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ARTICLE

The combination of *PTEN* deletion and 16p13.3 gain in prostate cancer provides additional prognostic information in patients treated with radical prostatectomy

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Abstract

Prostate cancer is a clinically heterogeneous disease and accurately risk-stratifying patients is a key clinical challenge. We hypothesized that the concurrent identification of the DNA copy number alterations 10q23.3 (*PTEN*) deletion and 16p13.3 (*PDPK1*) gain, related to the PI3K/AKT survival pathway, would improve prognostication. We assessed *PTEN* deletion status using fluorescence in situ hybridization (FISH) and evaluated its clinical significance in combination with the 16p13.3 gain in a set of 332 primary radical prostatectomy cases on a tissue microarray with clinical follow-up. The *PTEN* deletion was detected in 34% (97/287) of the evaluable tumors and was significantly associated with high Gleason grade group ($P < 0.0001$) and advanced pathological tumor stage (pT-stage, $P < 0.001$). The *PTEN* deletion emerged as a significant predictor of biochemical recurrence independent of the standard clinicopathologic parameters (hazard ratio: 3.00, 95% confidence interval: 1.81–4.98; $P < 0.0001$) and further stratified patients with low and intermediate risk of biochemical recurrence [Gleason grade group 1–2 (≤3 + 4), Gleason grade group 2 (3 + 4), pT2, prostate-specific antigen ≤ 10, low and intermediate CAPRA-S score; log-rank $P \leq 0.007$]. A *PTEN* deletion also increased the risk of distant metastasis (log-rank, $P = 0.001$), further supporting its role in prostate cancer progression. Combining both 16p13.3 gain and *PTEN* deletion improved biochemical recurrence risk stratification and provided prognostic information beyond the established CAPRA-S score (co-alteration: hazard ratio: 4.70, 95% confidence interval: 2.12–10.42; $P < 0.0001$). Our study demonstrates the potential clinical utility of *PTEN* genomic deletion in low-intermediate risk patients and highlights the enhanced prognostication achieved when assessed in combination with another genomic biomarker related to the PI3K/AKT pathway, thereby supporting their promising usefulness in clinical management of prostate cancer.

Introduction

Prostate cancer remains a major clinical burden, being the most prevalent cancer and one of the leading causes of cancer-specific deaths in North American men [1]. It is a clinically heterogeneous disease wherein the majority of cancers display a favorable outcome, while a subset affecting a considerable number of patients progress to metastatic and lethal stage [2]. Radical prostatectomy or radiation therapy is considered the standard primary treatment option for localized prostate cancer and more recently, active surveillance has emerged as a viable alternative for patients presenting favorable clinicopathologic features [3]. One of the key challenges in the clinical management of prostate cancer is to accurately distinguish indolent from aggressive tumors in order to avoid overtreatment of clinically insignificant cancers and undertreatment of tumors with metastatic potential [4].

Serum prostate-specific antigen levels, biopsy Gleason grade and clinical tumor stage (cT-stage) are used to risk stratify patients, but are not sufficient to accurately predict

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individual clinical outcome. Assessing the Gleason grade based on prostate biopsies is challenging and frequently leads to an underestimation of the actual grade of the entire tumor burden [5]. To address the shortcomings of the clinicopathologic predictors and to better capture the clinical heterogeneity of prostate cancer, biomarkers are being developed based on the underlying spectrum of molecular abnormalities of this disease. DNA copy number alterations are common in cancer and have been associated with molecular subtypes of prostate cancer, supporting the existence of alternative parallel pathways of tumorigenesis [6, 7]. Copy number alterations such as deletion of chromosome 10q23 (*PTEN*) and gain of 16p13.3 (*PDPK1*) are detected at higher frequency in lymph node metastases than in primary tumors, suggesting a role in prostate cancer progression [7, 8]. The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) protein is a tumor suppressor that negatively regulates the phosphatidylinositol 3-kinase/AKT survival pathway [9–11]. Encoded by *PDPK1*, 3-Phosphoinositide-dependent protein kinase-1 (PDK1) phosphorylates and activates the AGC kinase members regulated by phosphatidylinositol 3-kinase, including AKT [12]. In addition to its kinase activity on AKT, our laboratory has shown that PDK1 also has an important role in prostate cancer cell migration [13]. *PTEN* deletion has been shown to be associated with poor outcome in prostate cancer after radical prostatectomy [13–17]. Recently, we reported that the 16p13.3 gain was also associated with adverse outcome after radical prostatectomy [18]. In the current study, we demonstrated that the *PTEN* genomic deletion measured by FISH is a strong independent predictor of poor clinical outcome after radical prostatectomy, including in low-intermediate risk patients and showed that the combination of *PTEN* deletion and 16p13.3 gain status improved patient risk stratification.

Materials and methods

Study population and tissue microarray

This study was done in compliance with the REMARK guidelines [19] and approved by the Research Ethics Board of the McGill University Health Centre (BDM-10–115) with the written informed consent of the participants. A set of 332 de-identified formalin-fixed paraffin-embedded (FFPE) radical prostatectomy specimens collected between 1993 and 2008 at the McGill University Health Centre were represented on a tissue microarray by duplicate 1 mm cores extracted from the dominant tumor nodule. Dominant nodule was defined as generally the largest nodule. In cases in which a smaller nodule was considered to be prognostically more significant (higher grade or stage), this smaller nodule was considered to be dominant.

