¹⁹. Slides stained with the Epitomics antibody were incubated with 1:100 of the ERG primary antibody for one hour at room temperature or 15 minutes with the Ventana antibody. Primary antibody was detected using the ChromoMap DAB detection kit (VMSI, # 760-159) and the UltraMap anti-Rb HRP (VMSI, # 760-4315) secondary antibody was applied for 16 minutes at room temperature. Slides were counterstained with Hematoxylin II (VMSI, # 790-2208) for 8 minutes followed by

Bluing Reagent (VMSI, # 760-2037) for 4 minutes at 37°C. ERG nuclear staining and PTEN cytoplasmic staining were scored as positive or negative. Heterogeneity of ERG and PTEN staining was occasionally observed. In these cases the heterogeneity was recorded and the case was categorized based on the predominant pattern of staining.

FISH Methods

FISH (fluorescence *in situ* hybridization) was performed using four probes that hybridized to the following regions of the ERG gene: 5p ERG, 3p ERG, PTEN, and CEN10. The 5p ERG probe has ~370kb genomic coverage, and the 3pERG probe has 317kb genomic coverage. The PTEN probe covers ~765 kb of genomic coverage. The CEN10 probe is a plasmid probe purchased from ATCC (pA10RP8). The probes were labeled using haptenated nucleotides and were detected using the quantum dot anti-hapten antibody conjugates QD565 anti DIG (digoxigenin), QD655 anti DNP (dinitrophenyl), QD585 anti NP (nitropyrazole), and QD605 anti TS (thiazolesulfonamide). The four signals from each of the quantum dot labeled probes were observable through a fluorescence imaging system and were distinguishable through spectral de-convolution.²⁰ The detection systems and assays were carried out on formalin-fixed, paraffin-embedded (FFPE) tissue specimens using the automated BenchMark XT platform (Ventana Medical Systems, Inc.). Protease 2 (Ventana Medical Systems, Inc., # 780-4148) was used to enzymatically pre-treat the tissue. DAPI was used as the counterstain.

Statistical Methods

Mean and standard deviation were used to describe the baseline data. Chi-square tests were used to determine if ERG or PTEN expression was associated with Gleason sum scores. Multivariate logistic regression models adjusting for Gleason score were implemented. The capsular penetrated and non-penetrated lesions were assigned individual Gleason scores. These scores were included in the above logistic regression models. These models consisted of main effects for ERG and PTEN and also their interaction term. Odds ratios were calculated to determine if the odds of capsular penetration varied by ERG/PTEN expression profile. All statistical calculations were conducted using Stata10 statistical software (Statacorp, College town, Texas). A backward regression approach was used and p for exclusion was set at 0.3

RESULTS

Immunohistochemical staining revealed robust nuclear staining for ERG (Fig. 2) in 47 of 90 (52%) of the capsular penetrating lesions and 42 of 90 (46.7%) of the contralateral organ confined lesions. All normal glands revealed negative nuclear staining. Endothelial cell nuclei were positive as were lymphocytes present in focal areas of inflammatory response. There was heterogeneity of ERG expression in 8 of 90 (9%) of the capsular penetrating carcinoma lesions and 7 of 90 (8%) of the contralateral organ confined carcinoma lesions (Fig 3). ERG positive HGPIN lesions were associated with the carcinomas in 21 of 90 (23.3%) of the capsular penetrating lesions and 22 of 90 (24.4%) of the contralateral organ confined carcinoma lesions (Fig. 4).

All normal glands showed robust cytoplasmic staining for PTEN. Negative staining corresponding to lack of PTEN expression was observed in 31 of 90 (34%) of the capsular penetrating carcinoma lesions and 18 of 90 (20%) of the contralateral organ confined carcinoma lesions (Fig. 5). Again, the carcinoma lesions revealed heterogeneity of PTEN expression and was seen in 15 of 90 (16.6%) of the capsular penetrating carcinoma lesions and 11 of 90 (12.2%) of the contralateral organ confined carcinoma lesions (Fig. 5).

