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Fluorescence *in situ* hybridization study shows association of *PTEN* deletion with *ERG* rearrangement during prostate cancer progression

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The link between *ERG* rearrangement and *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) deletion is unclear in prostate cancer progression. Using fluorescence *in situ* hybridization, we systematically validated the frequency and distribution of *ERG* and *PTEN* aberrations in a cohort of 73 benign prostate tissues, 59 high-grade prostatic intraepithelial neoplasia (HGPIN) foci, 281 localized prostate cancer and 47 androgen-independent metastatic prostate cancer patients. Overall, *ERG* rearrangement was present in 15% (5/33) of HGPIN, 45% (121/267) of localized cancers and 35% (15/43) of metastases. By contrast, *PTEN* deletion was identified in 9% (3/33) of HGPIN, 17% (42/251) of localized cancers and 54% (22/41) of metastases, of which 0%, 40% (17/42) and 45% (10/22) were homozygous, respectively. Concomitance of *ERG* rearrangement and *PTEN* deletion was observed in a subset of HGPIN. Significantly, association between *PTEN* deletion and *ERG* rearrangement was present both in localized cancers ($P=0.0008$) and metastases ($P=0.02$). Further, immunohistochemistry revealed significant correlation of decreased PTEN protein expression with *PTEN* genomic deletion both in localized and metastatic cancer. Of note, *ERG* aberration, but not *PTEN* deletion, was consistently identical both in localized cancer and adjacent HGPIN. Similarly, whereas all metastases (41/41, 100%) shared the same *ERG* status across multiple sites from the same patient, 5% (2/41) of cases showed discordance for *PTEN* deletion status across multiple sites. Collectively, our data support *PTEN* deletion as a late genetic event in human prostate cancer, presumably a 'second hit' after *ERG* rearrangement. *PTEN* deletion and *ERG* rearrangement may cooperate, but contribute at different stages, in prostate cancer progression.
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Prostate cancer is the second leading cause of cancer-related death among North American men.[1] It proceeds through a putative precursor lesion, termed high-grade prostatic intraepithelial neoplasia (HGPIN), to hormone naive clinically localized cancer and finally to androgen-independent metastatic cancer.[1,2] Despite its high prevalence, the molecular basis of prostate cancer progression remains unclear.[2]

Recently, recurrent gene fusions involving the *ETS* family of transcription factors, *ERG, ETV1*, *ETV4* and *ETV5*, fused to *TMPRSS2* or other upstream partners, have been identified in the majority of prostate cancers.[3–10] Among these aberrations, *TMPRSS2–ERG* fusion is the most prevalent, occurring in approximately 50% of localized prostate cancers and 30% of androgen-independent

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metastatic cancers.[10–12] As *TMPRSS2* and *ERG* are located ~3 Mb apart on chromosome 21, the rearrangement between them occurs either through translocation or by an interstitial deletion.[13] Emerging data suggest that *TMPRSS2–ERG* fusion plays an important role in carcinogenesis *in vitro* and *in vivo*.[14,15] Clinically, *ERG* rearrangement has been observed in 10~20% of HGPIN.[16–18] Mosquera *et al*[18] showed that of 143 HGPIN cases, 16% (23 of 143) were *ERG* rearrangement positive, and in all cases the paired prostate cancer was *ERG* rearrangement positive through the same mechanism. These observations suggest that *ERG* rearrangement may be an early event in prostate cancer. Additionally, studies in watchful waiting cohorts suggest that *ETS* rearrangement cancers is associated with a more aggressive phenotype; however, conflicting reports have been described in prostatectomy series.[12,19–22]

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a key tumor suppressor gene in prostate cancer.[23] Loss of *PTEN* function results in increased PIP3 (Phosphatidylinositol (3,4,5)-triphosphate) levels and subsequent AKT phosphorylation and modulation of its downstream molecular oncogenic processes.[24] A series of *in vivo* studies have shown the role of *PTEN* in prostate carcinogenesis with prostate-specific deletion.[25,26] Clinically, deletion or mutation of at least one *PTEN* allele was reported to occur in 20–40% of localized cancers[27–29] and up to 60% of metastases.[30] Fluorescent *in situ* hybridization (FISH) and immunohistochemical studies showed that *PTEN* genomic deletion and absence of PTEN expression are associated with unfavorable clinical outcome measures.[29–31] Recent studies also showed that PTEN inactivation plays an important role in prostate cancer during progression to androgen-independence.[32,33]

Whereas *ERG* rearrangement and *PTEN* deletion are strongly implicated in prostate cancer development, little is known about the link between these two genomic events. Most recently, Yoshimoto *et al*[34] reported that *TMPRSS2–ERG* fusion could be accompanied by *PTEN* deletion in localized prostate cancer. However, there has been no systematic FISH validation on these genomic aberrations in the context of prostate cancer progression. Hence, we comprehensively evaluated a wide spectrum of benign tissues, premalignant and malignant lesions to characterize *ERG* rearrangement and *PTEN* deletion during prostate cancer progression.