Table 1 Clinicopathologic features of radical prostatectomy cases represented on the tissue microarray

Clinicopathologic variables	Category	*n* (%)
Total number of cases	*n*	332
Age (years)	Median	61
	Min–max	43–73
Preoperative serum prostate-specific antigen (ng/ml)	*n*[a]	327
	Mean (±SD)	8.66 (±8.27)
	PSA ≤ 10	253 (77%)
	PSA > 10	74 (23%)
Gleason grade groups at surgery	Group 1 (3 + 3)	70 (21%)
	Group 2 (3 + 4)	153 (46%)
	Group 3 (4 + 3)	78 (24%)
	Group 4 (8)	11 (3%)
	Group 5 (≥9)	20 (6%)
Pathological stage (T-stage)	pT2	219 (66%)
	pT3a	91 (27%)
	pT3b	22 (7%)
Surgical margin status	Positive	97 (29%)
Follow-up (months)	*n*[a]	297
	Median (min–max)	116 (1–253)
Biochemical recurrence	*n*[a]	297
	Positive	81 (27%)
Distant metastases	Positive	16/321[a] (5%)

[a]Values not available for all the 332 cases (*n* noted for each variable)

The clinical information was retrieved from the medical charts and the pathological correlates were obtained after re-review of all the radical prostatectomy cases by a single dedicated genitourinary pathologist (F.B.). The final Gleason grade was assigned according to the latest International Society of Urological Pathology/World Health Organization recommendations [20]. The clinicopathologic characteristics of 303 of the 332 cases were reported previously [18] and those of the entire expanded cohort are summarized in Table 1. The mean preoperative serum prostate-specific antigen level was 8.66 (±8.27) and the distribution of Gleason grade group 1 (Gleason score 6), 2 (Gleason score 3 + 4), 3 (Gleason score 4 + 3), 4 (Gleason score 8), and 5 (Gleason score ≥ 9) was 21%, 46%, 24%, 3%, and 6%, respectively. Sixty-six percent of patients were at stage pT2 while 34% belonged to stage pT3. Patients receiving neoadjuvant hormone therapy ($n = 6$) and cases with missing serum prostate-specific antigen data post-radical prostatectomy ($n = 15$) were not included in the biochemical recurrence analyses. Surgical failure cases ($n = 14$), for which the serum prostate-specific antigen did not fall to undetectable levels post-radical prostatectomy, were also excluded from the biochemical recurrence analyses. No patient had received adjuvant radiation therapy after surgery. The primary endpoint of the study was

biochemical recurrence and was defined by a serum prostate-specific antigen elevation of >0.2 ng/ml following radical prostatectomy (27%). The recurrence-free interval was defined as the time between the surgery date and the date of the first prostate-specific antigen increase above 0.2 ng/ml. Patients without biochemical recurrence event were censored at the last follow-up date with prostate-specific antigen measurement. The median follow-up for the cohort was 116 months (1–253 months, min–max). Metastasis status was evaluated and confirmed by imaging in patients with clinical symptoms ($n = 16$). The metastasis-free interval was defined as the period between the surgery date and the date of first metastasis detection and patients without signs/symptoms related to metastasis were censored at the last follow-up/prostate-specific antigen date. The CAPRA-S (Cancer of the Prostate Risk Assessment Post-Surgical) score was calculated from the status of six clinicopathologic variables [preoperative prostate-specific antigen, Gleason score, surgical margins, extracapsular extension, seminal vesicle invasion, lymph node invasion], and each patient was assigned to one of the three risk groups: low (0–2), intermediate [3–5], and high (≥6) according to Cooperberg et al. [21]. Of note, patients who did not undergo a lymph node dissection were considered to have negative lymph node for CAPRA-S score calculation as previously described [22]. The chromosome 16p13.3 gain data recently reported by our lab for this cohort [18] was used for the combinatorial approach.

Fluorescence in situ hybridization (FISH)

The BAC clone CTD-2557P6 (BACPAC Resources Center, Oakland, CA) mapping to the *PTEN* gene on the chromosome 10q23.3 region and commercially available CEP10 Spectrum Green probe (CEP 10, Abbott Molecular, Abbott Park, IL), which spans the 10p11.1-q11.1 centromeric region were used to perform dual-color FISH on the 5 µm tissue microarray sections as we described previously [13]. The CTD-2557P6 DNA was labeled with the Spectrum Orange-dUTP (Enzo Life Science, Farmingdale, NY) using the Nick Translation Reagent Kit (Abbott Molecular) as per the kit manual.

FISH data analysis

To evaluate the *PTEN* copy number status, fluorescent signals were counted in 100 non-overlapping interphase nuclei for each case (as identified on corresponding H&E) counterstained with ProLong® Gold antifade reagent with DAPI (Life Technology, CA), to delineate nuclei. The *PTEN* deletion was defined as ≥15% of tumor nuclei containing one or no *PTEN* locus signal and by the presence of two CEP10 signals as we previously reported [13]. A tumor was considered homozygous-deleted if ≥15% of tumor nuclei had no *PTEN* locus signals and two CEP10 signals. Images were acquired with an Olympus IX-81 inverted microscope at ×96 magnification, using Image-Pro Plus 7.0 software (Media Cybernetics, Rockville, MD).

Statistical analysis

The association between copy number alterations and the clinicopathologic indicators were assessed by Fisher's exact test for categorical variables and unpaired *t*-test for continuous variables. Kaplan–Meier curves were generated for biochemical recurrence-free and metastasis-free survival analysis. The log-rank test was used to evaluate the significance of differences between the stratified survival functions. Cox regression analyses were used to evaluate univariate hazard ratios and multivariate Cox proportional hazards regression analysis was performed to identify independent predictors of biochemical recurrence. The C-index was calculated as described by Harrel et al. [23]. Analyses were performed using SPSS, WinStat, and R (Version 3.3.2).