The distribution of the ERG and PTEN expression in the capsular penetrating and contralateral non penetrating lesions is shown in Table 2. These data demonstrate that after adjusting for Gleason score, the odds ratio (95% confidence intervals) and p-value for ERG +/PTEN-, ERG+,PTEN+ and ERG-/PTEN- are 5.19(1.37, 19.73)p = 0.015, 0.80(0.41, 1.56) p = 0.52 and 1.37(0.39, 4.78)p = 0.62 as compared to the wild-type (ERG-/PTEN+).

The FISH slide was searched until an informative cancer field for each lesion was identified (Fig. 6). The average selected field of view contained 73 cancer cells. Spectral imaging revealed an average of 7.2 informative cells for *ERG* gene status per case, and 5.4 informative cells for *PTEN* gene status per case. Of the 22 samples, there were three *ERG* and six *PTEN* samples which were non-informative. Of the 19 informative *ERG* FISH samples, 16 (84%) were concordant with the IHC result. The six cases with *ERG* rearrangements showed that five were deleted and one was inserted. Of the 16 informative *PTEN* FISH cases, 14 (88%) were concordant with the IHC result. Five cases revealed heterozygous deletion.

DISCUSSION

Prostate cancer remains a major health problem not only because of its frequency but also because of the variability of progression and the relative short term duration of response (3-5 months) to multiple chemotherapeutic, immunotherapeutic, small molecules, and new radiation methods. In part, this problem is related to the multiple separate neoplasms occurring in any individual and to the genetic instability and tumor heterogeneity of any given tumor.

In this study two separate lesions in the same patient were compared in a cohort of 90 men with pT3a pathologic stage disease. This comparison has the advantage of comparing two separate lesions with different local invasive potential in the same prostate. A comparison of the penetrating lesion and the contralateral organ confined lesion showed significant differences in Gleason sum scores as well as frequency of ERG and PTEN abnormalities of expression. Our findings showed that the odds of capsular penetration are 5.19 times higher in a lesion having ERG overexpression and PTEN deletion as compared to ERG negative and PTEN positive lesion. Recent studies sequencing the human prostate genome confirm the presence of genetic instability and the plethora of translocations, deletions and mutations²¹ One of the most common abnormalities, identified in 50-75% of primary prostate tumors²², is rearrangement of the ETS transcription factors *ERG*, *ETV1*, *ETV4*, and *ETV5*. Great interest has been generated by the discovery that many of these rearrangements have resulted in fusion of androgen response elements upstream of these transcription factors, creating a situation where androgen can regulate abnormal transcription. This in turn

has opened up multiple opportunities for intervention through therapies aimed at blockage of androgen stimulation, inhibition of ETS mediated transcription, or targeting of downstream pathways. Over expression of ERG can now be phenotypically detected by two new monoclonal antibodies validated by FISH in several large cohorts of prostate cancer with defined ERG gene rearrangement status.^{19,23} In one study the EPR clone 3864, investigated in this study had a 98% correlation with positive ERG protein expression and gene rearrangement.¹⁹ In our study of the 19 informative *ERG* FISH samples, 16 (84%) were concordant with the IHC result. Using these antibodies it has been shown that the rearrangement is an early event occurring in approximately 15-20% of HGPIN lesions.²⁴ In the current study HGPIN lesions were seen adjacent to the carcinoma lesions in 23.3% of capsular penetrating cases and 24.4% of the contralateral organ confined lesion. These findings support the concept that invasive carcinomas can arise from HGPIN lesions.²⁵

Current molecular studies of solid neoplasms have revealed that prostate cancer is often polyclonal.²⁶ The current analysis supports this concept with heterogeneous expression of ERG observed in both the capsular penetrating lesions (9%) and the contralateral organ confined lesions (8%). PTEN expression was also seen to be heterogeneous in 16.6% of the capsular penetrating lesions and 12.2% of the contralateral lesion. Of the 16 informative *PTEN* FISH cases, 14 (88%) were concordant with the IHC result.

