Received: 31 October 2023 | Accepted: 9 October 2024
DOI: 10.1002/pros.24815

ORIGINAL ARTICLE

Low SMARCD3 expression is associated with poor prognosis in patients with prostate cancer

Iris E. Ertl PhD[1] | Ursula Lemberger PhD[1] | Pawel Rajwa MD, PhD[1,2] | Patrik Petrov[1] | Stefan T. Mayer[1] | Gerald Timelthaler Mag[3] | Bernhard Englinger PhD[1,3] | Robert Brettner MSc[1] | Nathalie Garstka MD[1] | Eva Compérat[4] | Lukas Kenner[5,6,7,8] | Shahrokh F. Shariat[1,9,10,11,12,13,14]

[1]Department of Urology, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria
[2]Second Department of Urology, Centre of Postgraduate Medical Education, Warsaw, Poland
[3]Center for Cancer Research, Medical University of Vienna, Vienna, Austria
[4]Department of Pathology, Medical University of Vienna, Vienna, Austria
[5]Department for Experimental and Laboratory Animal Pathology, Clinical Institute of Pathology, Vienna, Austria
[6]Unit of Laboratory Animal Pathology, University of Veterinary Medicine Vienna, Vienna, Austria
[7]Christian Doppler Laboratory for Applied Metabolomics, Medical University Vienna, Vienna, Austria
[8]Center for Biomarker Research in Medicine (CBmed), Graz, Styria, Austria
[9]Department of Urology, Weill Cornell Medical College, New York, New York, USA
[10]Department of Urology, University of Texas Southwestern, Dallas, Texas, USA
[11]Department of Urology, Second Faculty of Medicine, Charles University, Prag, Czech Republic
[12]Karl Landsteiner Institute of Urology and Andrology, Vienna, Austria
[13]Research Center for Evidence Medicine, Urology Department, Tabriz University of Medical Sciences, Tabriz, Iran
[14]Division of Urology, Department of Special Surgery, Jordan University Hospital, The University of Jordan, Amman, Jordan

Correspondence
Iris E. Ertl, PhD, Department of Urology, Research Lab, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria.
Email: iris.ertl@meduniwien.ac.at

Funding information
"Margaretha-Hehberger-Stiftung", embedded in the "Medizinisch-Wissenschaftlicher Fonds des Bürgermeisters der Bundeshauptstadt Wien", Vienna, Austria, Grant/Award Number: 17083

Abstract

Backgrounds: SWI/SNF complexes represent a family of multi-subunit chromatin remodelers that are affected by alterations in >20% of human tumors. While mutations of SWI/SNF genes are relatively uncommon in prostate cancer (PCa), the literature suggests that deregulation of various subunits plays a role in prostate tumorigenesis. To assess SWI/SNF functions in a clinical context, we studied the mutually exclusive, paralogue accessory subunits SMARCD1, SMARCD2, and SMARCD3 that are included in every known complex and are sought to confer specificity.

Methods: Performing immunohistochemistry (IHC), the protein levels of the SMARCD family members were measured using a tissue microarray (TMA) comprising malignant samples and matching healthy tissue of non-metastatic PCa patients ($n = 168$). Moreover, IHC was performed in castration-resistant tumors ($n = 9$) and lymph node metastases ($n = 22$). To assess their potential role as molecular

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2024 The Author(s). *The Prostate* published by Wiley Periodicals LLC.

biomarkers, SMARCD1 and SMARCD3 protein levels were correlated with clinical parameters such as T stage, Gleason score, biochemical recurrence, and progression-free survival.

Results: SMARCD1 protein levels in non-metastatic primary tumors, lymph node metastases, and castration-resistant samples were significantly higher than in benign tissues. Likewise, SMARCD3 protein expression was elevated in tumor tissue and especially lymph node metastases compared to benign samples. While SMARCD1 levels in primary tumors did not exhibit significant associations with any of the tested clinical parameters, SMARCD3 exhibited an inverse correlation with pre-operative PSA levels. Moreover, low SMARCD3 expression was associated with progression to metastasis.