Results

Association of *PTEN* deletion status with adverse clinical outcome post-radical prostatectomy

The 10q23.3 (*PTEN*) deletion status was assessed using dual-color FISH on 332 radical prostatectomy specimens represented on a tissue microarray. The clinicopathologic features of these patients are summarized in Table 1. The *PTEN* deletion status could be successfully assessed in 287 tumors arrayed out, of which 97 (34%) harbored a *PTEN* genomic deletion. The *PTEN* deletion status was consistent across duplicate TMA cores evaluated. Of the cases with *PTEN* deletion, 80 (28%) were hemizygous deleted while 17 (6%) harbored a homozygous *PTEN* deletion (Fig. 1). Of note, 15 out the 17 cases that we identified as homozygous-deleted for *PTEN* also harbored a significant number of nuclei (≥15%) showing a hemizygous deletion within the tissue microarray core. Preliminary analysis indicated that these cases with homozygous deletion were not different than cases harboring only a hemizygous deletion in term of their association with adverse pathology and poor outcome (not shown). We therefore considered hemizygous and homozygous *PTEN* deletion cases together as a single group for the analyses presented in this report. As shown in Table 2, the *PTEN* deletion status was significantly associated with high Gleason grade group ($P = 0.0001$) and advanced pT-stage ($P = 0.001$).

The prognostic significance of *PTEN* genomic status was first evaluated using biochemical recurrence as a surrogate primary endpoint post-radical prostatectomy. *PTEN* FISH

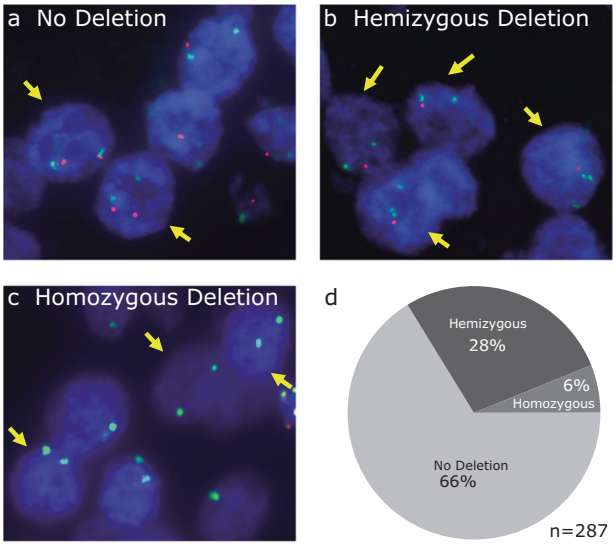


Fig. 1 Dual-color FISH analysis of *PTEN* (10q23) deletion in prostate cancer specimens. White arrows show **(a)** normal interphase nuclei with 2 green and 2 orange signals in a PCa tumor with no *PTEN* deletion; **(b)** 2 green and 1 orange signals in a tumor harboring hemizygous *PTEN* deletion; and **(c)** 2 green and 0 orange signals in a homozygous *PTEN*-deleted case. FISH analysis **(d)** detected hemizygous in 80/287 (28%), homozygous in 17/287 (6%), and no *PTEN* deletion in 189/287 (66%) of the primary radical prostatectomy samples on the McGill urology tissue microarray ($n = 287$)

Table 2 Association of *PTEN* deletion status with clinicopathologic features of aggressive prostate cancer

Clinicopathologic variables	Total cases *n* (%)	*PTEN* status		*P*-value
		No deletion	Deletion	
PTEN status	287	190 (66%)	97 (34%)	
Gleason grade groups	287			0.0001
Group 1 (GS 3 + 3)	61	52 (85%)	9 (15%)	
Group 2 (GS 3 + 4)	132	90 (68%)	42 (32%)	
Group 3 (GS 4 + 3)	67	37 (55%)	30 (45%)	
Group 4 and 5 (GS ≥ 8)	27	11 (41%)	16 (59%)	
Pathological T-stage	287			0.001
pT2	189	138 (73%)	51 (27%)	
pT3a	77	43 (56%)	34 (44%)	
pT3b	21	9 (43%)	12 (57%)	
Preoperative prostate-specific antigen (mean/ ±standard deviation)	282	7.95 (±7.60)	9.03 (±7.24)	0.25[a]
Surgical margin status	287			0.30
Negative	200	136 (68%)	64 (32%)	
Positive	87	54 (62%)	33 (38%)	

P-value calculated by Fisher exact test

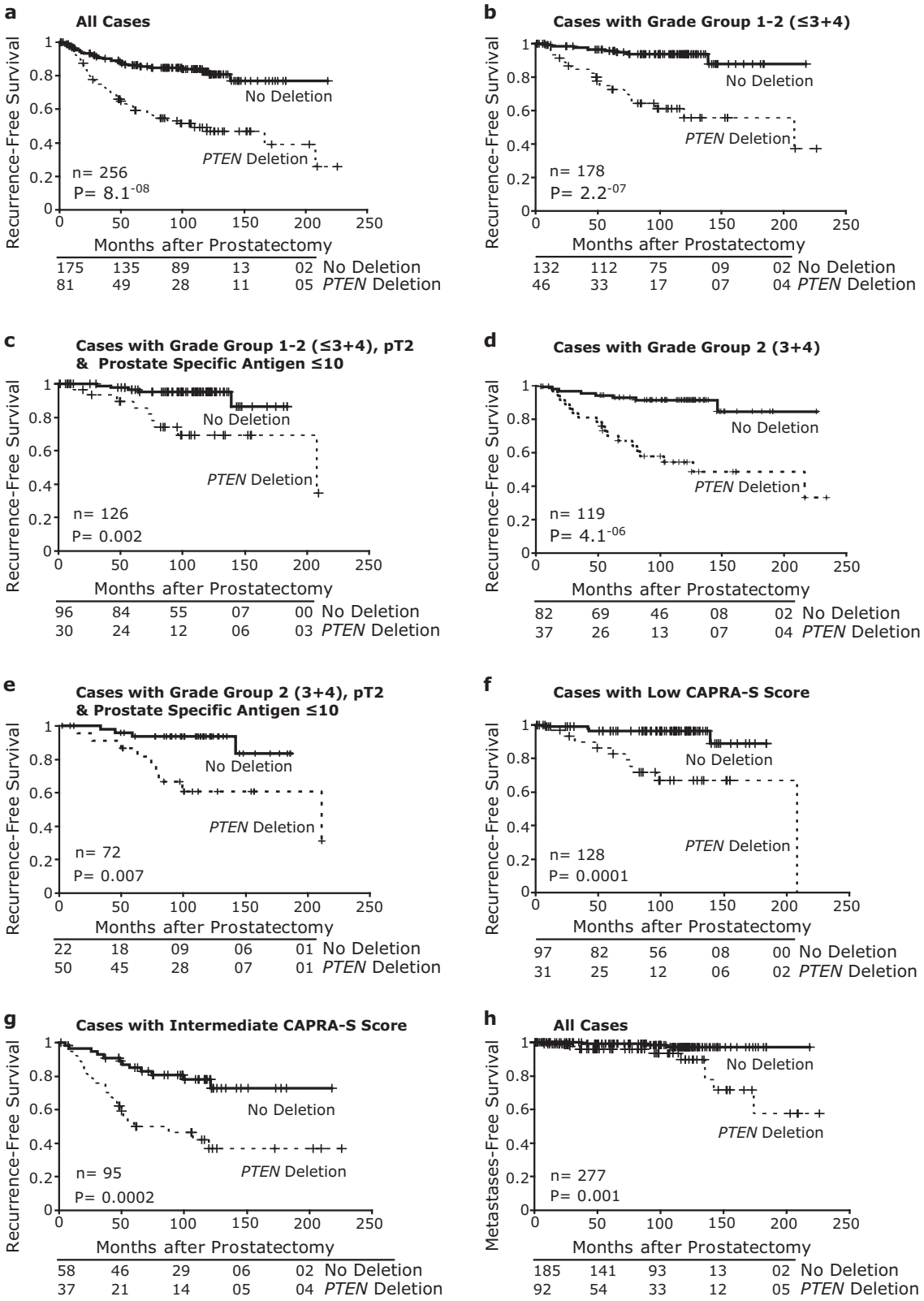
Number of cases that could be assessed (*n*) noted for each variable