Materials and methods

Study Population, Clinical Data and Tissue Microarray (TMA)

A total of six TMAs were interrogated in this study that represents: (1) 281 clinically localized prostate cancer patients who underwent radical prostatectomy as a monotherapy between 1995–1996 and 2004–2006 at the University of Michigan Hospital; (2) 47 androgen-independent metastatic prostate cancer patients with multiple metastatic sites and tumors in the prostate (when present) from a rapid autopsy program described earlier[12] and (3) 20 benign prostate hyperplasia, 18 atrophy and 35 benign prostate tissues derived from the peripheral zone of prostate containing cancer. Morphology was confirmed by three pathologists (BH, RBS and RM), and three cores (0.6 mm in diameter) were taken from each representative area of interest. Patient demographics of localized prostate cancer are shown in Supplementary Table 1. The detailed clinical, pathological and TMA data were maintained on a secure relational database as described earlier.[11] This study was approved by the Institutional Review Board at the University of Michigan Medical School and all the patients provided written informed consent. Both radical prostatectomy series and the rapid autopsy program were part of the University of Michigan Prostate Cancer Specialized Program of Research Excellence Tissue Core.

Case Selection for HGPIN

A total of 59 HGPIN present in prostatectomy specimens from 56 localized prostate cancers represented in this cohort were also included in the study. Initially, we randomly chose 34 HGPIN lesions from equal number of localized cancer patients to assess the frequency of *PTEN* deletion and/or *ERG* rearrangement. We further reviewed localized cancer patients that harbored known *ERG* and/or *PTEN* genomic aberrations and selected 20 cases containing HGPIN to analyze association of *PTEN* and *ERG* aberrations. All HGPIN lesions were selected by three pathologists (BH, RM and RBS) by consensus and divided into two categories: those adjacent to cancer (distance <3 mm from the edge of the cancer, HGPINadj) or those away from cancer (distance >3 mm from the closest cancer in any single section and 4 mm from the closest cancer on the adjacent section above or below, HGPINaway).[35] Although HGPINadj cases selected for the study may potentially represent an intraductal spread of prostate cancer, none of the HGPINadj lesions included in the study morphologically contained high-grade pleomorphic nuclei, which are $6\times$ size of normal nuclei and/or intraluminal comedonecrosis, features usually considered characteristic of an intraductal spread of cancer.[36]

Fluorescence *In Situ* Hybridization

Interphase FISH was carried out as described.[8,11] Bacterial artificial chromosomes (BACs) were obtained from the BACPAC Resource Center (Oakland, CA, USA), and probes were prepared as described.[3,11] For detection of *ERG* rearrangement,

RP11-95I21 (5′ to *ERG*) and RP11-476D17 (3′ to *ERG*) were used with a break-apart probe strategy.[11] To detect *PTEN* deletion, a combination of *PTEN* gene locus-specific probe (RP11-165M8) and 10q11.1-specific probe (RP11-351D16) for chromosome 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 ImagingZ1 microscope (Carl Zeiss, Oberkochen, Germany). FISH signals were scored manually ($\times$ 100 oil immersion) in morphologically intact and non-overlapping nuclei by two pathologists (BH and RM), 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 modification.[37] Briefly, on the basis of 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 (absolute deletion), or two signals of locus probe and ≥ 4 signals of reference probe (relative deletion). 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.[29,34,37] Representative FISH images of *PTEN* deletion and *ERG* rearrangement are shown in Figure 1b.