Conclusions: In congruence with previous literature, our results implicate that both SMARCD1 and SMARCD3 may exhibit relevant functions in the context of prostate tumorigenesis. Moreover, our approach suggests a potential role of SMARCD3 as a novel prognostic marker in clinically non-metastatic PCa.

KEYWORDS
prognostic marker, prostate cancer, SMARCD1, SMARCD3, SWI/SNF complex

1 | INTRODUCTION

SWI/SNF complexes represent a heterogeneous family of large multi-subunit chromatin remodelers acting as transcriptional regulators.[1,2] It was previously shown that SWI/SNF complexes are involved in various cellular processes including proliferation, differentiation, chromosomal stability, centromere function, and DNA repair.[2,3] In accordance, mutations of SWI/SNF genes were detected in ~20% of a wide spectrum of human tumor types.[4,5] Compared to other human malignancies, mutations of SWI/SNF genes are relatively uncommon in prostate cancer (PCa) patients.[6] However, previous studies implicate that deregulation of SWI/SNF subunits plays a role in prostate tumorigenesis, and suggest both tumor suppressor and oncogenic functions in this biological context.[7–11]

SMARCD1/BAF60A, SMARCD2/BAF60B, and SMARCD3/BAF60C are mutually exclusive accessory subunits conferring functional specificity.[12] Unlike other accessory subunits, the SMARCD proteins are not restricted to certain SWI/SNF complexes, but are components of all known subtypes (i.e., cBAF, PBAF, and ncBAF complexes).[13] Thus, to obtain a comprehensive overview on the role of SWI/SNF in prostate tumorigenesis, we previously conducted in vitro studies published elsewhere.[11] Amongst others, we demonstrated that the SMARCD proteins are involved in essential cellular processes such as maintenance of the nuclear and cellular morphology and hormone-dependent and -independent transcriptional regulation of numerous classical PCa genes.[11]

In this study, we sought to assess the clinical significance of the SMARCD family members by measuring their protein levels in primary tumors and matching healthy tissues of non-metastatic PCa patients ($n = 168$), as well as in castration-resistant primary tumors ($n = 9$) and lymph node metastases ($n = 22$). Our approach revealed that SMARCD1 and SMARCD3 expression was significantly higher in tumors compared to benign tissues, and that both proteins exhibited the highest levels in metastatic samples. We further found an inverse correlation of SMARCD3 expression with pre-operative PSA levels as well as an association with metastasis.

In summary, our findings support the hypothesis that SMARCD family members exhibit relevant functions in PCa and suggest a value to SMARCD3 tissue expression as a potential novel prognostic marker in clinically non-metastatic PCa.

2 | MATERIAL AND METHODS

2.1 | Cell culture

The cell line LnCAP was purchased from ATCC, and was cultivated in RPMI 1640 medium (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS) (Gibco™ Fetal Bovine Serum, Life Technologies) under standard conditions (37°C; 5% CO_2). The *Mycoplasma* contamination status was monitored using the MycoAlert™ Mycoplasm Detection Kit (Lonza).

2.2 | siRNA mediated knock-down

siRNA-mediated knockdown was performed using RNAiMAX Transfection Reagent (Thermofisher Scientific) according to the manufacturer's instruction. LnCAP cells were transfected with a scrambled control (Silencer® Select Negative Control No.1, Thermofisher

Scientific) or siRNAs targeting SMARCD1 (sc-72598, Santa Cruz Biotechnologies or Silencer® Select s13152, Thermofisher Scientific), SMARCD2 (sc-93762, Santa Cruz Biotechnologies; s13154, Thermofisher Scientific) or SMARCD3 (sc-89355, Santa Cruz Biotechnologies or Silencer® Select s13159; Thermofisher Scientific) and incubated for 48 h.