Studies have shown that ERG insertions comprise approximately 40% of ERG gene rearrangements, whereas ERG deletions comprise approximately 60%.²⁷ In this study six cases with *ERG* rearrangements showed that five were deleted and one was inserted. Recent studies have identified increased aggressiveness of ERG deletions compared to ERG insertions leading to higher tumor stage and lymph node metastasis.⁴ Additionally, ERG deletions have been shown to have high susceptibility to evolve into androgen-independent metastatic lesions compared with ERG insertions.^{28,29} Our findings that increase of ERG deletion relative to ERG insertion supports the trend that ERG deletions are more likely to identify patients at increased risk for higher tumor stage than an ERG insertion and are in agreement with findings reported by others^{30, 31}.

CONCLUSIONS

This study shows that the combination of ERG over expression and PTEN deletion is more common in aggressive capsular penetrating lesions than contra-lateral organ confined lesions. Both abnormalities are associated with genetic alteration and both are heterogeneous within individual lesions, indicating genetic instability. Finally, it is hypothesized that biopsy specimens showing the combination of ERG over-expression and PTEN deletion would be expected to indicate more aggressive disease perhaps independent of Gleason grade, which may identify patients who would benefit from a more aggressive surgical procedure. This latter finding will require additional studies, and likely include a prospective setting.

Acknowledgments

The authors thank Edward Abril for excellent technical support for IHC staining, and Kathy McDaniel for editing the manuscript. The authors also acknowledge the TACMASS Core (Tissue Acquisition and Cellular/Molecular

Analysis Shared Service) for use of staining equipment and for the support from staff. The TACMASS core is supported by the Arizona Cancer Center Support Grant, NIH CA023074.”

