

Upregulation of SPDEF is associated with poor prognosis in prostate cancer

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Received March 4, 2019; Accepted July 16, 2019

DOI: 10.3892/ol.2019.10885

Abstract. SAM pointed domain-containing Ets transcription factor (SPDEF), a member of the ETS transcription factor family, has been associated with prostate cancer development; however, its role in tumour development and progression is controversial. In the present study, SPDEF expression was analysed on a tissue microarray with >12,000 prostate cancer samples. SPDEF expression levels were higher in most prostate cancer samples than in normal prostate epithelium, suggesting SPDEF was upregulated in cancer. Nuclear SPDEF expression was identified in 80% of prostate cancer samples, and considered weak in 26.4%, moderate in 40.1% and strong in 13.5% of cases. SPDEF positivity was significantly associated with tumour stage, Gleason grade, lymph node metastasis and PSA recurrence (all $P < 0.0001$). SPDEF overexpression was more common in ERG positive (94%) than in ERG negative cancer (69%; $P < 0.0001$). Elevated SPDEF expression predicted poor

prognosis independent from established prognostic parameters, including Gleason grade, pT, pN, serum PSA level and nodal status ($P < 0.01$). In summary, SPDEF overexpression was associated with aggressive behaviour, particularly in ERG negative prostate cancer, and may have potential for clinical application.

Introduction

Prostate cancer is the most prevalent cancer in men in Western societies (1), but only a small subset is highly aggressive and needs extensive treatment (2,3). Predictive preoperative prognostic parameters are limited to Gleason score and tumour extent on biopsies, prostate-specific antigen (PSA) serum level and clinical stage. Thus it is hoped that additional biomarkers can be identified which will improve the prediction of an aggressive tumour course.

The E26 transformation-specific (ETS) family of transcription factors has been named after its evolutionarily conserved DNA-binding domain (4). ETS factors play important roles in many human tumour types. In prostate cancer, the ETS family member ERG is fused to the TMPRSS2 serine protease in approximately 50% of cases (5-7). Another ETS factor with relevance in prostate cancer is SAM pointed domain-containing Ets transcription factor (SPDEF). SPDEF is physiologically expressed in normal tissues of the prostate (8), breast (9), ovary (10), lung (10), brain (10) and gastrointestinal tract (11). Studies on breast (9,12-14), prostate (13,15), ovarian (16) and colon cancers (17) have described frequent dysregulation of SPDEF with conflicting results. In prostate cancer some authors see SPDEF as an oncogenic driver (8,13) while others claim a tumour metastasis suppressor role for SPDEF (15,18-22).

To better understand the role of SPDEF in prostate cancer, we took advantage of our large prostate cancer tissue

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Abbreviations: ERG, ETS-related gene; ETS, E26 transformation-specific; SPDEF, SAM pointed domain-containing Ets transcription factor; TMA, tissue microarray; TMPRSS2, transmembrane protease, serine 2; UICC, International Union Against Cancer

Key words: SAM pointed domain-containing Ets transcription factor, ETS-related gene, deletion, prostate cancer, tissue microarray

microarray (TMA) to study expression of SPDEF with the monoclonal antibody MAB9916 clone 4A5 by immunohistochemistry in more than 12,000 prostate cancer samples, and then compared SPDEF expression with relevant clinical and pathological parameters in a patient cohort which was castration-sensitive or naïve to androgenic deprivation therapy.

Materials and methods

Patients. Radical prostatectomy specimens were available from 12,427 patients, undergoing surgery between 1992 and 2012 at the Department of Urology and the Prostate Cancer Center Martini Clinic at the University Medical Center Hamburg-Eppendorf. All prostate specimens were analysed according to a standard procedure, including a complete embedding of the entire prostate for histological analysis (23). Histopathological data was retrieved from the patient files, including tumour stage, Gleason grade, nodal stage and status of the resection margin. In addition to the classical Gleason categories, 'quantitative' Gleason grading was performed by estimating the percentage of Gleason 4 patterns as previously described (24). Follow-up data were available for a total of 11,152 patients with a median follow-up of 60 months (range: 1 to 241 months; Table I). Prostate specific antigen (PSA) serum values were measured following surgery and PSA recurrence was defined as a postoperative serum PSA of at least 0.2 ng/ml and increasing at subsequent measurements. The TMA manufacturing process was described earlier in detail (25). In short, for each patient, a 0.6 mm diameter core was taken from a representative tissue block. The tissues were distributed among 27 TMA blocks, each containing 144 to 522 tumour samples. For internal controls, each TMA block also contained various control tissues, including normal prostate tissue. The use of leftover archived diagnostic tissues for manufacturing of tissue microarrays and their analysis for research purposes, as well as patient data analysis, has been approved by local laws (HmbKHG, §12a) and by the local ethics committee (Ethics Commission Hamburg, WF-049/09). The present study has been carried out in compliance with the Helsinki Declaration.

Immunohistochemistry. The freshly cut TMA sections were collectively immunostained in a single run. Slides were deparaffinized and exposed to heat-induced antigen retrieval (121°C, 5 min, pH 7.8 Tris-EDTA-citrate buffer). Primary SPDEF antibody (mouse monoclonal antibody, MAB9916, clone 4A5; Abnova Germany, dilution 1:4050) was applied (37°C, 60 min) and bound antibody was then visualized using the EnVision kit (Dako, Glostrup, Denmark) according to the manufacturer's directions. The SPDEF staining was typically nuclear and slightly cytoplasmic in prostate cancer and usually negative in benign prostate tissue. The staining intensity (0, 1+, 2+, and 3+) and the fraction of positive tumour cells were separately recorded for each tissue spot. A final score was assigned as described (26): Negative scores had a complete absence of staining; weak scores had a staining intensity of 1+ in ≤70% of the tumour cells or a staining intensity of 2+ in ≤30% of the tumour cells; moderate scores had a staining intensity of 1+ in >70% of tumour cells, a staining intensity of 2+ in >30% but in ≤70% of the tumour cells, or a staining intensity of 3+ in ≤30% of the tumour cells; and strong scores had a staining intensity

Table I. Pathological and clinical data of the arrayed prostate cancer samples.