2.3 | Western blotting

Protein extracts were generated using RIPA lysis buffer (ab156034, Abcam) supplied with protease inhibitors (cOmplete™ Mini Protease Inhibitor Cocktail Tablets, Roche). Protein concentrations were measured with the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific). Western blotting was performed using 10% Mini-PROTEAN® TGX Stain-Free™ Protein Gels and the TransBlot® Turbo™ Transfer System (Bio-Rad Laboratories). Membranes were blocked in 5% milk or BSA solution for 1 h at RT and incubated with the respective primary antibodies (α-SMARCD1: sc-135843, Santa Cruz Biotechnologies; α-SMARCD2: Clone 2F7, Novus Biologicals, Littleton; α-SMARCD3: 12838-1-AP, Proteintech Group) overnight at 4°C. Incubation with secondary antibodies (goat anti-rabbit IgG H&L (HRP), ab6721, Abcam or goat anti-mouse IgG H&L (HRP), ab6789, Abcam) was performed for 1 h at RT. Membranes were developed using Pierce™ ECL western blotting substrate (Thermofisher Scientific).

2.4 | Tissue microarray

2.4.1 | Patient cohort

The tissue microarray (TMA) was manufactured from radical prostatectomy (RP) specimens from 168 clinically non-metastatic patients treated at a single center. Informed consent was obtained before sample collection. All surgical specimens were processed according to standard pathologic procedures. Pathologic stage and grade were assigned by specialized uropathologists according to the tumor node and metastasis (TNM) staging system. From each patient, four cores from cancer areas and two cores from an adjacent benign area were selected. Only cores with a tumor content of ≥50% were included in the analysis. Human kidney tissue was included as control on each slide. Organ-confined (≤pT2c) and locally advanced tumors (≥pT3a) of different Gleason scores were included in the study. Biochemical recurrence (BCR) was defined as two consecutive PSA values ≥0.2 ng/ml after RP. Assessing BCR and progression to metastatic disease, only patients with a follow-up of at least 10 years were included, unless the event occurred within this time period. Histopathological characteristics of non-metastatic patients are shown in Table 1. In addition, the TMA comprised lymph node metastases and castration-resistant primary tumors of 22 and 9 independent cases, respectively. Similarly to the non-metastatic tumors, ≥4 cores of each patient were selected by specialized uropathologists and only cores with a tumor content of ≥50% were included in the analyses. The study was approved by the ethics commission (EKNr: 1934/2016).

2.4.2 | Immunohistochemistry

For IHC, the Epredia™ UltraVision Quanto HRP DAB kit (Thermofisher Scientific) was used according to the manufacturers' instructions. In brief, samples were pre-heated to 56°C for 2 h and incubated in xylol (2 × 10 min) and 100%, 96%, 70%, or 50% ethanol for 5 min, respectively. Afterwards the slides were cooked in Target Retrieval Solution (pH9; 121°C; 1 h), followed by incubation with hydrogen peroxide block for 15 min and Ultra V Block for 5 min. Incubation with primary antibodies (α-SMARCD1: sc-135843, Santa Cruz Biotechnologie; α-SMARCD2: Clone 2F7, Novus Biologicals; α-SMARCD3: 12838-1-AP, Proteintech Group) diluted in PBS + 0,05% Triton X-100 + 0.5% BSA was conducted over night at 4°C. Subsequently, the slides were incubated with primary antibody enhancer for 20 min, HRP Polymer for 30 min and ready-to-use AEC Single Solution for ~10 min. Samples were counterstained with hematoxylin and sealed using Aquatex® (Sigma-Aldrich).

2.4.3 | TMA analysis and statistical evaluations

Tissue slides were scanned with a SCAN II digital slide scanner (3d Histech) using a 20× objective. Digitized TMA cores were subsequently analyzed using the Definiens® TissueStudio® histomorphometry software. The software's automated tissue detection identified TMA cores, which were manually checked for artefacts or degraded tissue areas. Using representative areas and with the help of the software's cell detection algorithms, nuclei and cells were identified based on staining intensity, size, and other morphological features. Furthermore, the staining intensity of each identified cell in these areas was classified as negative, low, medium, or high. These positivity thresholds were then applied to all TMA cores of each marker respectively. To compare SMARCD1, SMARCD2, or SMARCD3 protein levels in various tissue types (i.e., benign tissue, non-metastatic PCa, lymph node metastases, and castration-resistant primary tumors) the percentage of positive cells of each evaluable core was determined and the median number of positive cells was calculated. Statistical significance was assessed performing Kruskal–Wallis and Dunn's multiple comparison tests using GraphPad Prism (GraphPad Software Inc.).