[a]Unpaired *t*-test

status and complete prostate-specific antigen follow-up data were available for 256 radical prostatectomy cases, out of which 69 (27%) experienced biochemical recurrence. The *PTEN* genomic deletion status emerged as a significant predictor of early biochemical recurrence following radical prostatectomy (log-rank $P < 0.0001$; Fig. 2a) independent of the standard clinicopathologic prognostic indicators like Gleason grade group, pT-stage, preoperative prostate-specific antigen level, and surgical margin status in a multivariate Cox analysis (hazard ratio: 3.00, 95% confidence interval: 1.81–4.99; $P < 0.0001$; Table 3A).

Clinical significance of *PTEN* genomic deletion in low-intermediate risk prostate cancer patients

We assessed the ability of the *PTEN* deletion status to predict biochemical recurrence risk in a clinically relevant subset of patients belonging to grade groups 1 and 2 (≤3 + 4) according to the latest International Society of Urological Pathology/World Health Organization Gleason grading recommendations [20]. The *PTEN* deletion status was significantly associated with biochemical recurrence in patients of grade group 1–2 (≤3 + 4, log-rank, $P < 0.0001$, Fig. 2b) including those that were also of stage pT2 and with preoperative prostate-specific antigen ≤ 10 (log-rank, $P = 0.002$, Fig. 2c). Furthermore, the *PTEN* deletion was significantly linked to biochemical recurrence in a subgroup of grade group 2 (3 + 4, log-rank, $P < 0.0001$, Fig. 2d) even with favorable stage pT2 and prostate-specific antigen ≤ 10 (log-rank, $P = 0.007$, Fig. 2e). There was an insufficient number of biochemical recurrence events ($n = 1$) in grade group 1 (Gleason score 6) to allow subgroup analysis and the *PTEN* deletion status did not further stratify grade group 3–5 (≥ 4 + 3, not shown). We then assessed if the *PTEN* genomic deletion status could further stratify the risk groups defined by the clinically validated clinicopathologic CAPRA-S score to predict biochemical recurrence post-radical prostatectomy [21]. The multivariate analysis showed that the *PTEN* deletion was a significant predictor of biochemical recurrence along CAPRA-S score risk groups (hazard ratio: 2.84, 95% confidence interval: 1.75–4.63; $P < 0.0001$, Table 3B). *PTEN* deletion identified a subset of patients with a greater risk of biochemical recurrence among those of low and intermediate CAPRA-S score risk groups (Fig. 2f–g, log-rank, $P = 0.0001$ and $P = 0.0002$, respectively). We further evaluated the association of *PTEN* deletion with bone or soft tissue metastases, an important adverse secondary endpoint. *PTEN* deletion status was indeed significantly associated with an increased risk of distant metastasis (log-rank $P = 0.001$, Fig. 2h), further supporting its potential clinical utility as a marker of prostate cancer progression.


a
All Cases
Recurrence-Free Survival
No Deletion
PTEN Deletion
n= 256
P= 8.1-08
Months after Prostatectomy
175 135 89 13 02 No Deletion
81 49 28 11 05 PTEN Deletion
b
Cases with Grade Group 1-2 (≤3+4)
Recurrence-Free Survival
No Deletion
PTEN Deletion
n= 178
P= 2.2-07
Months after Prostatectomy
132 112 75 09 02 No Deletion
46 33 17 07 04 PTEN Deletion
c
Cases with Grade Group 1-2 (≤3+4), pT2 & Prostate Specific Antigen ≤10
Recurrence-Free Survival
No Deletion
PTEN Deletion
n= 126
P= 0.002
Months after Prostatectomy
96 84 55 07 00 No Deletion
30 24 12 06 03 PTEN Deletion
d
Cases with Grade Group 2 (3+4)
Recurrence-Free Survival
No Deletion
PTEN Deletion
n= 119
P= 4.1-06
Months after Prostatectomy
82 69 46 08 02 No Deletion
37 26 13 07 04 PTEN Deletion
e
Cases with Grade Group 2 (3+4), pT2 & Prostate Specific Antigen ≤10
Recurrence-Free Survival
No Deletion
PTEN Deletion
n= 72
P= 0.007
Months after Prostatectomy
22 18 09 06 01 No Deletion
50 45 28 07 01 PTEN Deletion
f
Cases with Low CAPRA-S Score
Recurrence-Free Survival
No Deletion
PTEN Deletion
n= 128
P= 0.0001
Months after Prostatectomy
97 82 56 08 00 No Deletion
31 25 12 06 02 PTEN Deletion
g
Cases with Intermediate CAPRA-S Score
Recurrence-Free Survival
No Deletion
PTEN Deletion
n= 95
P= 0.0002
Months after Prostatectomy
58 46 29 06 02 No Deletion
37 21 14 05 04 PTEN Deletion
h
All Cases
Metastases-Free Survival
No Deletion
PTEN Deletion
n= 277
P= 0.001
Months after Prostatectomy
185 141 93 13 02 No Deletion
92 54 33 12 05 PTEN Deletion