Variables	Study cohort on TMA ^a , n	Biochemical relapse, n (%)
Follow-up	11,152	2,769 (24.8)
Mean/median (months)	64.4/60.0	-
Age (years)		
≤50	323	81 (25.1)
51-59	2,696	705 (26.1)
60-69	6,528	1,610 (24.7)
≥70	1,498	370 (24.7)
Pretreatment PSA (ng/ml)		
<4	1,585	242 (15.3)
4-10	7,480	1,355 (18.1)
>10-20	2,412	737 (30.6)
>20	812	397 (48.9)
pT stage (AJCC 2002)		
pT2	8,187	1,095 (13.4)
pT3a	2,660	817 (30.7)
pT3b	1,465	796 (54.3)
pT4	63	51 (81.0)
Gleason grade		
≤3+3	2,297	230 (10.0)
3+4	6,679	1,240 (18.6)
3+4 Tertiary 5	433	115 (26.6)
4+3	1,210	576 (47.6)
4+3 Tertiary 5	646	317 (49.1)
≥4+4	416	348 (83.7)
pN stage		
pN0	6,970	1,636 (23.5)
pN+	693	393 (56.7)
Surgical margin		
Negative	9,990	1,848 (18.5)
Positive	2,211	853 (38.6)

^aNumbers do not always add up to 12,432 in different categories because of cases with missing data. AJCC, American Joint Committee on Cancer; PSA, prostate-specific antigen; TMA, tissue microarray.

of 2+ in >70% of the tumour cells or a staining intensity of 3+ in >30% of the tumour cells. Ki-67 labelling index (Ki67-LI) data were taken from a previous publication (27).

FISH. Details of the method were previously published for ERG (26), 5q21 (CHD1) (28), 6q15 (MAP3K7) (29), PTEN (10q23) (30), and 3p13 (FOXPI) (31).

Cell culture and Western blotting. DU-145 (prostate cancer) cells were obtained from the Leibniz-Institute DSMZ-German collection of microorganisms and cell cultures (no.: ACC 261, Human) and grown in DMEM (Dulbecco's Modified Eagles Medium), enriched with 10% heat-inactivated and filtered foetal bovine serum, 1% penicillin-streptomycin, 1%

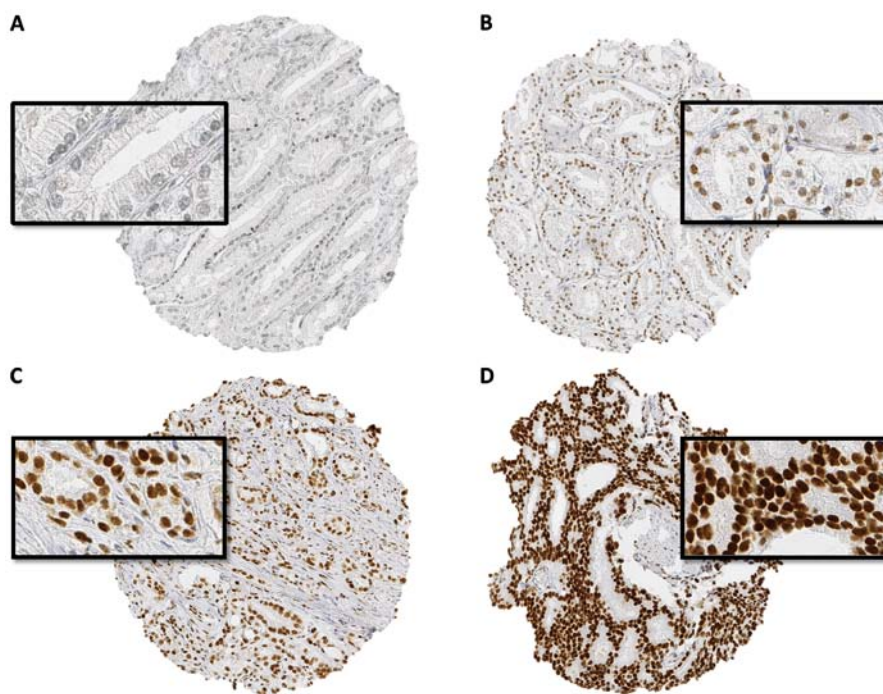


Figure 1. Representative images of 600- μ m tissue spots. (A) negative, (B) weak, (C) moderate and (D) strong SAM pointed domain-containing Ets transcription factor nuclear staining in prostate cancer. Magnification, x100 and x400.