To compare protein expression between tumor samples and matching healthy tissues of a given patient, corresponding cores were jointly analyzed and staining intensities were categorized as low (<5% positive cells), medium (5–20% positive cells), or high (>20% positive cells) (Figure 1A). Patients with higher or lower staining intensity categories in tumors compared to benign samples were defined to exhibit protein up- or downregulation, respectively. Only cases, for which ≥2 benign or malignant cores were available, were included in

TABLE 1 Clinicopathological features of non-metastatic PC patients represented on the tissue microarray.

	Complete TMA (*n* = 168)		SMARCD1 (*n* = 166)		SMARCD3 (*n* = 157)	
	Median	Range	Median	Range	Median	Range
Age	62.4	45.5–78	62.4	45.5–78	62.1	45.5–78
Follow-up months	139	0–272	139	0–272	140	0–272
Follow-up years	11.6	0–22.7	11.6	0–22.7	11.7	0–22.7
Pre-op PSA (ng/ml)	*n*	%	*n*	%	*n*	%
<4	21	12.5	21	12.7	20	12.7
4–10	99	58.9	99	59.6	93	59.2
>10	47	28	45	27.1	43	27.4
Missing	1	0.6	1	0.6	1	0.6
Gleason score	*n*	%	*n*	%	*n*	%
≤3 + 3	78	46.4	78	47	73	46.5
3 + 4	51	30.4	49	29.5	49	31.2
4 + 3	17	10.1	17	10.2	14	8.9
≥4 + 4	22	13.1	22	13.3	21	13.4
T stage	*n*	%	*n*	%	*n*	%
T2a	22	13.1	22	13.3	21	13.4
T2b	60	35.7	60	36.1	54	34.4
T2c	30	17.9	30	18.1	30	19.1
T3a	28	16.7	27	16.3	27	17.2
T3b	18	10.7	17	10.2	17	10.8
T4	10	6	10	6	8	5.1
Disease stage	*n*	%	*n*	%	*n*	%
Localized (≤pT2c)	112	66.7	112	67.5	105	66.9
Advanced (≥pT3a)	56	33.3	54	32.5	52	33.1
Surgical margins	*n*	%	*n*	%	*n*	%
Positive	73	43.5	72	43.4	66	42
Negative	95	56.5	94	56.6	91	58
PSA recurrence*	*n*	%	*n*	%	*n*	%
No	66	39.3	66	39.8	63	40.1
Yes	40	23.8	39	23.5	38	24.2
No info	62	36.9	61	36.7	56	35.7
Metastasis*	*n*	%	*n*	%	*n*	%
No	87	51.8	86	51.8	84	53.5
Yes	9	5.4	9	5.4	7	4.5
No info	72	42.9	71	42.8	66	42

Note: Healthy and tumor tissue of a total of 168 non-metastatic patients suffering from localized or advanced PCa were represented on the tissue microarray. SMARCD1 and SMARCD3 protein levels were evaluable in tumor tissue of 166 and 157 patients, respectively. PSA recurrence and progression to metastatic disease was assessed in patients with a follow-up of ≥10 years.

*Follow-up ≥ 10 years.