References

1. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science*. 2005; 310:644. [PubMed: 16254181]
2. Demichelis F, Fall K, Perner S, et al. TMPRSS2:ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. *Oncogene*. 2007; 26:4596. [PubMed: 17237811]
3. Perner S, Demichelis F, Beroukhi R, et al. TMPRSS2:ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer. *Cancer Res*. 2006; 66:8337. [PubMed: 16951139]
4. Attard G, Clark J, Ambrosini L, et al. Duplication of the fusion of TMPRSS2 to ERG sequences identifies fatal human prostate cancer. *Oncogene*. 2008; 27:253. [PubMed: 17637754]
5. Nam RK, Sugar L, Yang W, et al. Expression of the TMPRSS2:ERG fusion gene predicts cancer recurrence after surgery for localised prostate cancer. *Br J Cancer*. 2007; 97:1690. [PubMed: 17971772]
6. Mehra R, Han B, Tomlins SA, et al. Heterogeneity of TMPRSS2 gene rearrangements in multifocal prostate adenocarcinoma: molecular evidence for an independent group of diseases. *Cancer Res*. 2007; 67:7991. [PubMed: 17804708]
7. Lapointe J, Kim YH, Miller MA, et al. A variant TMPRSS2 isoform and ERG fusion product in prostate cancer with implications for molecular diagnosis. *Mod Pathol*. 2007; 20:467. [PubMed: 17334351]
8. Mehra R, Tomlins SA, Shen R, et al. Comprehensive assessment of TMPRSS2 and ETS family gene aberrations in clinically localized prostate cancer. *Mod Pathol*. 2007; 20:538. [PubMed: 17334343]
9. Darnel AD, Lafargue CJ, Vollmer RT, et al. TMPRSS2-ERG fusion is frequently observed in Gleason pattern 3 prostate cancer in a Canadian cohort. *Cancer Biol Ther*. 2009; 8:125. [PubMed: 19029822]
10. Petrovics G, Liu A, Shaheduzzaman S, et al. Frequent overexpression of ETS-related gene-1 (ERG1) in prostate cancer transcriptome. *Oncogene*. 2005; 24:3847. [PubMed: 15750627]
11. Saramaki OR, Harjula AE, Martikainen PM, et al. TMPRSS2:ERG fusion identifies a subgroup of prostate cancers with a favorable prognosis. *Clin Cancer Res*. 2008; 14:3395. [PubMed: 18519769]
12. Gray IC, Stewart LM, Phillips SM, et al. Mutation and expression analysis of the putative prostate tumour-suppressor gene PTEN. *Br J Cancer*. 1998; 78:1296. [PubMed: 9823969]
13. Whang YE, Wu X, Suzuki H, et al. Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. *Proc Natl Acad Sci U S A*. 1998; 95:5246. [PubMed: 9560261]
14. Carracedo A, Alimonti A, Pandolfi PP. PTEN level in tumor suppression: how much is too little? *Cancer Res*. 2011; 71:629. [PubMed: 21266353]
15. Yoshimoto M, Joshua AM, Cunha IW, et al. Absence of TMPRSS2:ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. *Mod Pathol*. 2008; 21:1451. [PubMed: 18500259]
16. Yoshimoto M, Cunha IW, Coudry RA, et al. FISH analysis of 107 prostate cancers shows that PTEN genomic deletion is associated with poor clinical outcome. *Br J Cancer*. 2007; 97:678. [PubMed: 17700571]
17. Reid AH, Attard G, Ambrosini L, et al. Molecular characterization of ERG, ETV1 and PTEN gene loci identifies patients at low and high risk of death from prostate cancer. *Br J Cancer*. 2010; 102:678. [PubMed: 20104229]
18. Han B, Mehra R, Lonigro RJ, et al. Fluorescence in situ hybridization study shows association of PTEN deletion with ERG rearrangement during prostate cancer progression. *Mod Pathol*. 2009; 22:1083. [PubMed: 19407851]
19. Park K, Tomlins SA, Mudaliar KM, et al. Antibody-based detection of ERG rearrangement-positive prostate cancer. *Neoplasia*. 2010; 12:590. [PubMed: 20651988]

20. Svensson MA, LaFargue CJ, MacDonald TY, et al. Testing mutual exclusivity of ETS rearranged prostate cancer. *Lab Invest.* 2011; 91:404. [PubMed: 20975660]
21. Berger MF, Lawrence MS, Demichelis F, et al. The genomic complexity of primary human prostate cancer. *Nature.* 2011; 470:214. [PubMed: 21307934]
22. Rubin MA, Maher CA, Chinnaiyan AM. Common gene rearrangements in prostate cancer. *J Clin Oncol.* 2011; 29:3659. [PubMed: 21859993]
23. Chaux A, Albadine R, Toubaji A, et al. Immunohistochemistry for ERG expression as a surrogate for TMPRSS2-ERG fusion detection in prostatic adenocarcinomas. *Am J Surg Pathol.* 2011; 35:1014. [PubMed: 21677539]
24. Mosquera JM, Perner S, Genega EM, et al. Characterization of TMPRSS2-ERG fusion high-grade prostatic intraepithelial neoplasia and potential clinical implications. *Clin Cancer Res.* 2008; 14:3380. [PubMed: 18519767]
25. Nagle RB, Cress AE. Metastasis Update: Human Prostate Carcinoma Invasion via Tubulogenesis. *Prostate Cancer.* 2011; 2011 249290.
26. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med.* 2012; 366:883. [PubMed: 22397650]
27. Perner S, Demichelis F, Beroukhi R, et al. TMPRSS2:ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer. *Cancer Res.* 2006; 66:8337. [PubMed: 16951139]
28. Mehra R, Tomlins SA, Yu J, et al. Characterization of TMPRSS2-ETS gene aberrations in androgen-independent metastatic prostate cancer. *Cancer Res.* 2008; 68:3584. [PubMed: 18483239]
29. Guo CC, Dancer JY, Wang Y, et al. TMPRSS2-ERG gene fusion in small cell carcinoma of the prostate. *Hum Pathol.* 2011; 42:11. [PubMed: 21040948]
30. Squire JA. TMPRSS2-ERG and PTEN loss in prostate cancer. *Nat Genet.* 2009 May; 41(5):509–10. [PubMed: 19399032]
31. Markert EK, Mizuno H, Vazquez A, Levine AJ. Molecular classification of prostate cancer using curated expression signatures. *Proc Natl Acad Sci U S A.* 2011 Dec 27; 108(52):21276–81. [PubMed: 22123976]