◀ **Fig. 2** Prognostic value of the *PTEN* genomic deletion in prostate tumors. Kaplan–Meier recurrence-free survival analysis of patients stratified on the basis of *PTEN* deletion status determined by FISH in (**a**) all radical prostatectomy patients with clinical follow-up for biochemical recurrence; (**b**) Gleason grade group 1–2 (≤3 + 4), (**c**) Gleason grade group 1–2 (≤3 + 4), stage pT2 and prostate-specific antigen ≤ 10 patients; (**d**) Gleason grade group 2 (3 + 4); (**e**) Gleason grade group 2 (3 + 4), stage pT2 and prostate-specific antigen ≤ 10 patients; (**f**) low CAPRA-S score (0–2) risk patients; and (**g**) intermediate CAPRA-S score [3–5] risk patients. Survival analysis based on *PTEN* deletion status shows (**h**) worse metastases-free survival. Censored data (tick marks), number of patients at risk at respective time points, and *P*-value (log-rank test) are indicated

Improved biochemical recurrence risk stratification upon combining 16p13.3 gain with *PTEN* deletion

16p13.3 genomic gain status has recently been shown to be associated with aggressive clinicopathologic features of prostate cancer [8, 18], as well as with poor clinical outcome in our cohort [18]. A set of 251 cases for which both 16p13.3 gain and *PTEN* deletion data were available was used for the combinatorial *PTEN*-16p13.3 co-alteration analyses. We first tested whether *PTEN* deletion status could further stratify patients without 16p13.3 gain. As shown in Fig. 3a, cases with *PTEN* deletion have an increased risk of biochemical recurrence among this subgroup (log-rank, $P < 0.0001$). Interestingly, amongst patients with no *PTEN* deletion, the 16p13.3 gain further identified a subset of patients at high risk of recurrence (Fig. 3b, log-rank, $P = 0.001$). We then grouped cases based on their *PTEN*-16p13.3 co-alteration status-(0) no *PTEN* deletion and no 16p13.3 gain, (1) *PTEN* deletion or 16p13.3 gain, and (2) *PTEN* deletion and 16p13.3 gain. Kaplan–Meier analysis demonstrated that the *PTEN*-16p13.3 co-alteration status further segregated prostate cancer cases in three distinct prognostic subgroups (log-rank $P < 0.0001$, Fig. 3c) stratified by the number of positive markers: the favorable prognostic group with no alterations in *PTEN* and 16p13.3, the intermediate prognostic group with one alteration in either *PTEN* or 16p13.3, and the worst prognostic group with two alterations (*PTEN* and 16p13.3). Moreover, in the multivariate Cox analysis adjusted for standard prognostic indicators, the *PTEN*-16p13.3 co-alteration status remained significant and conferred the highest risk of biochemical recurrence (hazard ratio: 4.18, 95% confidence interval: 1.82–9.59; $P = 0.001$; Table 4A). Similarly, a *PTEN* deletion along a 16p13.3 gain increased the risk of recurrence significantly even after adjusting for the CAPRA-S score risk groups (hazard ratio: 4.70, 95% confidence interval: 2.12–10.42, $P < 0.0001$, Table 4B). To estimate the potential prognostic benefit of assessing both genomic alterations, we calculated the C-index for each of *PTEN* deletion and 16p13.3 gain alone and in combination using biochemical recurrence as an endpoint in Cox model. The C-index was higher by using both alterations than 16p13.3 gain or *PTEN* deletion alone (0.69 vs. 0.62 and 0.63, respectively) and each of these alterations improved the C-index of the CAPRA-S score reaching a maximum when both were included (0.78, Fig. 3d).

Discussion

In this study, we confirmed the association of *PTEN* deletion with poor outcome in prostate cancer and demonstrated its potential at further stratifying low-intermediate risk patients treated by radical prostatectomy. In addition, we showed that its prognostic value can be improved by considering the gain of 16p13.3. We detected *PTEN* deletion by FISH in 34% of the 287 radical prostatectomy specimens examined, a frequency falling within the range of 17–42% reported by other previously published studies using FISH and including over hundred samples [14–17, 24, 25]. The majority of deletions observed were hemizygous (28% vs. 6% of homozygous) in agreement with most of the previous publications on radical prostatectomy cases. In contrast, Krohn et al. [14] reported 12% of homozygous and 8% of hemizygous deletion in their cohort while Troyer et al. [16] observed 9% homozygous and 9% hemizygous deletion in their samples. The variation in frequency of *PTEN* deletion and in proportion of hemizygous vs. homozygous deletion observed among the studies possibly reflects differences of cohort sizes and clinicopathologic features, but also likely differences in tissue preparation and FISH scoring method. The presence of homozygous-deleted and hemizygous-deleted nuclei in most of tumor classified as *PTEN* homozygous-deleted in our study likely reflect intratumoral heterogeneity and possibly disease progression.