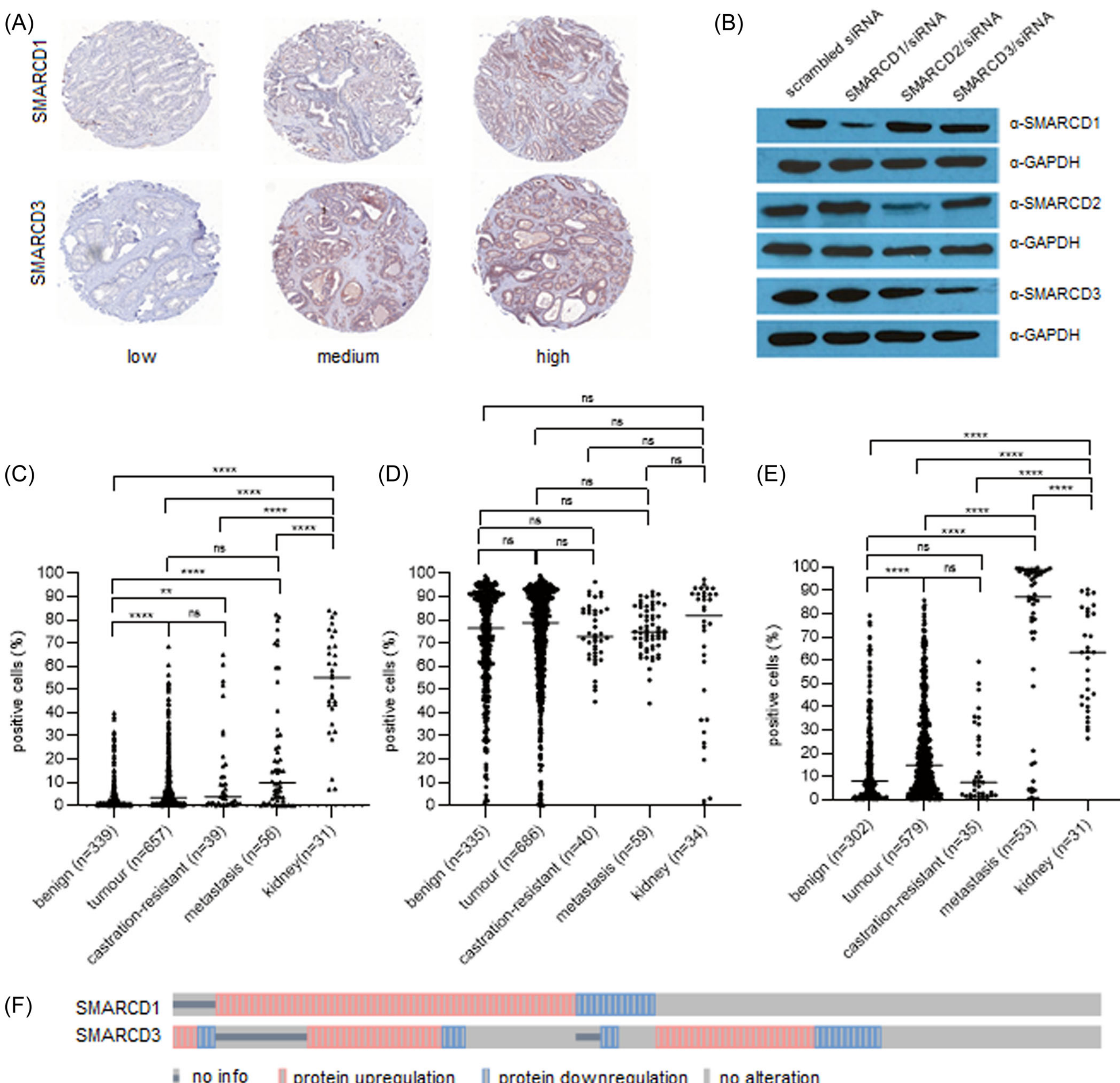

FIGURE 1 SMARCD protein expression in PCa patient samples. (A) Examples for cores representing primary tumors of non-metastatic patients with low (<5% positive cells), medium (5–20% positive cells), or high (>20% positive cells) staining intensities of SMARCD1 (upper panel) or SMARCD3 (lower panel). (B) Validation of specificity of antibodies targeting SMARCD1, SMARCD2, and SMARCD3 by siRNA-mediated knockdown and Western blotting. (C) The median numbers of SMARCD1, (D) SMARCD2, and (E) SMARCD3 positive cells were assessed in all evaluable cores representing benign prostate tissue, non-metastatic primary tumors, lymph node metastases, and castration-resistant primary tumors. Statistical significance was assessed performing Kurskal–Wallis and Dunn's multiple comparison tests. ns, not significant; $^{**}p \le 0.01$; $^{****}p \le 0.0001$. (F) Cores representing tumors and matching healthy tissues of non-metastatic patients were jointly analyzed and staining intensities were categorized as low, medium, or high. Patients with higher or lower staining intensity categories in tumors compared to benign samples were defined to exhibit protein up- or downregulation, respectively. [Color figure can be viewed at wileyonlinelibrary.com]