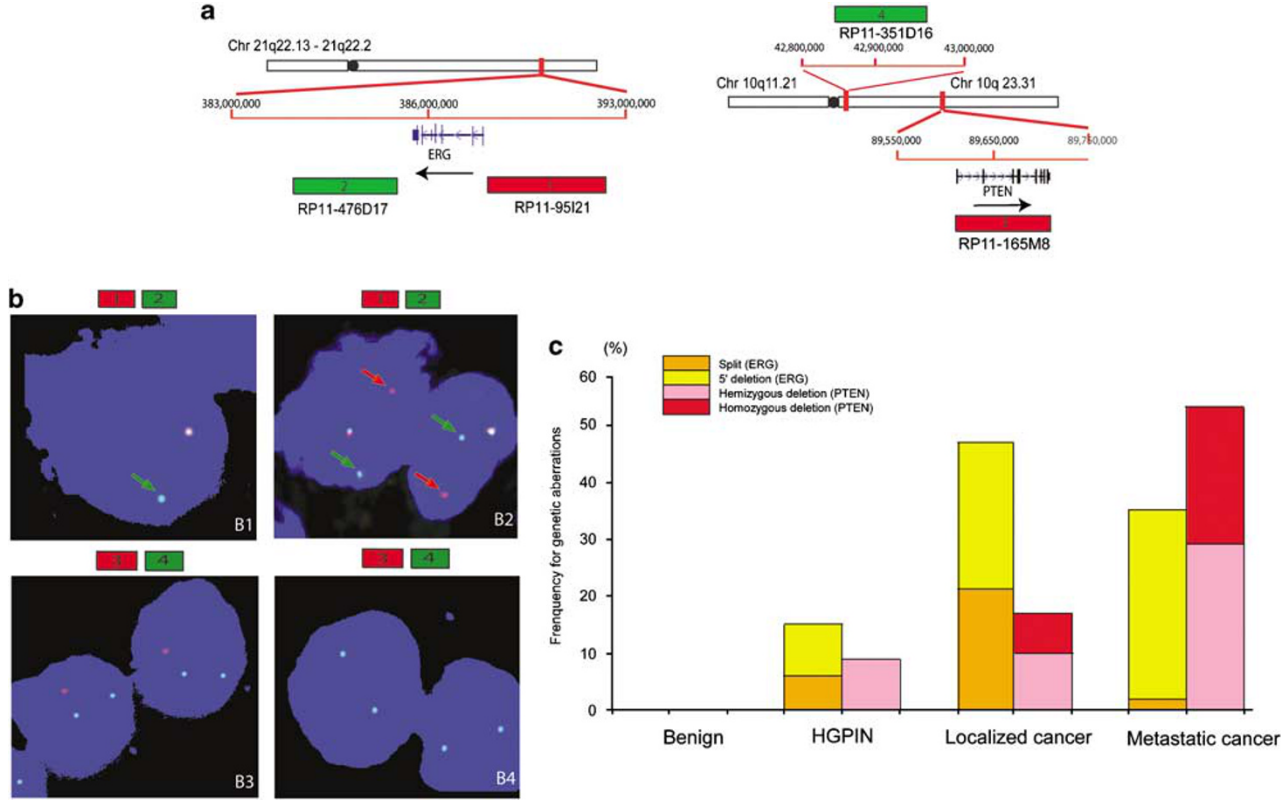

Figure 1 Fluorescent *in situ* hybridization (FISH) probe design and representative *ERG* aberrations and *PTEN* deletions detected in prostate cancer. (**a**) Schematic of bacterial artificial chromosomes (BACs) located 5′ and 3′ to *ERG* and locus/control for *PTEN* used as probes for interphase FISH. 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**) (**b1, b2**) FISH was carried out using BACs as indicated with the corresponding fluorescent label on formalin-fixed paraffin-embedded tissue sections for break-apart FISH of the *ERG* gene. Green and red arrows showed individual signals, whereas yellow signals were indicated as colocalized probes. (**b1**) *ERG* rearrangement-positive (with deletion) case showed loss of one red labeled probe 5′ to *ERG*. (**b2**) *ERG* rearrangement positive (translocation) case showed one pair of split 5′ and 3′ signals. (**b3, b4**) Representative images of hemizygous and homozygous *PTEN* deletion in prostate cancer. (**b3**) Representative case with *PTEN* hemizygous deletion showed one red signal (10q23/*PTEN* locus) and pairs of green signals (10q11.1) in tumor cells. (**b4**) Representative case with *PTEN* homozygous deletion showed absence of red signals (10q23/*PTEN* locus), but retained pairs of green signals (*PTEN* control). For all assays, at least 50 cancer cell nuclei were evaluated. (**c**) Frequency of *PTEN* deletion and *ERG* rearrangement of benign tissues, high-grade prostatic intraepithelial neoplasia (HGPIN), localized and metastatic prostate cancers is indicated. Color legend signifies respective aberrations.

Immunohistochemistry (IHC)

Immunohistochemistry was carried out using a rabbit polyclonal antibody against PTEN (Ab9552, Cell Signaling, MA, USA) on TMA using 1:100 dilution, and incubated overnight at 4°C following standard LSAB immunohistochemical staining protocol.[38] The slides were evaluated blindly by three independent observers (BH, KS and RM). Triplicate cores from each specimen were scored separately, and the presence of tumor tissue in at least two interpretable cores was required to include a case for analysis. According to the cytoplasmic staining intensity, the tumors were divided into three categories as described earlier:[39,40] grade 2 showed increased or equal staining intensity compared with the corresponding normal tissue; grade 1 had decreased staining intensity and grade 0 showed complete absence of staining.

Statistical Analysis

Fisher's exact test was used to test the statistical significance of associations between *PTEN* deletion status and *ERG* rearrangement status, as well as the association between *PTEN* genomic deletion status and PTEN protein expression level, with *P*-values <0.05 being considered statistically significant. Statistical analyses were carried out using the R software package, version 2.7.2 (http://www.r-project.org).

Results

Frequency of *PTEN* Deletion and *ERG* Rearrangement

To determine the frequency of *PTEN* and *ERG* genomic aberrations in different prostate tissue types, we screened a wide spectrum of prostate lesions and benign prostate tissues represented on multiple TMAs. As shown in Table 1, *PTEN* deletion was found in 17% (42/251) of the localized prostate cancer patients, out of which 60% (25/42) exhibited hemizygous deletion. In androgen-independent metastatic prostate cancer, *PTEN* deletion was present in 54% (22/41) of cases, among which 55% (12/22) showed hemizygous deletion. By contrast, only 9% (3/33) of HGPIN showed *PTEN* deletion, all of which were hemizygous.

Overall, *ERG* was rearranged in 45% (121/267) of the localized prostate cancer cases, of which 55% (67/121) showed deletion of the 5′ end of *ERG* (Table 1). A similar frequency of *ERG* rearrangement (35%, 15/43) was observed in androgen-independent metastatic cancers as reported earlier,[12] the majority showing deletion of the 5′ end of *ERG*. By contrast, *ERG* aberrations were identified in 15% (5/33) of HGPIN, and as expected, were not detected at all in non-neoplastic prostate tissues. Of note, part of our FISH data of *ERG* aberrations represented on three TMAs have been published before as part of the University of Michigan cohort.[9,11,12]

Association of *PTEN* Deletion and *ERG* Rearrangement in Localized and Androgen-Independent Metastatic Prostate Cancer

As *ERG* rearrangement and *PTEN* deletion are among the most common genomic aberrations in prostate cancer,[10,23] we next explored the association of these two genomic events in this cohort. As shown in Table 2, the *ERG* rearrangement was present in approximately 71% (29/41) of localized cancers with *PTEN* deletion (hemizygous or homozygous). Likewise, *PTEN* deletion occurred more frequently in cases that harbored *ERG* rearrangement (26%, 29/110) as compared with those *ERG* rearrangement negative cases (9%, 12/127). Of the androgen-independent metastases, co-existence of the *PTEN* deletion and *ERG* rearrangement was present in 28% (11/39) of cases. Overall, a significant association between *PTEN* deletion and *ERG* rearrangement was observed both in localized prostate cancer ($P=0.0008$) and androgen-independent metastatic prostate cancer ($P=0.02$) (Table 2). Interestingly, for localized cancers with *PTEN* deletion, *ERG* fusion-positive cases were more likely to show homozygous deletion of *PTEN*, although this association did not reach statistical significance ($P=0.08$). It is to be noted that there was a total of

