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Association of ERG/PTEN Status with Biochemical Recurrence after Radical Prostatectomy for Clinically Localized Prostate Cancer

Rohit Mehra^{1,2,4,#}, Simpa S. Salami^{3,4}, Robert Lonigro¹, Ritu Bhalla⁵, Javed Siddiqui², Xuhong Cao², Daniel E. Spratt^{4,6}, Ganesh S. Palapattu^{3,4}, Nallasivam Palanisamy⁷, John T. Wei³, Arul M. Chinnaiyan^{1,2,3}, and Scott A. Tomlins^{1,2,#}

¹Department of Pathology, University of Michigan Health System, Ann Arbor, MI

²Michigan Center for Translational Pathology, Ann Arbor, MI

³Department of Urology, University of Michigan Health System, Ann Arbor, MI

⁴University of Michigan Rogel Cancer Center, Ann Arbor, MI

⁵Howard Hughes Medical Institute, Ann Arbor, MI; LSU Health Sciences Center, New Orleans, LA

⁶Department of Radiation Oncology, University of Michigan Health System, Ann Arbor, MI

⁷Department of Urology, Vattikuti Urology Institute, Henry Ford Health System, Detroit, MI

Abstract

Background and objective: We have previously demonstrated a significant correlative relationship between *PTEN* deletion and *ERG* rearrangement both in development of clinically localized prostate cancers and metastases. Herein, we evaluate the cooperative role of ERG and PTEN in oncological outcome after radical prostatectomy for clinically localized prostate cancer.

Methods: We evaluated *ERG* and *PTEN* status using three previously described cohorts. The first cohort included 235 clinically localized prostate cancer cases represented on tissue microarrays (TMA), evaluated using previously validated FISH assays for *ERG* and *PTEN*. The second cohort included 167 cases of clinically localized prostate cancer on TMAs evaluated for *PTEN* by FISH, and for PTEN and ERG by dual IHC. The third cohort comprised 59 clinically localized prostate cancer cases assessed by array comparative genomic hybridization (aCGH). Kaplan Meir plots and long rank tests were used to assess the association of *ERG* and *PTEN* status with biochemical recurrence after radical prostatectomy for clinically localized prostate cancer.

Results: Of the 317 cases eligible for analyses with evaluable *ERG* and *PTEN* status, 88 (27.8%) patients developed biochemical recurrence over a median follow-up of 5.7 years. Overall, 45%

#Co-corresponding authors: Rohit Mehra, M.D., 2G332 UH, 1500 E. Medical Center Drive, Ann Arbor, MI 48109, Phone: 734-647-9125, Fax: 734-763-4095, mrohit@med.umich.edu; Scott A. Tomlins, M.D., Ph.D., 1500 E. Medical Center Dr., 7322 Cancer Center, SPC 5948, Ann Arbor, Michigan 48109-5948, Phone: 734-764-1549, Fax: 734-647-7950, tomlinss@umich.edu.

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(142/317) of cases demonstrated *ERG* rearrangement and 20% (62/317) of cases demonstrated *PTEN* loss. Hemizygous and homozygous deletion of *PTEN* was seen in 10% (18/175) and 3% (5/175) of *ERG* negative cases, respectively. In contrast, hemizygous and homozygous deletion of *PTEN* was seen in 11% (15/142) and 17% (24/123) of *ERG* positive cases, respectively. *PTEN* loss (heterozygous or homozygous) was significantly associated with shorter time to biochemical recurrence compared to no *PTEN* loss ($p<0.001$). However, *ERG* rearrangement vs. no rearrangement was not associated with time to PSA recurrence ($p=0.15$). Patients who exhibited *ERG* rearrangement and loss of *PTEN* had no significant difference in time to recurrence compared to patients with wild type *ERG* and loss of *PTEN* ($p=0.30$).

Conclusion: Our findings confirm a mutual cooperative role of *ERG* and *PTEN* in the pathogenesis of prostate cancer, particularly for homozygous *PTEN* deletion. *ERG* did not stratify outcome either alone or in combination with *PTEN* in this cohort.