NEAA, 1% pyruvate. The cells were incubated in 75 cm² flasks at 37°C and 5% CO₂. For electrophoresis and blotting about 10x10⁶ cells were harvested after being washed once with 1x PBS in 150 μ l lysis buffer. The cells were scratched with a cell scraper and transferred into a precooled 1.5 ml Eppendorf tube, rested 30 min on ice, and were centrifuged at 14,000 rpm for 5 min at 4°C. The amount of protein was measured with the Qubit fluorometer at 550 nm. Four-times Laemmli-buffer (BioRad) and β -mercaptoethanol (1:10 dilution) were added to the DU-145 lysate sample and water control, heated at 95°C for 5 min, and loaded together with the size marker Precision Plus Protein™ dual colour standard (BioRad) to a 4-15% polyacrylamide gel. The gel was run for 30 min at 180 V in a mini-protean Tetra cell system in 1x Tris-glycin-SDS buffer (BioRAD). The proteins were transferred at 2A in 7 min to a polyvinylidene fluoride membrane, washed with PBS-Tween 20x (PBS + 0.5% Tween 20), and incubated for 1 h with blocking buffer (5% milk powder in PBS-Tween 20, filtered and boiled). MAB9916 clone 4A5 (1:1,400 in blocking buffer) was added overnight at 4°C, washed 3x 15 min with PBS-Tween and incubated with secondary antibody (peroxidase goat anti mouse, diluted 1:1,000, Dianova) for 40 min at room temperature on a shaker. The membrane was developed with enhanced chemiluminescence substrate (Bio Rad) and recorded on a ChemiDoc™ imaging system.

Statistical analysis. Contingency tables and the χ^2 -test were performed to search for associations between molecular parameters and tumour phenotype. Ki67 labelling data were tested by ANOVA. Kaplan-Meier curves were tested by log-rank for differences between groups and Cox proportional hazards regression analysis was performed to test for independence and significance between pathological, molecular

and clinical variables. Separate models were calculated with different sets of parameters, according to their availability before or after the prostatectomy. Statistical calculations were done with JMP 10 (SAS Institute Inc., NC, USA).

Results

SPDEF-staining. A total of 9403 (76%) of tumour samples were interpretable in our TMA analysis. Reasons for non-informative cases (3024, 24%) included lack of tissue samples or absence of unequivocal cancer tissue in the TMA spot.

SPDEF expression in normal and cancerous prostate tissues. Normal prostate tissue only rarely showed weak nuclear staining, which was limited to epithelial cells. In cancer, nuclear SPDEF immunostaining was found in the majority (7522 of 9403; 80%) of interpretable prostate cancers and was considered weak in 26%, moderate in 40% and strong in 14% of cases. Representative images of SPDEF staining are given in Fig. 1.

Association with *TMPRSS2:ERG* fusion status and *ERG* protein expression. Data on *TMPRSS2:ERG* fusion status previously obtained by FISH were available from 5634 and by immunohistochemistry from 8304 tumours with evaluable SPDEF immunostaining [18,19]. Data on both ERG FISH and IHC were available from 5421 cancers, and concordant results (ERG IHC positive and break by FISH or ERG IHC negative and missing break by FISH) were found in 5171 of 5421 (95.4%) cancers. The SPDEF staining score was associated with *TMPRSS2:ERG* rearrangement and ERG positivity. For example, SPDEF immunostaining was seen in 94 and 96% of cancers with *TMPRSS2:ERG*

Table II. Association between SPDEF expression and prostate cancer phenotype.

Parameters	Number, n	SPDEF (%)				P-value
		Negative	Weak	Moderate	Strong	
All cancer samples	9,403	20.0	26.4	40.1	13.5	
Tumour stage ^a						<0.0001
pT2	5,987	22.9	27.9	37.7	11.5	
pT3a	2,148	16.2	24.2	42.1	17.6	
pT3b	1,179	12.6	23.7	48.1	15.6	
pT4	53	13.2	9.4	50.9	26.4	
Gleason grade ^a						<0.0001
≤3+3	2,126	28.9	32.1	31.7	7.2	
3+4	5,302	18.8	26.0	41.5	13.8	
4+3	1,468	13.8	20.9	45.5	19.8	
≥4+4	460	12.4	23.0	45.4	19.1	
Lymph node metastasis ^a						<0.0001
N0	5,384	17.6	24.6	42.6	15.2	
N+	560	13.0	18.6	48.6	19.8	
Preoperative PSA level (ng/ml) ^a						0.0394
<4	1,132	16.5	27.6	43.2	12.7	
4-10	5,602	20.1	26.6	39.8	13.4	
>10-20	1,885	21.3	25.7	38.6	14.5	
>20	676	21.0	24.1	41.6	13.3	
Surgical margin ^a						0.0105
Negative	7,419	20.4	26.9	39.6	13.1	
Positive	1,811	18.2	25.1	41.9	14.9	

^aCategory with some missing data. The χ^2 -test was used to calculate P-values. PSA, prostate-specific antigen; SPDEF, SAM pointed domain-containing Ets transcription factor.

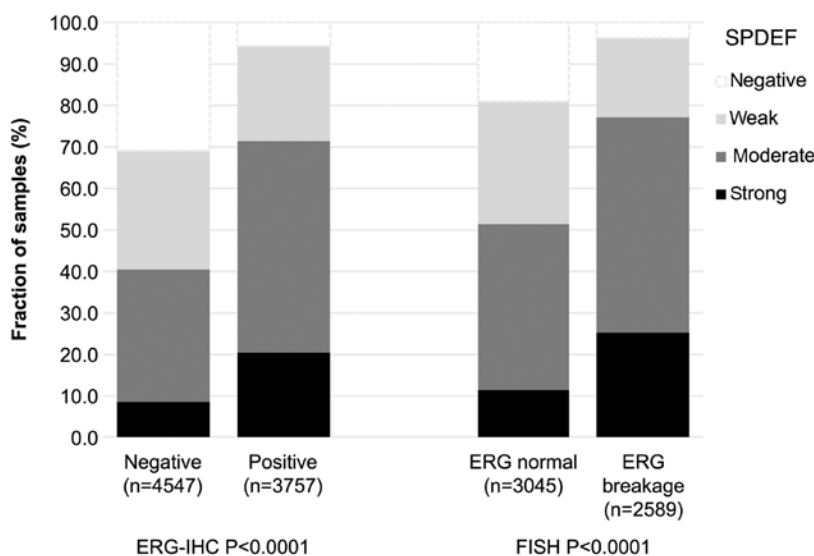


Figure 2. Association of SPDEF expression with ERG fusion probed by IHC and FISH. ERG, ETS-related gene; FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; SPDEF, SAM pointed domain-containing Ets transcription factor.