Table 1 Summary of *ERG* rearrangement and *PTEN* deletion status[a]

	ERG			*PTEN*		
Tissue types	*No rearrangement*	*Translocation*	*5′ deletion*	*Not deleted*	*Hemizygous*	*Homozygous*
Normal prostate tissue	35 (100%)	0 (0%)	0 (0%)	32 (100%)	0 (0%)	0 (0%)
BPH	20 (100%)	0 (0%)	0 (0%)	19 (100%)	0 (0%)	0 (0%)
Atrophy	18 (100%)	0 (0%)	0 (0%)	18 (100%)	0 (0%)	0 (0%)
HGPIN	28 (85%)	2 (6%)	3 (9%)	30 (91%)	0 (0%)	3 (9%)
Localized prostate cancer	146 (55%)	54 (20%)	67 (25%)	209 (83%)	25 (10%)	17 (7%)
Androgen-independent metastasis	28 (65%)	1 (2%)	14 (33%)	19 (46%)	12 (29%)	10 (25%)

BPH, benign prostate hyperplasia; HGPIN, high-grade prostatic intraepithelial neoplasia.
[a]Not all the cases included are informative for both *ERG* rearrangement and *PTEN* deletion status.

Table 2 Distribution of *ERG* rearrangement and *PTEN* deletion status in localized and metastatic prostate cancer patients

PTEN	*ERG*					
	*Localized cancer**			*Metastatic cancer***		
	No rearrangement	*Translocation*	*5′ deletion*	*No rearrangement*	*Translocation*	*5′ deletion*
No deletion	115	38	43	15	1	2
Hemizygous	10	5	9	7	0	5
Homozygous	2	5	10	3	0	6

*$P = 0.0008$.
**$P = 0.02$.

12 localized cancer cases harboring rearrangement of *ETV1* or *ETV4* in the current study. Overall, eight of them are informative for *PTEN* genetic status, in which 50% (4/8) are with *PTEN* deletions.

PTEN Deletion and *ERG* Rearrangement in HGPIN

High-grade prostatic intraepithelial neoplasia is a putative precancerous lesion, and earlier studies have shown that *PTEN* genomic deletion as well as *ERG* rearrangement could occur in subset of HGPIN. [16–18,41] Additionally, Perner *et al*[16] showed that *ERG* rearrangement displayed a different picture in HGPINadj *vs* HGPINaway. However, it is unclear whether the *PTEN* deletion and *ERG* rearrangement could co-exist in HGPIN and what their association is to cancer. To address this, we analyzed 25 HGPIN on prostatectomy specimens from 20 selected localized cancers, which harbored genomic aberrations of *ERG* and/or *PTEN*. As shown in Figure 2a,100% (11/11) HGPINadj shared the same *ERG* aberrations with the paired cancer foci (T1-T9, T13, T20), which is comparable with earlier studies.[16,18] By contrast, 60% (6/10) HGPINadj shared the same *PTEN* genomic aberrations with the paired cancer foci (T7-T11, T13), whereas the remaining four cases were not (T6, T12, T19-20). Of note, concomitance of *PTEN* deletion and *ERG* rearrangement were observed in HGPINadj intermingling with cancer foci from four localized cancer patients (T7-T9, T13). Interestingly, no *PTEN* deletion or *ERG* rearrangement was observed in HGPINaway. Figure 2b represents a reconstructed map of the prostatectomy sections in index cases T6 and T9. In total, these findings suggest co-existence of *ERG* rearrangement and *PTEN* deletion in a subset of HGPINadj.

Homogeneity of *ERG* Rearrangement, but not *PTEN* Deletion in Multiple Metastatic Sites of Androgen-Independent Prostate Cancer

Earlier, we validated *TMPRSS2–ETS* aberrations in 30 androgen-independent metastatic prostate cancer patients. In patients exhibiting *ERG* aberrations, we observed that multiple metastatic sites from an individual case harbored the same *TMPRSS2–ERG* rearrangement, all of which occurred through intrachromosomal deletion.[12] In this study, we extrapolated our initial findings to 47 metastatic prostate cancer patients representing the University of Michigan warm autopsy cohort and evaluated *ERG* status in 156 tumor foci from different organs as well as the prostate (when present). Similar to our earlier study,[12] *ERG* was rearranged in 35% (15/43) of cases, and cases were rearranged through deletion of 5′ end of *ERG,* except M39, in which *ERG* split (translocation) was observed (Figure 3a). Notably, homogeneous *ERG* aberrations were present in all metastatic sites and primary tumors (when present) within an individual patient. These results support the concept that *ERG* rearrangement occurs at the clinically localized stage before progression to an androgen-independent metastatic stage in prostate cancer.

We further analyzed *PTEN* deletion status across all metastatic sites in these 47 cases. As shown in Figure 3b, of 41 interpretable warm autopsy cases, 39 showed concordant *PTEN* deletion status across all metastatic sites and primary tumors in prostate (when present). By contrast, in two cases (M1 and M5), hemizygous *PTEN* deletion was identified in metastatic foci of soft tissue (M1) or the liver (M5), but no deletion was present in the prostate and other metastatic sites. We further examined all available paraffin tissue specimens for all the metastatic sites and primary tumors, but did not find any additional cancer foci harboring *PTEN* deletion in these two cases (data not shown). Thus, these results suggested that *PTEN* deletion could occur after dissemination of tumor in a small subset of androgen-independent metastatic cancer patients.