Supporting a role for *PTEN* alteration in prostate cancer progression, our study showed that its deletion was significantly associated with the aggressive clinicopathologic features of high Gleason grade group and advanced surgical stage pT3, a finding consistent with previous reports of *PTEN* FISH on large radical prostatectomy sets [14, 16]. In agreement with our prior report on a separate sample set [13] as well as with previous studies of other groups [14–17], we have also shown that *PTEN* deletion assessed by FISH was associated with biochemical recurrence after radical prostatectomy. Moreover, the prognostic value of the deletion was independent of standard clinicopathologic markers. In our study, homozygous deletion was not associated with a higher risk of biochemical recurrence than hemizygous deletion in agreement with the report of Krohn et al. [14], but in contrast to Yoshimoto et al. and Troyer et al. [16, 17]. Our data indicate that the loss of one copy was sufficient to increase significantly the risk of

Table 3 Univariate and multivariate Cox proportional hazard analysis predicting biochemical recurrence for *PTEN* deletion status adjusted for standard clinicopathologic parameters (A) and CAPRA-S score (B)

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% confidence interval)	*P*-value	Hazard ratio (95% confidence interval)	*P*-value
(A) Standard clinicopathologic parameters				
PTEN status (deleted vs. non-deleted)	3.47 (2.14–5.63)	<0.0001	3.00 (1.81–4.99)	<0.0001
Preoperative prostate-specific antigen[a]	1.06 (1.04–1.09)	<0.0001	1.05 (1.01–1.08)	0.005
Gleason grade				
Group 3–5 (≥4 + 3) vs. group 1–2 (≤ 3 + 4)	4.75 (2.91–7.78)	<0.0001	2.60 (1.44–4.67)	0.001
pT-stage (T3 vs. T2)	3.32 (2.05–5.38)	<0.0001	1.53 (0.87–2.67)	0.137
Surgical margin (positive vs. negative)	2.30 (1.43–3.72)	0.001	1.88 (1.12–3.13)	0.016
Age at surgery[a]	1.02 (0.98–1.07)	0.256	0.98 (0.94–1.03)	0.494
(B) CAPRA-S score				
PTEN status (deleted vs. non-deleted)	3.47 (2.14–5.63)	<0.0001	2.84 (1.75–4.63)	<0.0001
CAPRA-S risk				
Low (0–2) reference	—	<0.0001	—	<0.0001
Intermediate (3–5)	3.40 (1.81–6.36)	<0.0001	3.00 (1.60–5.64)	0.001
High (≥ 6)	10.65 (5.42–20.91)	<0.0001	8.95 (4.53–17.67)	<0.0001

[a]Analyzed as a continuous variable; *P*-value: Wald test

biochemical recurrence, which is consistent with *PTEN* haploinsufficiency demonstrated in prostate cancer animal models [26]. It is also possible that the second allele has been inactivated by alternative mechanisms [27], which was not investigated in our study. Interestingly, the *PTEN* deletion status could further stratify patients of low-intermediate risk grade group 1–2 (≤3 + 4), pT2, and prostate-specific antigen < 10, a finding not reported in previous studies. The revised Gleason scoring system applied to our cohort offers more refinement over previous iterations by splitting Gleason score 7 into two groups of distinct prognoses: grade group 2 (3 + 4) and grade group 3 (4 + 3). While the grade group 2 has the best outcome, it was further stratified by *PTEN* FISH. Since the percentage of Gleason pattern 4 was not recorded in this cohort, it is unclear if and how it would correlate with the *PTEN* status. Similarly, *PTEN* FISH was able to sub-classify cases belonging to low and intermediate CAPRA-S risk groups, thus emphasizing the potential complementary role of this marker to clinicopathologic assessment for outcome prediction.

PTEN deletions detected by FISH are known to be enriched in metastatic as compared to primary prostate tumors [25, 28]. To our knowledge, one *PTEN* FISH study reported metastasis outcome on radical prostatectomy specimens, but did not find any significant association with *PTEN* deletion [16]. Here, we have shown that patients with a *PTEN* deletion in their radical prostatectomy sample were at a higher risk of experiencing distant metastases. Our results are in agreement with Lotan et al. [29] who used a selected high-risk radical prostatectomy cohort (all patients experienced a biochemical recurrence) to demonstrate that the loss of PTEN protein expression was associated with a shorter time to distant metastasis. While further validation on different cohorts is needed, our findings highlight the potential of *PTEN* deletion as a marker of disease progression to advanced metastatic disease.

We recently reported that the 16p13.3 gain was associated with aggressive clinicopathologic features of prostate cancer as well as an increased risk of biochemical recurrence and distant metastases in the same radical prostatectomy specimens surveyed here for *PTEN* deletion [18]. Moreover, the 16p13.3 gain status improved the stratification of patients with intermediate and high risk of disease progression based on their CAPRA-S score. The analysis of the combined data presented here exemplified the advantages of considering both *PTEN* and 16p13.3 CNAs for biochemical recurrence risk stratification. Cases that were negative for *PTEN* deletion were further stratified by the 16p13.3 gain status and vice versa, thus allowing the identification of patients that have a reduced risk of biochemical recurrence. A maximum risk of biochemical recurrence was reached for patients whose tumors harbored both *PTEN* deletion and 16p13.3 gain. The advantage of this combinatorial approach was further evidenced by an increase of the C-index, which reached its maximum when