fusion detected by IHC and FISH. In contrast, only 69% of cancers without ERG staining and 81% of cancers without ERG rearrangements were SPDEF positive ($P<0.0001$ each; Fig. 2).

Table III. Association between SPDEF expression and prostate cancer phenotype in the ERG negative subset.

Parameters	Number, n	SPDEF (%)				P-value
		Negative	Weak	Moderate	Strong	
All cancer samples	4,547	31.0	28.5	32.2	8.4	
Tumour stage						<0.0001
pT2	3,015	33.8	29.5	30.0	6.6	
pT3a	928	28.8	27.4	33.0	10.9	
pT3b	567	19.2	26.3	40.9	13.6	
pT4	24	25.0	12.5	54.2	8.3	
Gleason grade						<0.0001
≤3+3	953	44.5	31.2	21.5	2.8	
3+4	2,552	30.2	29.2	32.6	8.1	
4+3	757	20.9	24.4	40.6	14.1	
≥4+4	267	18.4	25.8	41.2	14.6	
Lymph node metastasis						0.0002
N0	2,659	27.6	28.8	34.0	9.6	
N+	262	20.2	22.1	43.1	14.5	
Preoperative PSA level (ng/ml)						0.6675
<4	466	27.5	28.8	35.4	8.4	
4-10	2,671	31.3	28.4	32.3	8.0	
>10-20	998	31.5	29.0	30.4	9.2	
>20	373	30.6	27.3	32.7	9.4	
Surgical margin						0.4234
Negative	3,597	31.3	28.7	31.9	8.1	
Positive	870	29.2	28.5	32.9	9.4	

The χ^2 -test was used to calculate P-values. ERG, ETS-related gene; PSA, prostate-specific antigen; SPDEF, SAM pointed domain-containing Ets transcription factor.

Associations with tumour phenotype. High-level SPDEF immunostaining was associated with advanced pT stage, higher Gleason grade, lymph node metastasis ($P < 0.0001$ each) and positive surgical margin ($P = 0.0105$; Table II). The associations with pT stage and high Gleason grade held true for both the ERG negative and ERG positive cancer cohorts ($P < 0.0001$ each; Tables III and IV).

Association to key genomic deletions. To examine whether SPDEF expression might be associated with one or several of the most common genomic deletions, SPDEF data were compared to pre-existing data on *PTEN* (10q23), 3p13 (*FOXPI*), 6q15 (*MAP3K7*) and 5q21 (*CHD1*) deletions (Fig. 3A-C). Strong SPDEF expression was associated with all analysed deletions in ERG negative cancers ($P \leq 0.0008$). In contrast, SPDEF expression was only linked to *PTEN* deletions ($P < 0.0001$) and 3p13 deletions ($P = 0.0096$) but not to the other genomic deletions in ERG positive samples.

Association with Ki67-labelling index (LI). High levels of SPDEF immunostaining were significantly associated with accelerated cellular proliferation as measured by Ki67-LI (Table V; $P < 0.0001$). This also applied for all analysed

subgroups of tumours with identical Gleason scores ($P < 0.0001$ for Gleason 3+3, 3+4, 4+3 and $\geq 4+4$).

Association with PSA recurrence. Follow-up data were available for 8787 patients with interpretable SPDEF immunostaining on the TMA. A highly significant association was seen between PSA recurrence and elevated SPDEF expression ($P < 0.0001$; Fig. 4A). Subset analysis further revealed that the prognostic impact was stronger in the subgroup of ERG negative ($P < 0.0001$; Fig. 4B) than in ERG positive cancers ($P = 0.0001$; Fig. 4C). Further subset analyses of cancers with identical classical and quantitative Gleason grade revealed a prognostic impact of SPDEF immunostaining beyond classical Gleason grade for the 4+3 and $\geq 4+4$ score groups (Fig. 5A) and the quantitative Gleason grade group 31-49% Gleason 4 (Fig. 5B-J).

Multivariate analysis. To evaluate the clinical relevance of SPDEF expression in different scenarios, four different types of multivariable analyses were performed, as previously described (32). In brief, scenario 1 included postoperative parameters (pathological tumour stage, pathological lymph node status (pN), surgical margin status, preoperative PSA

Table IV. Association between SPDEF expression and prostate cancer phenotype in the ERG positive subset.