Comparison of *PTEN* Genomic Deletion and PTEN Protein Expression by IHC

We earlier reported that >95% of prostate cancer cases with *ERG* overexpression harbor *TMPRSS2–ERG* gene fusions.[3] By contrast, multiple mechanisms account for loss of PTEN protein expression, including genomic deletion, mutation and promoter

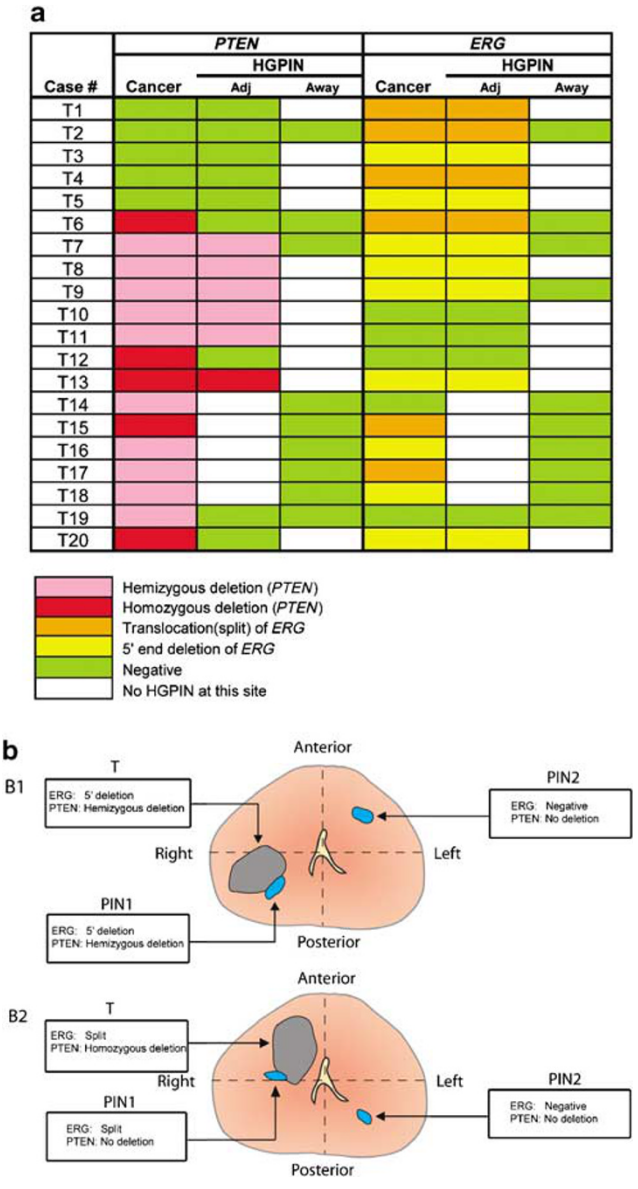

Figure 2 Genomic aberrations of *ERG* and *PTEN* in high-grade prostatic intraepithelial neoplasia (HGPIN). (**a**) Matrix representation of *ERG* and *PTEN* genomic aberrations in selected localized prostate cancer patients with paired HGPIN (Adj, HGPIN adjacent to cancer; Away, HGPIN away from cancer) assessed by fluorescent *in situ* hybridization (FISH). Patient case numbers are shown on the left of the matrix map. Each column represents one case and each row represents FISH evaluation for aberrations of *ERG* or *PTEN*. Color legend signifies respective aberrations or availability. (**b**) Representative reconstructed maps of the prostectomy sections in case T9 (**b1**) and T6 (**b2**). Tumor is represented as T; HGPINadj and HGPINaway are represented as PIN1 and PIN2, respectively. A summary of genomic aberrations of *ERG* and *PTEN* for each of these foci is presented in the boxes.

methylation.[23] To explore the association of *PTEN* gene status with PTEN protein expression, we analyzed 207 prostate cancer patients in this cohort. Predominantly cytoplasmic, with occasional nuclear, staining of *PTEN* was observed by IHC (Figure 4a). As shown in Table 3, statistically significant associations were observed between *PTEN* deletion and decreased PTEN protein expression in both localized ($P<0.0001$) and metastatic ($P=0.045$) cancer. Of note, 26 (19%) localized cancer cases revealed decreased PTEN protein expression, but were negative for *PTEN* genomic deletion, suggesting that reduced PTEN expression might be because of other mechanisms. Additionally, we detected PTEN protein expression in selected HGPINadj with known *PTEN* genomic aberrations in localized cancer T7–T11 and T13 (Figure 2a). As expected, all but one (HGPINadj in T9) exhibited decreased or absent PTEN expression.

Concordance between *PTEN* deletion status and decrease PTEN protein expression was also observed in 28 out of 39 (72%) androgen-independent metastases (Table 3). Out of 11 discordant cases, decreased (grade 1) or absence (grade 0) of PTEN protein expression was observed in eight cases that were negative for *PTEN* deletion. In the remaining three cases, normal PTEN protein expression was identified, although *PTEN* hemizygous deletion was observed.