Figure 1. Schematic diagram of the prostate

Schematic diagram of the study design for 90 prostatectomies selected for this study, each with a unilateral capsular penetrating (CPL) lesion and a contra-lateral organ confined lesion (COC).

Figure 2. Immunohistochemistry of a positive ERG expressing carcinoma
IHC stained slide shows positive ERG stain of neoplasm compared to negative stain of normal tissue. Note positive nuclear ERG stain of normal endothelial cells in the normal area. 94 $\times$.

Figure 3. IHC of ERG showing heterogeneity of expression

IHC ERG stain of a Gleason Sum Score 3+3 prostate adenocarcinoma shows heterogeneous ERG expression. 94×

Figure 4. IHC ERG positive stain of carcinoma with adjacent HGPIN
ERG IHC stain shows positive in prostate adenocarcinoma (Cancer) with a Gleason Sum Score of 4+4, as well as in the adjacent high grade PIN lesion (HGPIN). 23 $\times$.

Figure 5. IHC PTEN stain of prostate

(A) Positive cytoplasmic stain of PTEN in normal glands (N) and negative staining of PTEN in prostate adenocarcinoma (Ca) with a Gleason Sum Score 4+4 is shown. (B) Heterogeneous expression of PTEN is shown within a Gleason Sum Score 3+3 prostate adenocarcinoma. This sample exhibits a negative stain for PTEN in adjacent areas of the same neoplasm.

Table 1**Baseline descriptive data for subjects selected for the final study**

Variable			
Age at surgery, years, Mean (SD)	62.74 (11.6)		
Follow-up, years, Mean (SD)	5.74 (3.5)		
	Range	N	Percentage
PSA pre-surgical, ng/ml	4	7	7.8%
	4-10	63	70.0%
	10	20	22.2%
Gleason score	6	20	22.0%
	7	44	48.9%
	>7	26	28.9%

Table 2
ERG/PTEN profile by lesion type

ERG-PTEN profile	Capsular Penetrated Lesion	Non-capsular Penetrated Lesion	Odds Ratio (95% confidence intervals)	p-value
ERG-/PTEN+	42 (47%)	44 (49%)	Reference	
ERG+/PTEN-	14 (16%)	3 (3%)	5.19(1.37, 19.73)	0.015
ERG+/PTEN+	29 (32%)	36 (40%)	0.80(0.41, 1.56)	0.52
ERG-/PTEN-	5 (6%)	7 (8%)	1.37(0.39, 4.78)	0.62
Total	90 (100%)	90 (100%)		

After adjusting for Gleason score, the odds of capsular penetration are 5.19 times higher in lesions expressing ERG+/PTEN- profile as compared to lesions expressing ERG-/PTEN+ (wild type)

Table 3

Marker status by Gleason score

Marker	Status	Gleason sum scores, N (%)				Total	p-value
		6	7	8	10		
ERG	Negative	22(46.1)	13(27.7)	12(25.5)	0	47(100)	0.65
	Positive	22(51.2)	12(27.9)	8(18.6)	1(2.3)	43(100)	
PTEN	Negative	3(15.8)	7(36.8)	8(42.1)	1(5.3)	19(100)	0.003
	Positive	41(57.8)	18(25.4)	12(16.9)	0	71(100)	

Positive PTEN is associated with statistically significant lower Gleason sum score