Parameters	Number, n	SPDEF (%)				P-value
		Negative	Weak	Moderate	Strong	
All cancer samples	3,757	5.8	23.0	50.7	20.5	
Tumor stage						<0.0001
pT2	2,200	6.8	24.7	49.4	19.2	
pT3a	1,019	4.6	20.9	50.7	23.7	
pT3b	499	4.2	21.0	56.7	18.0	
pT4	22	4.5	4.5	40.9	50.0	
Gleason grade						<0.0001
≤3+3	803	9.5	31.9	45.7	13.0	
3+4	2,211	5.2	22.2	51.8	20.9	
4+3	578	4.2	15.6	52.4	27.9	
≥4+4	143	2.8	16.8	53.8	26.6	
Lymph node metastasis						0.4186
N0	2,179	4.7	19.7	52.8	22.8	
N+	241	5.0	15.4	56.0	23.7	
Preoperative PSA level (ng/ml)						0.1717
<4	502	5.4	24.1	52.8	17.7	
4-10	2,278	5.7	23.8	49.9	20.5	
>10-20	688	6.4	20.6	49.7	23.3	
>20	237	5.9	18.1	56.5	19.4	
Surgical margin						0.1312
Negative	2,918	6.2	23.7	50.1	20.0	
Positive	767	4.7	21.1	52.7	21.5	

The χ^2 -test was used to calculate P-values. ERG, ETS-related gene; PSA, prostate-specific antigen; SPDEF, SAM pointed domain-containing Ets transcription factor.

value, pathological Gleason grade). In scenario 2, all postoperatively available parameters with exception of nodal status were included. This takes into account that the indication and extent of lymph node dissection is not standardized in the surgical therapy of prostate cancer. Two additional scenarios had the purpose to model the preoperative situation: Scenario 3 included preoperative PSA, clinical tumour stage (cT stage) and Gleason grade obtained on the prostatectomy specimen. Since postoperative determination of a tumour's Gleason grade is of higher quality than the preoperatively determined Gleason grade [subject to sampling errors and consequently under-grading in more than one third of the cases (33)], another multivariable analysis was added. In scenario 4, the preoperative Gleason grade obtained on the original biopsy was combined with preoperative PSA, cT stage and SPDEF expression. In all four scenarios, the multivariate analysis demonstrated that SPDEF expression provides independent prognostic information in the subset of ERG negative cancers and also in the non-stratified cohort of all cancers. In the ERG positive subset, only the preoperative model 4 showed a significant prognostic effect (P=0.0024, Table VI). The overall univariate Cox proportional hazard ratio for strong versus negative SPDEF expression was weak (1.99, 95% CI 1.72-2.20, Table SI).

Discussion

The results of our study identify high SPDEF expression as an independent predictor of poor prognosis in prostate cancers. The increased SPDEF staining in the analysed prostate cancers, compared to the negative to weak SPDEF expression in normal prostate tissue, suggests for role of SPDEF in prostate cancer development. These observations fit well with a recent study by Situ *et al* (34) describing low or absent SPDEF staining in normal prostate tissue but strong up regulation in cancer. Sood *et al* (13) also described a slightly increasing rate of SPDEF positivity from normal (27%) to high-grade prostatic intraepithelial neoplasia (33%) and invasive prostate cancer (40%). Within our 9,403 interpretable prostate carcinomas, elevated SPDEF expression levels were strikingly linked to advanced tumour stage, high Gleason grade, rapid cell proliferation and early PSA recurrence. These findings fit well with a role for SPDEF as a potential oncogenic driver in prostate cancer. Other authors had also reported increasing SPDEF expression from intermediate to high Gleason grade in prostate cancer (13,34). A link between high SPDEF expression and cancer development and progression was also found in breast (12,35,36) and ovarian (16,37) cancers. It is of note that others have reported loss of SPDEF expression during tumour