Keywords

ERG; PTEN; prostate cancer; fluorescent in situ hybridization (FISH); immunohistochemistry

Introduction

In a world being driven by precision medicine¹, there is currently an impetus to identify biologically relevant subgroups of prostate cancer patients where therapy can be developed and targeted judiciously. Current and future studies need to identify which patients need to be treated, when the patients need the treatment, and how these patients should be treated. To address these unanswered questions, we need biomarkers that can delineate patients with prostate cancer who are at risk of disease recurrence, progression and/or metastasis (prognostic markers), and which appropriate therapy can be given to these high risk patients (predictive markers).

PTEN loss and *ERG* rearrangement might have a potential combined prognostic and predictive role. *PTEN* is a membrane-associated phosphatase encoded by a gene on chromosome 10q23, which acts as a tumor suppressor by inhibiting the pro-proliferation PI3K/AKT pathway.² *PTEN* is an antagonist of PI3K (phosphatidylinositol 3-kinase) signaling and impaired *PTEN* function leads to phosphatidylinositol-3,4,5 trisphosphate (PIP3) accumulation in cells and to unrestrained activation of its downstream signals.³ Current estimates suggest that PI3K/AKT signaling is upregulated in 30-50% of prostate cancers, often through loss of *PTEN*.⁴ Recurrent gene fusions involving the *ETS* family of transcription factors (*ERG*, *ETV1*, *ETV4* and *ETV5*) and *TMPRSS2* occurs in the majority of prostate cancers.⁵⁻¹⁰ Among these aberrations, *TMPRSS2:ERG* fusion is the most prevalent, occurring in approximately 50% of localized prostate cancers and 30% of castration resistant metastatic cancers in PSA screened predominantly Caucasian cohorts.^{9,10} Emerging data suggest that *TMPRSS2:ERG* fusion plays an important role in carcinogenesis in vitro and in vivo.^{11,12} Although most post-prostatectomy studies report no or marginal association of *ERG* rearrangements with prostate cancer outcome, there are other cohorts where *ERG* deletion has been associated with a significantly poor survival.^{13,14}

In murine models, *ERG* rearrangements and *PTEN* deletions interact to induce prostate cancer.^{13–15} Several studies have confirmed a significant association between *ERG* gene rearrangements and *PTEN* genomic deletions in a subset of human prostatic adenocarcinomas, and most studies show an association of *PTEN* loss and poor outcome, with conflicting results on the importance of *ERG* status for the prognostic ability of *PTEN* status.^{16–20} Hence, in the current study, we examine the association of *ERG* rearrangement and *PTEN* loss with biochemical recurrence after radical prostatectomy in a large cohort of patients with clinically localized prostate cancer.

Materials and Methods

This study was approved by the Institutional Review Board at the University of Michigan Medical School (patient consent was deemed not necessary as part of the ethical review that was conducted). We evaluated *ERG* and *PTEN* status using three cohorts. The first cohort included 235 clinically localized prostate cancer cases represented on tissue microarrays (TMA), evaluated using previously validated FISH assays for *ERG* and *PTEN*. The second cohort included 167 cases of clinically localized prostate cancer on TMAs evaluated for *PTEN* by FISH, and for *PTEN* and *ERG* by dual IHC. The third cohort comprised 59 clinically localized PCa assessed by array comparative genomic hybridization (aCGH). For these analyses, we excluded any overlapping cases in the cohorts (n=25), those from patients treated with pre- or adjuvant hormone therapy or radiation (n=9), and those with no follow up information available (n=49). After excluding those without evaluable status for both *ERG* and *PTEN*, our final cohort consisted of a total of 371 unique patients.