Discussion

ERG rearrangement and *PTEN* deletion are two of the most common genomic events in human prostate cancer. In this initial study, we used rearrangements in *ERG* as a marker for *ETS* rearrangements in prostate cancer, as >90% of all *ETS* rearrangements involve *ERG*.[10] Additionally, as only *TMPRSS2* and *SLC45A3* have been identified as 5′ fusion partners of *ERG*, with *SLC45A3-ERG* being extremely rare in our PSA-screened radical prostatectomy series,[9] we can conclude that most of the *ERG*-rearranged prostate cancers are *TMPRSS2–ERG* fusions (>97%). In the current study, for the first time, we have observed significant association of *ERG* rearrangement and *PTEN* deletion in clinically localized ($n=281$) and androgen-independent metastatic prostate cancers ($n=47$). Biologically, we and others have reported that overexpression of ERG resulted in increased cellular invasion *in vitro*.[14,15] By contrast, *PTEN* deletion was associated with a gain of transformation potential and marked increase in cellular proliferation in prostate cancer through its negative regulation of the PI3K pathway.[24] Of note, many studies have shown that *PTEN* can synergize with other oncogenic factors or related genes in mouse models, including *NKx3.1*, *p27* and *p53* to promote cancer development and androgen independence.[25,42,43] Although it is unknown whether cross-talk exists between *ERG* and the PTEN–PI3K pathway, we hypothesize that the interactions between these two genomic aberrations may be synergic or additive. Indeed, while this study was in preparation, Yoshimoto *et al*[34] reported that concurrent *PTEN* deletion and *TMPRSS2–ERG* fusions was present in a subset of prostate cancer cases and associated with an unfavorable outcome.

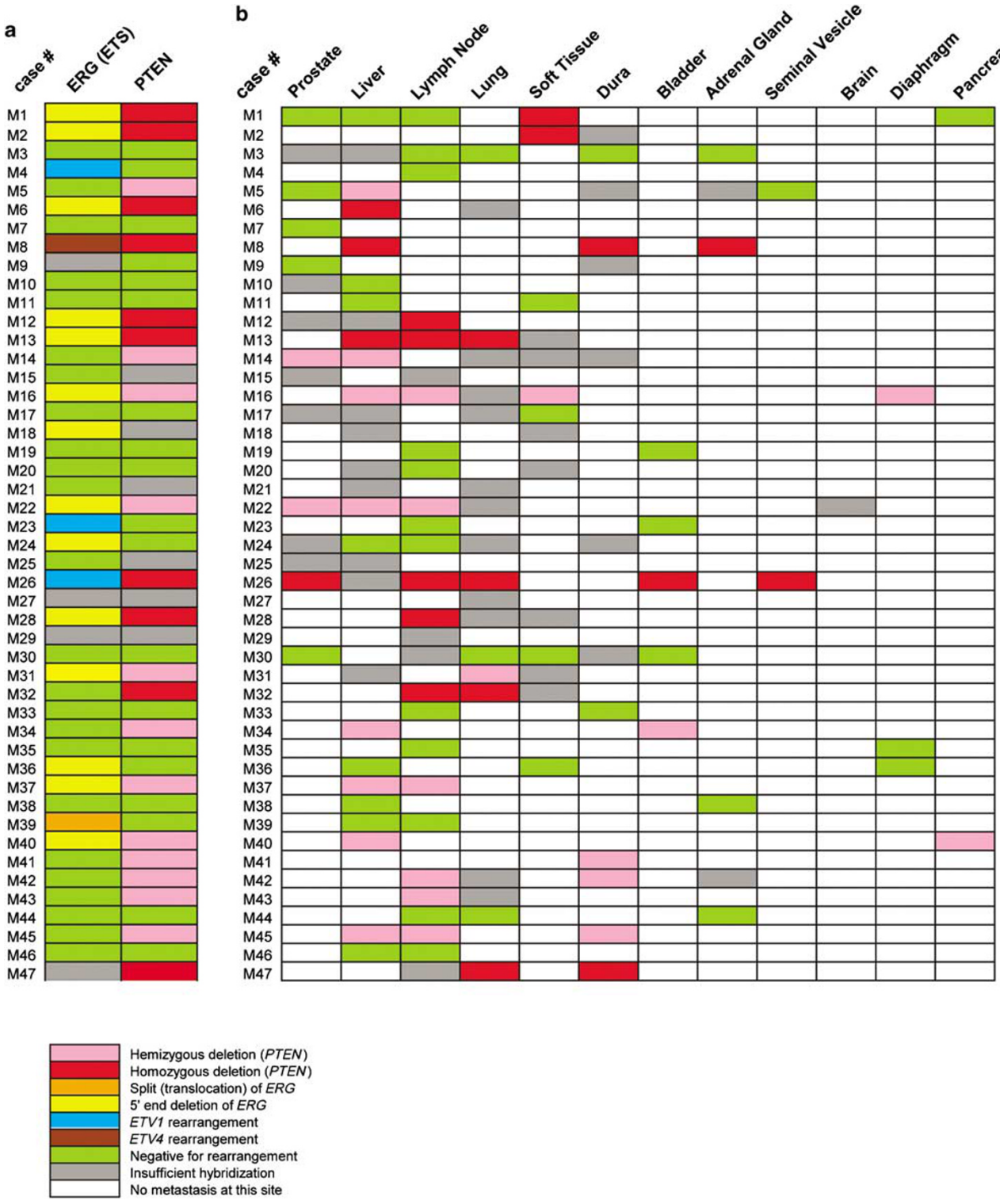

Figure 3 Genomic aberrations of *ERG* and *PTEN* in androgen-independent metastatic prostate cancer. (**a**) Matrix representation of the genomic aberrations of *ERG* and *PTEN* in metastatic prostate cancers. Patient case numbers are shown on the left of the map. Each column represents one case and each row represents fluorescent *in situ* hybridization (FISH) evaluation for *ERG* or *PTEN* aberration. Rearrangements of *ETV1* and *ETV4* in this cohort that were earlier published are indicated. (**b**) Matrix representation of *PTEN* deletion status of the metastatic sites and residual tumor in the prostate (when present) as evaluated in this cohort. Patient case numbers are shown on the left of the map. Each column represents one case and each row represents FISH evaluation for *PTEN* deletion at each organ site. Color legend signifies respective aberrations or availability.