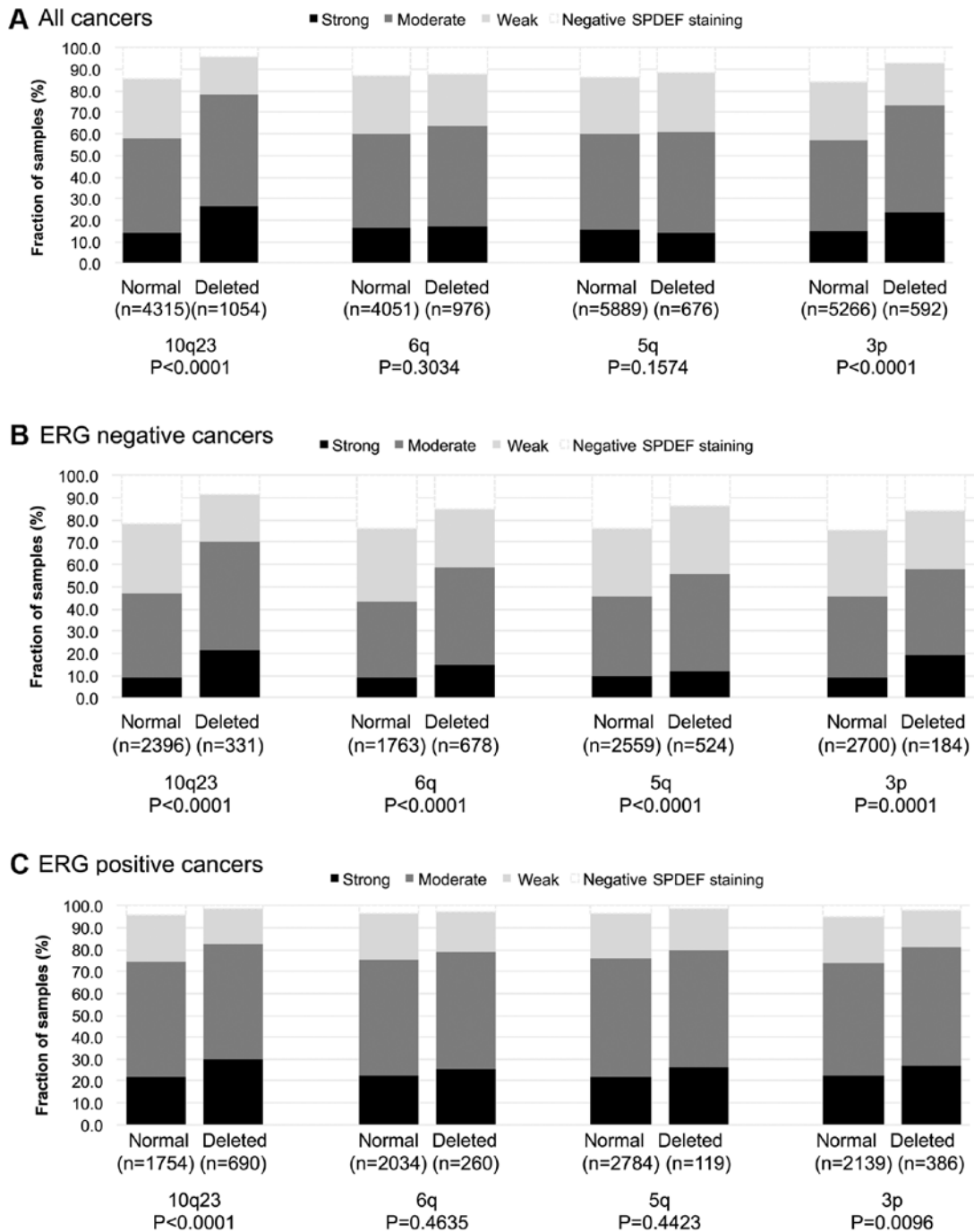


Figure 3. SPDEF expression vs. 10q23, 6q15, 5q21 and 3p13 deletion probed by fluorescence *in situ* hybridization analysis in (A) all cancer samples, (B) ERG negative subset and (C) ERG positive subset. ERG, ETS-related gene; SPDEF, SAM pointed domain-containing Ets transcription factor.

progression, and found reduced levels of SPDEF in prostate cancer as compared to normal epithelium in cohorts of 40 and 73 patients (15,20). Similar findings were also reported for breast (14), bladder (38) and colon (17) cancer. Such discrepancies are most likely due to differences in the reagents used, and possibly also due to patient cohort selection issues. We are confident in our reagents used. The monoclonal antibody (MAB9916 clone 4A5 from Abnova) used in this study was tested by Western blot, where it showed a single band of the predicted molecular mass of 37.5 kDa (Fig. S1) (8).

The prognostic effect of SPDEF expression was seen in both ERG positive and ERG negative cancers, but was stronger

in the latter group. It is well known that aberrant expression of the ERG transcription factor leads to dysregulation of at least 1,600 genes in affected prostate cancer cells, and that these changes may impact the role of various prognostic molecular features. In earlier studies on the same set of tumours, prognostic factors that were restricted to either ERG positive (39,40) or ERG negative cancers (29,41) were often found. Given the different average SPDEF expression levels between ERG positive and ERG negative cancers, it is possible that our immunohistochemistry protocol was better suited to distinguish expression differences in cancers with somewhat lower expression levels, such as in ERG negative cancers, than

Table V. Association between SPDEF expression and Ki67LI.

Gleason	SPDEF IHC	Number, n	Ki67LI, mean \pm SEM	P-value
Total	Negative	1,247	1.44 \pm 0.07	<0.0001
	Weak	1,502	2.75 \pm 0.07	
	Moderate	2,163	3.24 \pm 0.06	
	Strong	689	3.80 \pm 0.10	
\leq 3+3	Negative	369	1.25 \pm 0.10	<0.0001
	Weak	386	2.46 \pm 0.10	
	Moderate	374	2.65 \pm 0.10	
	Strong	80	3.16 \pm 0.22	
3+4	Negative	647	1.43 \pm 0.09	<0.0001
	Weak	801	2.50 \pm 0.08	
	Moderate	1,240	3.10 \pm 0.06	
	Strong	409	3.43 \pm 0.11	
4+3	Negative	97	1.41 \pm 0.33	<0.0001
	Weak	134	4.01 \pm 0.28	
	Moderate	218	3.47 \pm 0.22	
	Strong	98	4.23 \pm 0.33	
\geq 4+4	Negative	39	2.05 \pm 0.74	<0.0001
	Weak	60	3.85 \pm 0.60	
	Moderate	98	4.95 \pm 0.47	
	Strong	41	7.73 \pm 0.72	

ANOVA was used to calculate P-values. LI, labeling index; SPDEF, SAM pointed domain-containing Ets transcription factor.