Fluorescence *In Situ* Hybridization (FISH)

We performed interphase FISH as previously described by our group^{7,9}. Briefly, bacterial artificial chromosomes (BACs) were obtained from the BACPAC Resource Center (Oakland, CA, USA), and probes were prepared as described^{5,9}. For detection of *ERG* rearrangement, RP11-95I21 (5' to *ERG*) and RP11-476D17 (3' to *ERG*) were used with a break-apart probe strategy⁹. To detect *PTEN* deletion, a combination of *PTEN* gene locus-specific probe (RP11-165M8) and 10q11.1-specific probe (RP11-351D16) for chromosome 10 copy number identification were utilized. Schematic BACs for *ERG* and *PTEN* are shown in Figure 1a. The integrity and correct localization of all probes were verified by hybridization to metaphase spreads of normal peripheral lymphocytes. Slides were examined using an imaging Z1 microscope (Carl Zeiss, Oberkochen, Germany). FISH signals were scored manually (100x oil immersion) in morphologically intact and non-overlapping nuclei, and a minimum of 50 cancer cells from each site were recorded. Cancer sites with very weak or no signals were recorded as insufficiently hybridized. Cases lacking tumor tissue in all three cores were excluded. For validation of *PTEN* deletion, we utilized an earlier documented method with minor modification²¹. Briefly, based on hybridization in five control cores (data not shown), hemizygous deletion of *PTEN* gene was defined as >50% nuclei (mean±3 standard deviations in nonneoplastic controls) containing either one signal of locus probe and 2 signals of reference probe, or two signals of locus probe and 4 signals of reference probe. Homozygous deletion of *PTEN* was exhibited by the simultaneous lack of the both *PTEN* locus signals and the presence of control signals in >30% of cells.^{21–23}

Representative FISH images of *PTEN* deletion and *ERG* rearrangement are shown in Figures 1b and c.

Immunohistochemistry (IHC)

We have recently designed and validated a protocol for evaluating PTEN expression by immunohistochemistry (IHC).²⁰ ERG–PTEN dual IHC was performed using anti-ERG (EPR3864) rabbit monoclonal primary antibody (1:100; Cat no. 790-4576, Ventana Medical Systems, Tucson, AZ, USA) and a rabbit monoclonal primary antibody against PTEN (1:25; 138G6- Cell Signaling Technology, Danvers, MA, USA). Dual IHC was performed using an automated protocol developed for the DISCOVERY XT automated slide staining system (Ventana Medical Systems) using UltraMap antirabbit HRP (Cat no. 760-4315, Ventana Medical Systems) for ERG and UltraMap anti-rabbit AP (Cat no. 760-4314, Ventana Medical Systems) for PTEN as secondary antibodies and were detected using ChromoMap DAB (Cat no. 760-159, Ventana Medical Systems) and ChromoMap Blue (Cat no. 760-161, Ventana Medical Systems) for ERG and PTEN, respectively. Nuclear Fast Red counterstain (Cat no. 780-2186 Ventana Medical Systems) was used as the counterstain. Hematoxylin II (Cat no. 790-2208 Ventana-Roche) was used as counterstain. Examples of ERG/PTEN staining in prostate cancer are shown in Figure 2.

Array Comparative Genomic Hybridization (aCGH)

aCGH of 59 localized prostate cancers was performed using gDNA on Agilent's 105K or 244K aCGH microarrays (Human Genome CGH 105K or 244K Oligo Microarray) using Agilent's standard Direct Method protocol and Wash Procedure B as reported previously.²⁴ Briefly, 1.5 - 3 µg of gDNA from prostate specimens was restriction digested with AluI and RsaI, labeled with Cy-5 (test channel), purified using Microcon YM-30 columns and hybridized with an equal amount of Cy-3 (reference channel) labeled Human Male Genomic DNA (Promega) for 40 hours at 65 °C. Post-hybridization wash was performed with acetonitrile wash and Agilent Stabilization and Drying Solution wash according to the manufacturer's instructions. Scanning was performed on an Agilent scanner Model G2505B (5-micron scan with software v7.0), and data was extracted using Agilent Feature Extraction software v9.5 using protocol CGH-v4_95_Feb07.