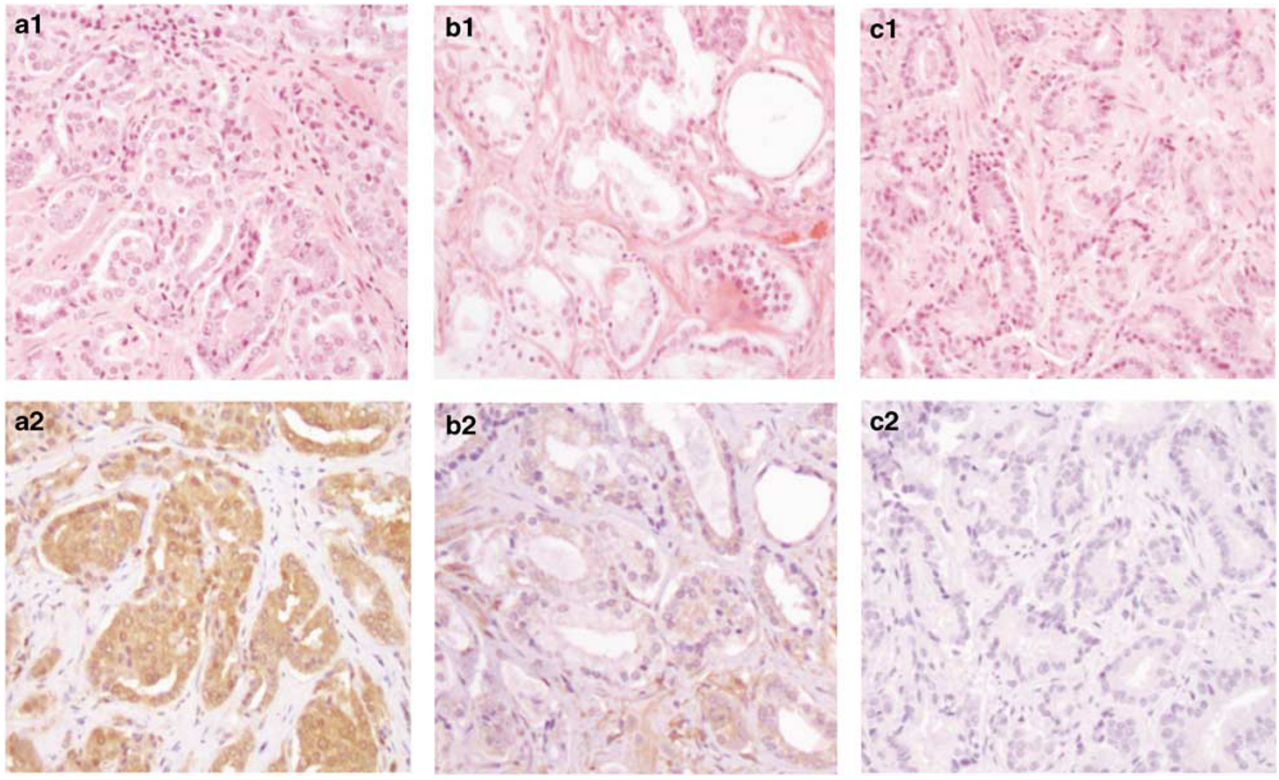

Figure 4 PTEN expression in prostate cancer by immunohistochemistry. (**a**) Representative case of prostate cancer exhibits positive staining for PTEN graded as 2 (greater than or equivalent to normal adjacent tissues in the same section) ((**a1**) H&E stained section. (**a2**) Immunohistochemical staining). Original magnification, ×200. (**b**) Representative case of prostate cancer exhibits weak staining for PTEN graded as 1 ((**b1**) H&E staining. (**b2**) Immunohistochemical staining). (**c**) Representative case of prostate cancer exhibits absence staining for PTEN graded as 0. ((**c1**) H&E staining. (**c2**) Immunohistochemical staining). Original magnification, ×200.

Table 3 The correlation between *PTEN* deletion status and PTEN protein expression in prostate cancer

	PTEN protein (IHC)			P-*value*
PTEN gene	*0*	*1+*	*2+*	
Localized prostate cancer				
No deletion	6	20	93	$P<0.0001$
Hemizygous	7	3	1	
Homozygous	4	6	0	
Metastatic prostate cancer				
No deletion	3	5	9	$P=0.045$
Hemizygous	3	7	2	
Homozygous	4	5	1	

Using Oncomine, they further attempted to explore the potential signaling pathways involved for synergy between these genomic events. How ERG overexpression interacts with *PTEN* deletion is still unclear and compound transgenic mice recapitulating these lesions will likely be ideal models to further explore this relationship. Using IHC, we also identified significant correlation between decreased PTEN expression and *PTEN* genomic deletion status, supporting deletion as a major mechanism leading to decreased PTEN protein expression.