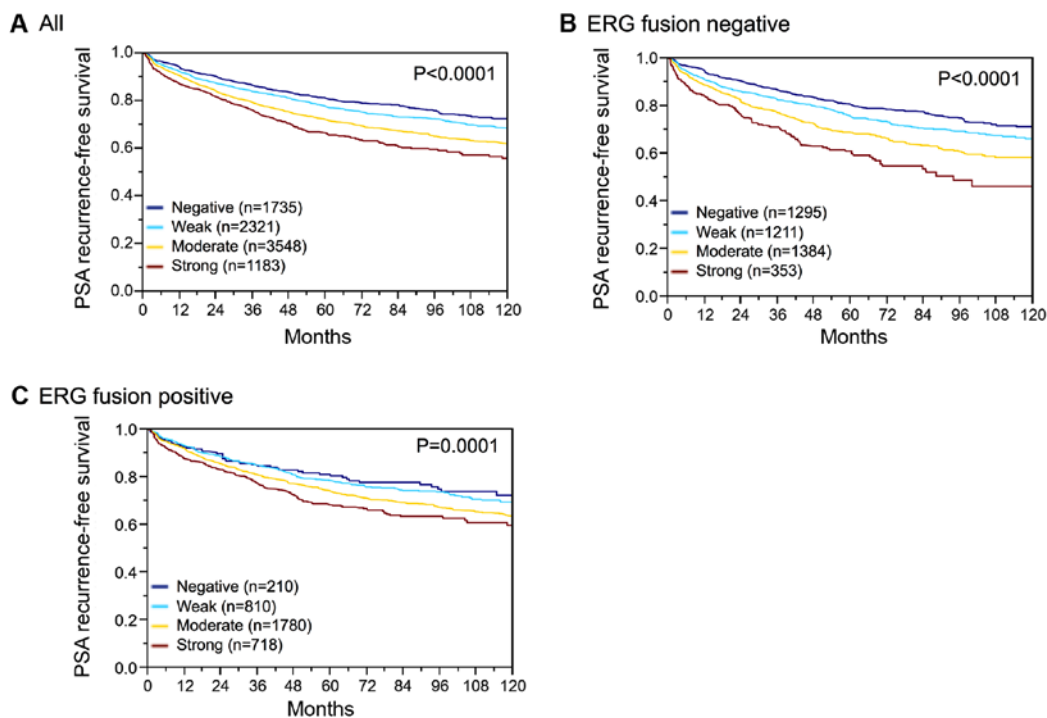


Figure 4. Kaplan-Meier analysis of PSA recurrence-free survival and negative, weak, moderate or strong SAM pointed domain-containing Ets transcription factor expression in (A) all cancer samples, (B) ERG fusion negative and (C) ERG fusion positive prostate cancer. Overall P-values are presented for all groups (log-rank test). ERG, ETS-related gene; PSA, prostate-specific antigen.

in tumours with higher expression, such as in ERG positive cancers. Most chromosomal deletions are decidedly more

frequent in either ERG positive (3p, PTEN) or ERG negative (5q, 6q) cancers (29,31,39,42). Because SPDEF is also