For data analysis, probes on all arrays were limited to those on the 105K array. Log(2) ratios for each probe were determined as $rProcessedSignal/gProcessedSignal$. The final dataset was uploaded into a custom instance of Oncomine (www.oncomine.com) for automated copy number analysis. In Oncomine, circular binary segmentation was performed on the dataset using the DNACopy package (v1.18) available via the Bioconductor package. Agilent Probe IDs are mapped to segments and reporter values are used to generate segment values (mean of reporters). Resulting segments are mapped to hg18 (NCBI 36.1) RefSeq coordinates (UCSC refGene) as provided by UCSC (UCSC refGene, July 2009, hg18, NCBI 36.1, March 2006) and segment values are assigned to each gene. Copy number profiles were visualized using Oncomine Power Tools. ERG rearrangement status was determined from qRT-PCR and DNA microarray expression profiling as reported previously.²⁵ Examples of cases with PTEN deletions and ERG rearrangement through deletion (assessable by aCGH) are shown in Figure 3.

Clinical Data Collection

We reviewed patients' medical records to abstract clinical and pathological information. Follow up data were collected including serum prostatic surface antigen (PSA). Biochemical recurrence was defined as serum PSA ≥ 0.2 ng/mL following radical prostatectomy.

Statistical Analysis

Descriptive statistics were used to summarize all demographic and clinicopathologic data. Fisher's exact test was used to test the associations between *PTEN* deletion and *ERG* rearrangement status with biochemical recurrence, with *p*-values 0.05 considered to be statistically significant. Statistical analyses were carried out using the R software package, version 2.7.2 (<http://www.r-project.org>).

Results

A total of 317 men with clinically localized prostate cancer who underwent radical prostatectomy, with long-term follow-up information, were evaluable for both *ERG* and *PTEN* evaluation. The demographic, clinical and pathological characteristics of the cohort are presented in Table 1. The median age and PSA were 61 years and 6.9 ng/mL respectively. Of the 317 eligible cases, 88 (27.8%) cases developed biochemical recurrence over a median follow-up of 5.7 years (range: 3.0 – 11.0 years). The median biochemical recurrence-free survival (RFS) was 5 years.

Overall, 44.8% (142/317) of cases demonstrated *ERG* rearrangement and 19.6% (62/317) of cases demonstrated *PTEN* loss. We observed a significant association between *PTEN* deletion and *ERG* rearrangement ($p = 0.0014$). Hemizygous and homozygous deletion of *PTEN* was seen in 10.3% (18/175) and 2.9% (5/175) of *ERG* negative cases, respectively. In contrast, hemizygous and homozygous deletion of *PTEN* was seen in 10.6% (15/142) and 19.5% (24/142) of *ERG* positive cases, respectively (Table 2). *ERG* positive cases were more likely to exhibit homozygous deletion of *PTEN* compared with *ERG* negative cases (19.5% vs. 2.9%; $p < 0.0001$).

PTEN loss (heterozygous or homozygous) was significantly associated with time to biochemical recurrence compared to no *PTEN* loss (Logrank test, $p = 0.0007$; Figure 4a). *ERG* rearrangement vs. no rearrangement, on the other hand, was not associated with time to biochemical recurrence ($p = 0.15$; Figure 4b). When *PTEN* deletion was stratified into homozygous versus heterozygous deletion, there appeared to be no difference in time to recurrence between the wild type *PTEN* and heterozygous deletion. Homozygous deletion, however, was associated with shorter time to biochemical recurrence compared with the wild type *PTEN* or heterozygous *PTEN* deletion status ($p < 0.0001$; Figure 4c).

Combining the *PTEN* with *ERG* rearrangement status as depicted in Figure 4d, wild type *PTEN* and positive *ERG* rearrangement status was associated with the best biochemical RFS estimates in this cohort. Patients with prostate cancer harboring *PTEN* deletion and negative *ERG* rearrangement status suffered the worst outcome. *PTEN* status appears to be the driver of biochemical RFS, with wild type *PTEN* having an overall better biochemical RFS estimates compared with *PTEN* deletion, regardless of *ERG* rearrangement status (Figure

4d). Although the combination of *PTEN* and *ERG* status stratified patients by time to biochemical recurrence (overall 4 combinations, $p=0.001$), patients who exhibited *ERG* rearrangement and loss of *PTEN* had no significant difference in time to recurrence compared to patient with wild type *ERG* and loss of *PTEN* ($p=0.30$; Figure 4d).