Further, we attempted to investigate the roles of *ERG* and *PTEN* genomic aberrations in prostate cancer progression. Of note, in line with earlier studies, no benign prostate glands, atrophy or BPH harbored *ERG* rearrangements.[13,16] *ERG* rearrangement was present in ~15% of HGPIN lesions in this series. In addition, in 11 selected *ERG*-rearranged cancer cases, 100% (11/11) HGPIN shared the same *ERG* rearrangement status with the paired cancer foci, and all of these HGPIN foci were adjacent to the cancer. Although we can not exclude the possibility that HGPINadj lesions may represent an intraepithelial spread from the adjacent invasive cancer or evolved together temporally and developed shared genetic abnormities at the same time, our data, in line with earlier studies,[16] strongly supported *ERG* aberrations as an early molecular event in prostate cancer development. Earlier, we and other groups have reported that *TMPRSS2–ERG* gene fusion could induce HGPIN in transgenic mice.[14,15] It is still not clear whether aberrant ERG overexpression in human induces HGPIN or may drive cancer progression during the transition from HGPIN to localized cancer in human. Further functional characterization, especially using animal models, would be helpful in addressing this issue. By contrast, *PTEN* deletion was not consistently identical both in localized cancer and adjacent HGPIN.

These findings suggested that *PTEN* deletion may not play a significant role in a subset of HGPIN lesions, which develop to localized cancer eventually. In comparison with localized prostate cancers, the prevalence of *ERG* aberration was similar in androgen-independent metastatic cancer (35% *vs* 47%) in this study. If *ERG* aberrations were a later genomic event in prostate cancer progression, one would expect a higher percentage of metastatic prostate cancers to be rearrangement positive. Consistent with earlier findings, all metastatic foci from an individual case were uniformly *ERG* rearrangement negative or positive, indicating that *ERG* rearrangement occurred before progression to metastatic disease.[12]

Although a series of *in vivo* studies have showed that *PTEN* haploinsufficiency results in the HGPIN in transgenic mouse,[23,24,42,43] in the current study, only 9% (3/33) of human HGPIN lesions harbored *PTEN* hemizygous deletion. By contrast, we observed a strikingly increase of prevalence for *PTEN* deletion in androgen-independent metastatic cancer (54%), which is comparable with those reported in recent studies.[31,33] These findings suggest that *PTEN* deletion is more likely a late event in prostate cancer progression, although this genetic aberration occurs earlier in tumor evolution in a subset of cases. Further, discordance of *PTEN* status in different organ sites was observed in a subset of androgen-independent metastatic cancers. That is, *PTEN* genomic deletion was detected in only one of multiple metastases in cases M1 and M5, but absent in the primary tumor and other organs. This suggests that *PTEN* deletion occurred during or after the formation of metastatic foci, possibly because of more generalized genomic instability seen in disseminated cancer. Similarly, Suzuki *et al*[30] has also observed heterogeneity of *PTEN* genetic aberrations between different metastatic sites within the same patient. Considering the important role of PTEN and high frequency of *PTEN* aberration in late-stage prostate cancer, one could assume that *PTEN* deletion may be a critical 'second hit' after *ERG* rearrangement in a subset of *ERG* fusion prostate cancer cases. Supporting this concept, by array CGH, Lapointe *et al*[44] have reported that *TMRPSS2–ERG* fusions seems to occur before *PTEN* genomic deletion.

It has been reported that *PTEN* dose is a key determinant in prostate cancer progression in mouse models.[26] However, our study did not observe a significant difference of *PTEN* deletion patterns (hemizygous *vs* homozygous) between localized and metastatic cancers. Therefore, these data suggest that human localized and metastatic prostate cancers do not seem to select for homozygous deletion. Alternatively, hemizygous deletion of *PTEN* may cooperate with other oncogenic factors including *ERG* rearrangements during prostate cancer progression, manifesting a phenotype that is equivalent to *PTEN* homozygous deletion.

Progression to androgen independence is a complex process including clonal selection and adaptation. Recent evidence suggested that *PTEN* deletion was associated with androgen independence and could functionally control 'two' hits: cell transformation and androgen-independent growth.[32,33] In the current study, we showed that almost all androgen-independent metastatic cancers harboring *ERG* rearrangement were associated with the 5′ deletion of *ERG*. Thus, we could expect that concomitance of *PTEN* deletion and *ERG* rearrangement (through 5′ end deletion) may select for more aggressive cancers that are able to progress to androgen-independent metastatic cancer. Notably, the co-existence of the *PTEN* deletion and the *ERG* rearrangement was present in nearly one-third (11/39) of metastatic cases, underscoring the combinatorial roles of aberrant ERG and PTEN in metastatic cancer.

Collectively, our data support *PTEN* deletion as a late genetic event in human prostate cancer, possibly as a 'second hit' after *ERG* rearrangement. *ERG* rearrangement and *PTEN* deletion may cooperate, but contribute at different stages in prostate cancer progression. Although future work is needed to test this notion, understanding the molecular cross-talk between these two genetic events may provide insight into understanding prostate cancer development. Also, it suggests that simultaneous therapeutic targeting of *PTEN* and *ETS* gene fusions may be important for treating a subset of advanced prostate cancers.

Disclosure

The University of Michigan has filed a patent on *ETS* gene rearrangements in prostate cancer, on which RM, SAT and AMC are co-inventors, and the diagnostic field of use has been licensed to Gen-Probe Incorporated. Gen-Probe has not played a role in the design and conduct of the study, nor in the collection, analysis, or interpretation of the data, and no involvement in the preparation, review or approval of the paper. AMC serves as a consultant to Gen-Probe Inc.

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Supplementary Information accompanies the paper on Modern Pathology website (http://www.nature.com/modpathol)