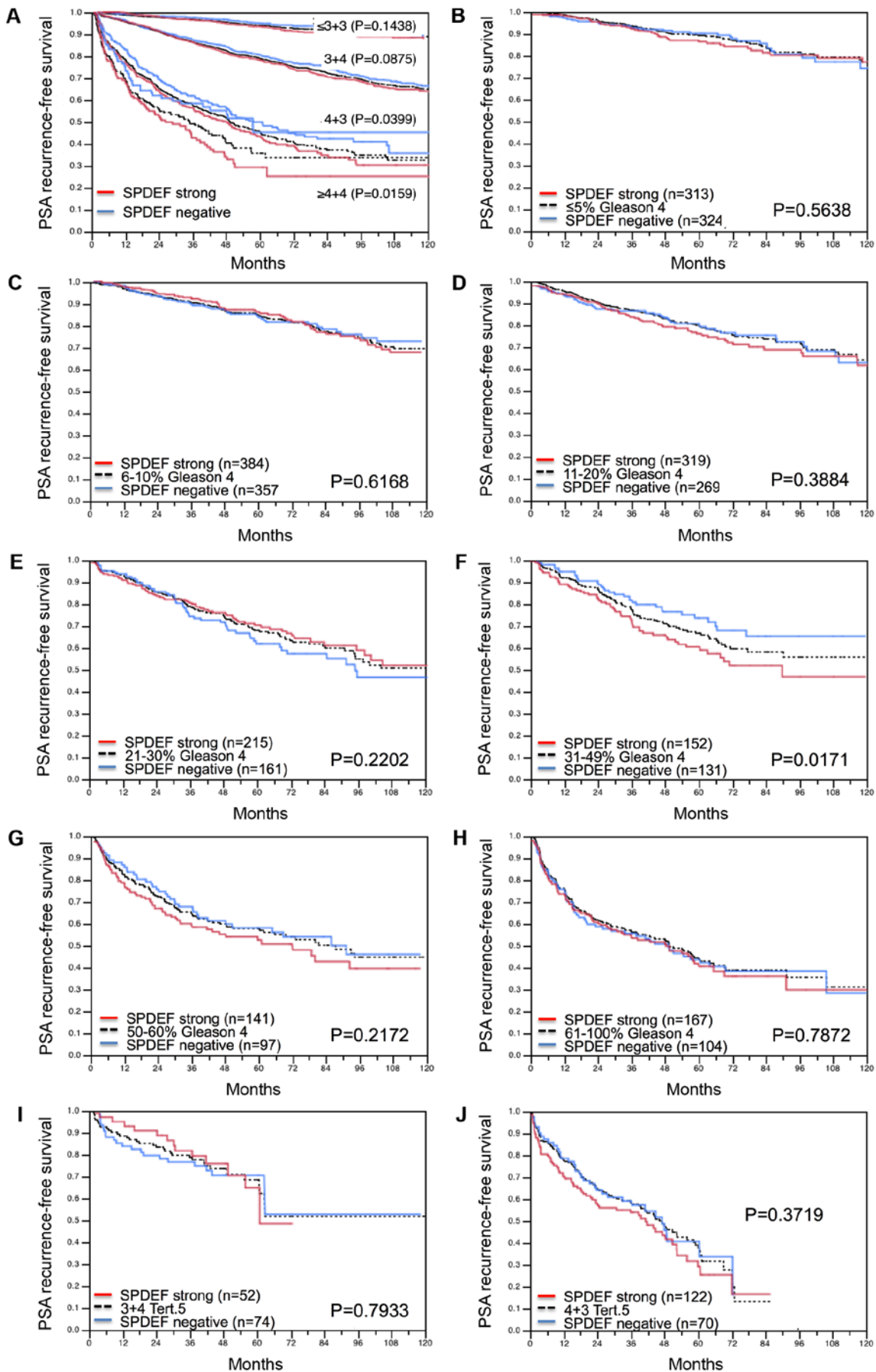


Figure 5. Prognostic impact of SPDEF expression in a subsets of cancer defined by (A) classical Gleason score categories, the quantitative Gleason score categories defined by the percentage of (B) $\leq 5\%$, (C) 6-10%, (D) 11-20%, (E) 21-30%, (F) 31-49%, (G) 50-60% and (H) 61-100% Gleason 4 patterns, and with a tertiary Gleason 5 pattern of (I) 3+4 tertiary grade 5 and (J) 4+3 tertiary grade 5. Overall P-values are presented for all groups (log-rank test). SPDEF, SAM pointed domain-containing Ets transcription factor; Tert. 5, tertiary grade 5.

Table VI. Multivariate analysis of Cox proportional hazards for biochemical RPE in various models and ERG subsets.

ERG subset	Model	Number, n	P-value of χ^2 test							
			Preoperative PSA-Level	pT stage	cT stage	RPE Gleason	Gleason Biopsy	Nodal stage	R status	SPDEF expression
Total	1	5,203	<0.0001	<0.0001	-	<0.0001	-	<0.0001	0.0006	0.0074
	2	8,265	<0.0001	<0.0001	-	<0.0001	-	-	<0.0001	0.0020
	3	8,131	<0.0001	-	<0.0001	<0.0001	-	-	-	<0.0001
	4	8,021	<0.0001	-	<0.0001	-	<0.0001	-	-	<0.0001
Negative	1	2,564	0.0002	<0.0001	-	<0.0001	-	<0.0001	0.1299	0.0013
	2	3,986	<0.0001	<0.0001	-	<0.0001	-	-	0.0014	<0.0001
	3	3,942	<0.0001	-	<0.0001	<0.0001	-	-	-	<0.0001
	4	3,891	<0.0001	-	<0.0001	-	<0.0001	-	-	<0.0001
Positive	1	2,116	0.0013	<0.0001	-	<0.0001	-	0.0041	0.0113	0.4037
	2	3,307	<0.0001	<0.0001	-	<0.0001	-	-	0.0002	0.6837
	3	3,230	<0.0001	-	<0.0001	<0.0001	-	-	-	0.4474
	4	3,182	<0.0001	-	0.0031	-	<0.0001	-	-	0.0024

Model 1 and 2 included postoperatively available parameters. Model 3 and 4 are mixed models with post- and preoperatively available parameters. ERG, ETS-related gene; PSA, prostate-specific antigen; RPE, relapse after prostatectomy; SPDEF, SAM pointed domain-containing Ets transcription factor.

associated with ERG status, a positive association with 3p and PTEN deletions and an inverse association with 5q and 6q deletions was expected in unselected prostate cancer cohorts. The association of high SPDEF expression with the cell proliferation marker Ki-67 fits well with the role of SPDEF as an oncogenic activator and with previous reports (8,13).

Our data are restricted to naïve prostate cancer patients, who had not received androgen deprivation therapy (ADT). Under ADT and in patients with castration resistant prostate cancer after ADT as well as in transgenic mouse models and prostate cancer cell lines it has been shown that SPDEF suppresses tumour metastasis (15,18-20,22). While ADT reduces SPDEF expression and cell proliferation, it relieves repression of TGFBI, CCL2, and MMP9 key drivers of metastasis. This provides an example of how a therapy which blocks growth of the primary tumour, may paradoxically promote metastasis (43).

The data of this study suggest that the SPDEF protein level may constitute a clinically useful marker in prostate cancer. SPDEF expression exerted a prognostic impact that was independent of established prognostic parameters. It is of note that the most critical clinical need in prostate cancer is not finding prognostic markers that are independent of established parameters. Most of all, parameters are needed that are more reproducible and reliable than the established ones. The Gleason grade, the strongest established prognostic parameter, suffers from very substantial interobserver variability reaching up to 40% in individual biopsies (44). Based on the data from this study, we assume that SPDEF expression measurement has potential to become part of a future multiparametric prognostic test for prostate cancer prognosis assessment.

In summary, these data show that SPDEF is a weak to moderate prognostic parameter in prostate cancer, especially in the ERG negative subset. Our data are consistent with a

particularly strong up regulation of SPDEF in response to accelerated cell proliferation in the subset of PTEN deficient and genetically unstable prostate cancer.

Acknowledgements

The authors would like to thank Dr Wilfried Fehrlé (Department of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany) for help in revision of the manuscript, and are grateful to Mrs. Sünje Seekamp and Mrs. Inge Brandt (Department of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany) for excellent technical assistance.

Funding

No funding was received.

Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

JM, FB, RS and GS designed the study, and drafted the manuscript. TS, MG, JRI, HHe and HHu participated in study design. KS, CB, CG, AH, VR and SW performed IHC analysis and scoring. FJ, CMK, TM, NCB, FL, FV, ML, CF and SM participated in pathology data analysis. CH-M, NCB and RS performed statistical analysis. SB, KM, and DH participated in data interpretation, and helped to draft the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee of the Ärztekammer Hamburg approved the study protocol (approval no. WF-049/09). According to local laws (HmbKHG §12a), patient informed consent was not required. Patient records/information were anonymized and de-identified prior to analysis. All procedures were performed in compliance with the principles outlined in the Helsinki Declaration.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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