Discussion

The interaction between *PTEN* deletion and *ERG* rearrangement may play a significant role in the pathogenesis and clinical course of prostate cancer.^{16–20,26,27} In our study, we report *PTEN* deletion in 20% of cases which was associated with worse biochemical RFS. We also found that homozygous *PTEN* deletion appeared to be the driver of biochemical recurrence, compared with heterozygous deletion. Although *ERG* rearrangement was found in 45% of cases, it was not significantly associated with recurrence. However, when stratified by *PTEN* status, negative *ERG* rearrangement was associated with worse RFS in each stratum. In addition, a significant association between *PTEN* deletion and *ERG* rearrangement was observed in our cohort.

ERG rearrangement and *PTEN* loss are frequent genetic alterations in prostate cancer. Bismar et al., demonstrated that *ERG* rearrangements and *PTEN* deletions were detected in 41.5% and 63.6% of castration resistant prostate cancer, with the majority (68.1%) of *ERG* rearrangements occurring through deletions.²⁸ The authors also reported that, *PTEN* deletions were significantly associated with *ERG* rearrangements occurring by deletions, which is consistent with our findings. In contrast, Liu *et al.*, detected *ERG* rearrangements in 13% of cases of unsuspected prostate cancers of the transition zone with 66% occurring by deletion.²⁹ *PTEN* deletions were detected in 19% of cases in this cohort with only 6% having both *PTEN* losses and *ERG* rearrangements, compared with 12.3% observed in our cohort. These authors suggested that majority of transition zone tumors harbor either *ERG* rearrangement or *PTEN* deletions, compared to peripheral zone prostate cancers which are more likely to harbor both aberrations simultaneously. These differences in incidence of *ERG* and *PTEN* aberrations are likely related to different zonal origins of prostate cancer.

Genomic *PTEN* loss is associated with prostate cancer aggressiveness, progression or poor prognosis.^{8,10,18,30} Decreased *PTEN* expression is linked with increased risk of recurrence in patients with clinically localized prostate cancer.^{6–8,31} In a recent study by Lotan et al., cytoplasmic *PTEN* loss was identified in 84% of intraductal carcinomas of the prostate and provides a plausible molecular explanation for why intraductal carcinoma is associated with poor prognosis.³² Krohn *et al.*, recently demonstrated that genomic deletion of *PTEN* is associated with tumor progression and early PSA recurrence in both *ERG* fusion-positive and *ERG* fusion-negative prostate cancers, and characterizes a particularly aggressive subset of metastatic and castration resistant prostate cancer.¹⁶ In our study, we found that *PTEN* status appears to be the driver of biochemical RFS after radical prostatectomy for clinically-localized prostate cancer, with wild type *PTEN* having an overall better biochemical RFS estimates compared with *PTEN* deletion, regardless of *ERG* rearrangement status (Figure 4d). Our findings support the recent report that *PTEN* loss is associated with poor disease specific survival after radical prostatectomy.³⁰ Although we did not evaluate *PTEN* levels and its correlation with genomic *PTEN* loss in this study, we have previously reported a

significant correlation between decreased PTEN expression and genomic *PTEN* deletion status.¹⁷ Further, in animal and human studies, a clear dose-response relationship exists between decreased PTEN levels and prostate carcinogenesis and biological behavior, supporting our observation that only homozygous *PTEN* loss was associated with worse biochemical RFS.^{31,33}

A strong association between *ERG* rearrangement and *PTEN* deletion suggest an interactive or cooperative role in the biology of prostate cancer.^{13–20,26,27} *ERG* rearrangement was prevalent in our cohort (45%), with a strong association with *PTEN* deletion, but was not associated with biochemical recurrence. Similar to our findings, Yoshimoto et al., found a strong relationship of *TMPRSS2:ERG* fusions with *PTEN* deletions, however *TMPRSS2:ERG* fusions did not show correlation with Gleason grade.²⁰ Further studies are needed to characterize the cooperative role of *ERG* rearrangement and *PTEN* deletion in the pathogenesis and progression of prostate cancer, and its potential as a therapeutic target.