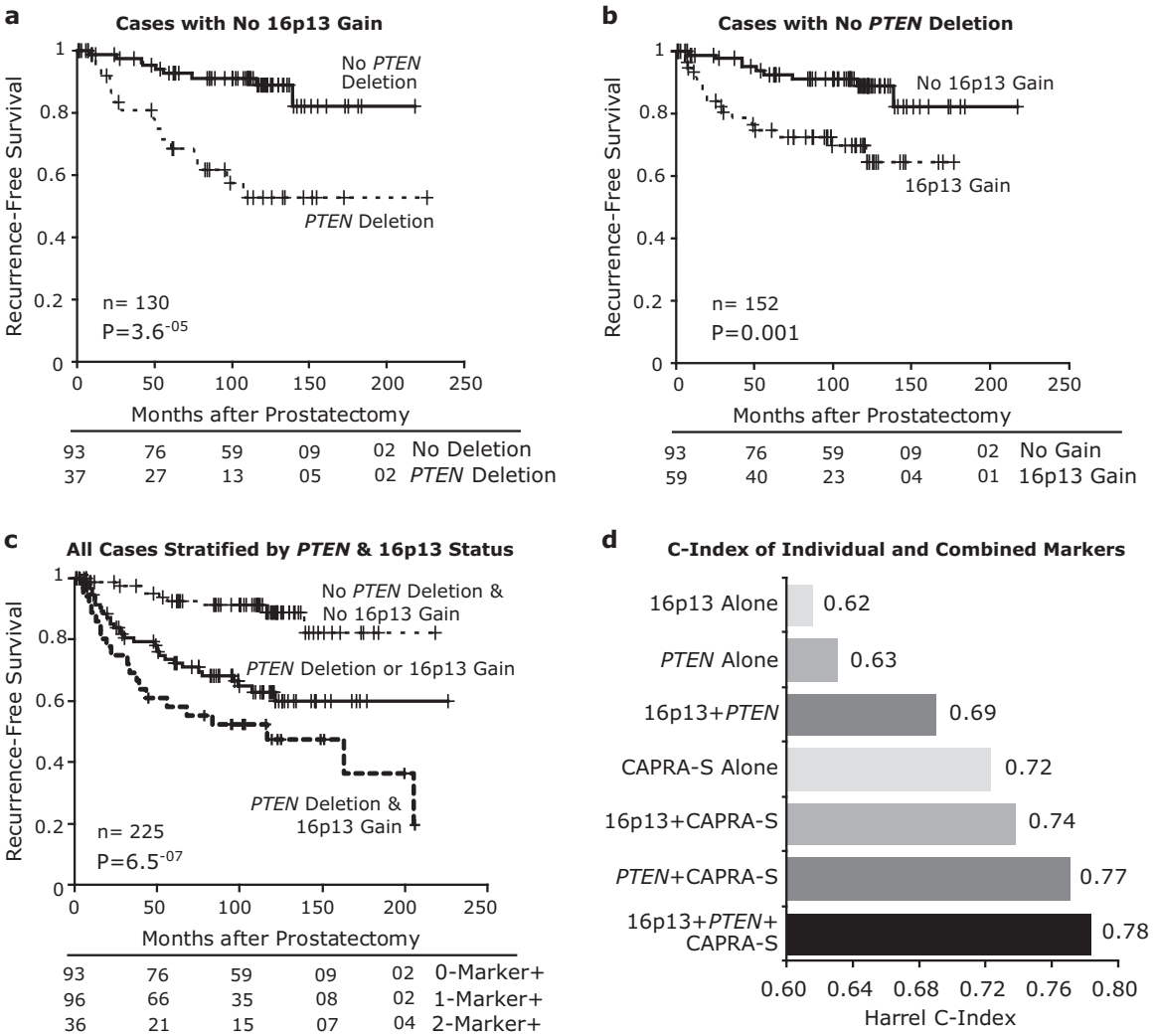


Fig. 3 Improved risk stratification upon combination of 16p13.3 gain and *PTEN* genomic deletion. Kaplan–Meier recurrence-free survival analysis of prostate cancer patients stratified on the basis of (**a**) *PTEN* deletion status in cases harboring no 16p13 gain; (**b**) 16p13 gain status in cases harboring no *PTEN* deletion; and (**c**) in patients with none, either or both genomic alterations. Censored data (tick marks), number of patients at risk at respective time points, and *P*-value (log-rank test) are indicated. Harrel C-index is improved (**d**) when combining 16p13 gain, *PTEN* deletion and CAPRA-S score risk group, compared to individual markers or either of the three

coupled with the CAPRA-S score risk groups. Interestingly, the *PTEN* deletion status identified patients of CAPRA-S low-risk group who have an increased risk of biochemical recurrence, while the 16p13.3 gain status alone did not further stratify the low-risk group as we reported [18]. These results are in agreement with previous studies showing that combinations of genomic features such as gene expression changes and copy number alterations, including *PTEN* deletion status, can add prognostic information to the CAPRA-S score [30, 31]. Owing to the relatively small number of secondary adverse events like metastases and prostate cancer-specific deaths in our cohort, future studies should focus on assessing the clinical significance of this *PTEN*-16p13.3 co-alteration status with respect to these adverse clinical end-points in large independent cohorts with long clinical follow-up.

Given the enhanced risk of biochemical recurrence associated with co-alteration of *PTEN* and 16p13.3, it is possible that patients with such tumors would benefit of adjuvant treatments. In prostate cancer, the usefulness of adjuvant systemic therapy such as chemotherapy post-surgery remains to be established [32]. The lack of demonstrated effectiveness may be explained in part by differences of tumor biology among patients. Molecular biomarkers such as *PTEN*/16p13.3 FISH may help patient selection and thus improve the success of future clinical trials. The assessment of these markers retrospectively in samples of patients who have received adjuvant therapy may provide data to support this hypothesis.