ETS gene fusion and members of the PI3K pathway may provide actionable targets for therapeutic intervention.³⁴ First, the interaction between the *TMPRSS2:ERG* gene fusion product and PARP1 (involved in DNA repair) could be harnessed for targeted therapy. As shown in preclinical studies, the inhibition of PARP1 may result in ETS-induced DNA damage leading to cell death.^{35,36} In the first human study, a randomized phase II trial (NCT01576172) of Abiraterone plus Prednisone with or without Veliparib (a PARP1 inhibitor) in treating patients with metastatic hormone-resistant prostate cancer, the final results for which are currently awaited.³⁷ Second, the PI3K/Akt/mTOR pathway is involved in tumorigenic processes such as cellular proliferation, cell growth, and angiogenesis.^{2,4} The tumor suppressor gene, *PTEN*, is the primary negative regulator of this pathway and its loss results in over activation of this pathway in human cancers such as brain, breast, and prostate.^{38,39} Early results of a Phase I/IIa study (NCT01458067) of GSK2636771 in *PTEN* deficient patients with advanced solid tumors are promising and the final report of the trial will be informative.^{40,41} Given the apparent cooperation or interaction between *ERG* rearrangement and *PTEN* deletion, the combined results of these two trials may provide a basis for combining these agents in a clinical trial.

Our study has several limitations. First, most of tissue-based outcome/prognostic studies for prostate cancer within the literature (including the current study) have employed TMAs where tissue cores from the index nodule have been used to construct the TMA. This is based upon the assumption that the index tumor nodule (defined as largest tumor nodule) is biologically the most representative (or most aggressive) of the disease within a single patient. Though this is true in most patients, there is a significant number of cases where the index tumor nodule indeed does not represent the highest Gleason score within a single patient. This phenomenon might explain partly why *ERG* fusions were not associated with poor outcome in our study, but it needs to be addressed within studies, that project use of biomarkers for clinical use. Second, we do not have data for local recurrence or metastasis in our cohort. But, PSA is a well-established marker of recurrence after radical prostatectomy for clinically localized prostate cancer. Third, there is a possibility of selection bias due to the retrospective nature of our study, however technicians who did the laboratory assessments of *ERG* and *PTEN* were blinded to the clinical or recurrence status of the

patients. Nevertheless, the strength of our study lies in the exploration of *ERG* and *PTEN* in combination, and their role in long-term biochemical recurrence in a large cohort of patients who underwent radical prostatectomy for clinically localized prostate cancer.

Conclusions

Using FISH, IHC and aCGH technologies, we confirm a mutual co-operative role of *ERG* and *PTEN* in the pathogenesis of prostate cancer, particularly for homozygous *PTEN* deletion. Although *ERG* did not stratify the outcome of clinically localized prostate cancer either alone or in combination with *PTEN*, further studies are needed to further characterize the role of *ERG* in prostate cancer pathogenesis and progression. These findings improve our understanding of the underlying biology of prostate cancer and the mechanism underlying a poor outcome in clinically localized disease.

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Figure 1. Fluorescent in situ hybridization (FISH) probe design and representative *ERG* aberrations and *PTEN* deletions detected in prostate cancer.

(A) Schematic of bacterial artificial chromosomes (BACs)- locus/control for *PTEN* and located 5' and 3' to *ERG* used as probes for interphase FISH. Chromosomal coordinates are from the March 2006 build of the human genome using the UCSC Genome Browser. BACs are indicated as numbered rectangles, with the number identifying the BAC as described below and the color indicating the probe color in the accompanying images. Genes are shown with the direction of transcription indicated by the arrowhead and exons indicated by bars. (B and C) FISH was carried out using BACs as indicated with the corresponding fluorescent label on formalin-fixed paraffin-embedded tissue sections. B. Locus/control FISH for *PTEN*. B1. Representative case with retained *PTEN* showing two red signals (10q23/*PTEN* locus) and two green signals (10q11.1) in tumor cells. B2. Representative case

with *PTEN* hemizygous deletion showed one red signal and pairs of green signals in tumor cells. B3. Representative case with *PTEN* homozygous deletion showed absence of red signals (10q23/*PTEN* locus), but retained pairs of green signals (*PTEN* control). C. Break-apart FISH for *ERG* gene. Green and red colors imply individual signals, whereas yellow signals were indicated as colocalized probes. C1. C2. *ERG* rearrangement-positive (with deletion) case showed loss of one green labeled probe 5' to *ERG*. C3. *ERG* rearrangement positive (translocation) case showed one pair of split 5' and 3' signals. For all assays, at least 50 cancer cell nuclei were evaluated.

Figure 2. Immunohistochemistry evaluation of ERG and PTEN in prostate cancer tissues. ERG–PTEN dual immunohistochemistry was performed to evaluate ERG and PTEN status, with results shown from a representative case. Hematoxylin and eosin photomicrograph (H&E, top) showing prostatic adenocarcinoma (black arrow), benign prostatic glands (green arrow) and vessels (blue arrow). Dual ERG/PTEN staining (middle) showing positive ERG (brown chromogen) expression and loss of PTEN (purple chromogen) expression in the prostatic adenocarcinoma. Note the positive internal control for ERG antibody staining in endothelial cells and retained PTEN expression in benign prostatic glands. PTEN loss

exclusively in the prostatic adenocarcinoma was confirmed by PTEN only staining (bottom). All images original magnification 20x.

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Figure 3. aCGH profiles of prostate cancer with *PTEN* deletion and *TMPRSS2:ERG* gene fusions through deletion.

aCGH profiling of 59 primary prostate cancers was performed as described previously²⁴ and uploaded into OncoPrint for analysis and visualization using the OncoPrint Power Tools DNA Copy Number Browser. The segmented, log2 copy number for all genes is plotted in genomic order (from chromosome 1 to X) for four representative samples showing wild type *PTEN* and *TMPRSS2* to *ERG* (top panel), wild type *PTEN* and *TMPRSS2:ERG* fusion through deletion (second panel from top), homozygous *PTEN* deletion and wild type

TMPRSS2 to *ERG*, and homozygous *PTEN* deletion and *TMPRSS2:ERG* fusion through deletion.

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Figure 4. Kaplan-Meier plots for *PTEN* and/or *ERG* status and time to biochemical recurrence.

A. Cases (n=317) were evaluated for *PTEN* status by FISH or aCGH, and classified as *PTEN* wild type or deleted. B. Cases (n = 317) were evaluated for *ERG* status by FISH, aCGH or IHC, and classified as *ERG* rearrangement negative or positive. C. As in A, except *PTEN* deleted cases were stratified by hemizygous or homozygous deletion status. D. Combined stratification by *PTEN-ERG* status as in A and C. Biochemical recurrence was censored at 10 years follow up. Log Rank *p*-values are indicated.

Table 1.

Demographics, clinical and pathologic features of the cohort

Variable		Value*
Age (years)		61 [55 – 65]
Race	White	266 (84)
	Black	25 (8)
	Unknown/Other	26 (8)
Gland size (grams)		45 [37 – 54]
PSA (ng/mL)		6.9 [4.9 – 10.2]
Gleason score	6	71 (22)
	7	209 (66)
	7 with 5	12 (4)
	8	11 (3)
	9	14 (4)
Maximum tumor dimension (cm)		1.5 (1.2 – 2.0)
pT stage	T2a	33 (10)
	T2b	195 (62)
	T3a	64 (20)
	T3b	18 (6)
	T4	7 (2)
pN stage	N0	243 (77)
	N1	7 (2)
	Nx	67 (21)
Positive surgical margin		101 (32)
Follow up (years)		5.7 [3.0 – 11.0]

* Median [Interquartile range] and frequency (percentages) are presented for continuous and categorical variables respectively.

Abbreviations: PSA – Prostate specific antigen.

Table 2.

ERG and PTEN Status of the Cohort

PTEN Status	ERG Status		Total
	Negative	Positive	
Negative	152	103	255
Positive	23	39	62
Total	175	142	317

PTEN Status	ERG Status		Total
	Negative	Positive	
Negative	152	103	255
Hemizygous	18	15	33
Heterozygous	5	24	29
Total	175	142	317