PTEN inactivation occurs predominantly via genomic deletion at 10q23 [6, 33], which results in increased levels of phosphatidylinositol [3–5]-trisphosphate (PIP3). PIP3

Table 4 Univariate and multivariate Cox proportional hazard analysis predicting biochemical recurrence for the *PTEN*-16p13 co-alteration status adjusted for standard clinicopathologic parameters (A) and CAPRA-S score (B)

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% confidence interval)	*P*-value	Hazard ratio (95% confidence interval)	*P*-value
(A) Standard clinicopathologic parameters				
PTEN-16p13 co-alteration status				
No *PTEN* and No 16p13 (reference)	—	<0.0001	—	0.003
PTEN or 16p13	3.93 (1.88–8.25)	<0.0001	2.90 (1.35–6.22)	0.006
PTEN and 16p13	6.88 (3.14–15.08)	<0.0001	4.18 (1.82–9.59)	0.001
Preoperative prostate-specific antigen[a]	1.08 (1.05–1.10)	<0.0001	1.05 (1.02–1.08)	0.002
Gleason grade				
Group 3–5 (≥4 + 3) vs. group 1–2 (≤3 + 4)	4.70 (2.79–7.90)	<0.0001	2.16 (1.14–4.12)	0.019
pT-stage (T3 vs. T2)	3.29 (1.98–5.48)	<0.0001	1.46 (0.80–2.67)	0.217
Surgical margin (positive vs. negative)	2.25 (1.37–3.72)	0.002	1.50 (0.87–2.57)	0.143
Age at surgery[a]	1.03 (0.98–1.07)	0.263	0.98 (0.96–1.05)	0.924
(B) CAPRA-S score				
PTEN-16p13 co-alteration status				
No *PTEN* and no 16p13 (reference)	—	<0.0001	—	0.001
PTEN or 16p13	3.93 (1.88–8.25)	<0.0001	3.55 (1.69–7.46)	0.001
PTEN and 16p13	6.88 (3.14–15.08)	<0.0001	4.70 (2.12–10.42)	<0.0001
CAPRA-S risk				
Low (0–2) reference	—	<0.0001	—	<0.0001
Intermediate (3–5)	3.21 (1.66–6.19)	0.001	2.70 (1.40–5.23)	0.003
High (≥6)	9.25 (4.58–18.69)	<0.0001	7.34 (3.57–15.07)	<0.0001

[a]Analyzed as a continuous variable; *P*-value: Wald test

triggers the phosphorylation and activation of AKT by PDK1 leading to the stimulation of pathways related to cell growth and survival [34]. Our laboratory has identified *PDPK1* encoding PDK1 at 16p13.3 as a potential driver of the gain and found that PDK1 stimulates prostate cancer cell migration in vitro [8]. Increasing data from the literature indicate that PDK1 not only phosphorylates AKT, but is also directly involved in cell invasion and migration [35]. It is possible that an overexpression of PDK1 resulting from 16p13.3 gain would potentialize the PIK3/AKT pathway activation and/or provide additional advantages relevant to tumor progression such as an increased cell motility leading to tumor cell spreading beyond the prostate, which may explain the augmented risk of tumor recurrence associated with both *PTEN* and 16p13.3 copy number alterations. Assessment of the in situ expression and activation status of the proteins involved in the PIK3/AKT pathway as well as further work on animal models are warranted to elucidate the role of PDK1 in PCa and validate this hypothesis.

Genomic instability, as reflected by the percentage of tumor genome harboring copy number alterations, has been shown to be associated with adverse outcome [36, 37]. It is thus possible that the higher risk of biochemical recurrence associated with *PTEN*-16p13.3 co-alteration reflects an increased genomic instability. PTEN has been shown to contribute to genomic instability leading to aggressive prostate cancer in animal models [38]. In our previous CNA analysis, we identified 8q24 gain (*MYC*) and 16q23 deletion along *PTEN* deletion and 16p13 gain as the four most common alterations enriched in lymph node metastases [7]. It has been shown that the co-deletion of *PTEN* and 16q23 was associated with poor outcome after radical prostatectomy [39]. It would be interesting to explore whether these other key copy number alterations would provide additional prognostic value, in particular MYC that synergizes with PTEN for tumor initiation and progression in animal models [38].

A potential use for such a panel of copy number alterations would be as FISH biomarkers on diagnostic biopsies

to identify more effectively patients suitable for active surveillance, ultimately improving the pretreatment prognostication given that accurate Gleason grade on biopsies can be challenging to obtain. *PTEN* deletion detected in prostate needle biopsies of Gleason score 6 (grade group 1) has been shown to be associated with upgrading to Gleason score 7+ (grade group 2 and up) at radical prostatectomy [40], which suggests that molecular biomarkers may overcome some of the limitations associated with the standard histopathologic evaluation of biopsy specimens. Whether the improved patient risk stratification afforded by combining *PTEN*/16p13.3 in our study would hold true in biopsy specimens from an active surveillance cohort remains to be demonstrated.

FISH is regarded as the gold standard for the assessment of copy number alterations in tissue specimens owing to its ability to delineate specific genomic events with a spatial resolution facilitating a sensitive evaluation of individual cancer foci at a single-cell level [41, 42]. Assessing the expression of encoded proteins by immunohistochemistry is considered as an alternative approach to capture the prognostic value associated with copy number alterations. Previous PTEN immunohistochemistry assays applied to large cohorts have yield variable results in terms of outcome prediction and correlation with FISH [14, 43]. An improved PTEN immunohistochemistry assay was applied recently to one of the cohorts previously analyzed by immunohistochemistry and FISH mentioned above [14] and the authors reported a sensitivity of 83% and 67% to respectively detect homozygous and hemizygous deletion [44]. While PTEN protein loss assessed by immunohistochemistry was associated with increased risk of biochemical recurrence, a further risk stratification was achieved by combining FISH with immunohistochemistry results, substantiating the enhanced value of *PTEN* FISH in outcome prediction. Future studies comparing both FISH and immunohistochemistry on additional cohorts, including ours, would be important to confirm these observations.

In summary, the results of our study support the prognostic value of *PTEN* deletion in prostate cancer, which can be further improved in combination with 16p13.3 gain status, suggesting that these genomic alterations may cooperatively contribute to prostate cancer progression. DNA copy number analysis of *PTEN* and 16p13.3 could be of important clinical value particularly for preoperative risk assessment of the clinically most challenging group of low-grade and intermediate-grade prostate cancer.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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