the analysis. For visualization and mutual exclusivity analysis, the online software Oncoprinter was used.[14,15] To assess a potential clinical significance of SMARCD1 and SMARCD3, all cores representing tumor tissue of a given patient were jointly analyzed and the percentage of positive cells or staining intensities (low: 5% positive cells; medium: 5–20% positive cells, high: >20% positive cells) were correlated with clinical parameters. Depending on the nature of the clinical variables (categorical or metric), Chi-squared tests or Pearson correlation analyses were performed using SPSS Statistics (IBM). Associations of clinical parameters with progression-free survival

(PFS) were assessed using the Kaplan–Meier method and Log-Rank (Mantel–Cox) tests using the same software. All tests were two-sided. Statistical significance was considered at $p < 0.05$.

3 | RESULTS

3.1 | SMARCD1 and SMARCD3 protein levels are elevated in malignant tissues

To assess SMARCD1, SMARCD2, and SMARCD3 protein levels in human prostate tumors, we used a TMA containing malignant samples and matching healthy tissue of 168 non-metastatic PCa patients (Figure 1A; Table 1). Moreover, IHC was performed in samples of castration-resistant tumors ($n = 9$) and lymph node metastases ($n = 22$). Given the high similarity between SMARCD1, SMARCD2, and SMARCD3, we evaluated the specificity of each antibody by siRNA-mediated knockdown and Western blotting prior to IHC (Figure 1B).

As stated above, four cores of malignant tissue and two benign samples of each non-metastatic patient were included in the TMA. Beyond that, ≥4 cores representing lymph node metastases and castrations-tumors of independent cases were available. After rigorous quality control, we assessed the median number of SMARCD1/2/3 positive cells in all evaluable cores of a given tissue type (i.e., benign tissue, non-metastatic PCa, lymph node metastases, and castration-resistant primary tumors). In case of SMARCD1, we found significantly higher protein levels in cores representing non-metastatic primary tumors (3.5%; $n = 657$), as well as in lymph node metastases (3.9%; $n = 56$) and castration-resistant samples (9.8%; $n = 39$) compared to benign tissues (1.3%; $n = 339$) (Figure 1C). For SMARCD2, no significant differences between benign (76.5%; $n = 335$), malignant (78.7%; $n = 686$), metastatic tissues (74.8%; $n = 59$), castration-resistant tumors (72.9%; $n = 40$), and kidney samples included as control (81.9%; $n = 34$) were observed (Figure 1D). We, thus, excluded this paralogue from further analyses. The median number of SMARCD3 positive cells in cores representing non-metastatic primary tumors (14.8%; $n = 579$) and especially lymph node metastases (87.3%; $n = 53$) was significantly higher than in benign samples (8%; $n = 302$). However, no difference between castration-resistant tumors (7.4%; $n = 35$) and non-malignant tissues was detected (Figure 1E).

To compare SMARCD1 or SMARCD3 protein levels in primary tumors and matching benign tissues of single patients, the corresponding cores were jointly analyzed, whereby only cases for which ≥2 benign or malignant cores were evaluable, were included in this analysis. We found that SMARCD1 levels were higher or lower in tumors of 38.8% and 8.5% of patients ($n = 166$), respectively (Figure 1F). SMARCD3 was elevated in 37.7% of cases ($n = 157$), while 15.2% exhibited lower SMARCD3 protein levels in tumors compared to matching healthy samples (Figure 1F). Mutual exclusivity analysis revealed a co-occurrence of SMARCD1 and SMARCD3 alterations ($p = 0.014$).

3.2 | SMARCD3 protein levels are associated with pre-operative PSA levels and metastasis

To confirm the validity of our clinical data, we evaluated relations between various clinical parameters (e.g., T stage, Gleason score, BCR, progression-free survival), thereby demonstrating numerous associations that were previously reported (Table 2; Figure 2).[16–19]

We further assessed potential correlations of clinical parameters with SMARCD1 and SMARCD3 protein levels in tumor tissue of non-metastatic PCa patients ($n = 166$ and $n = 157$, respectively) (Table 1). In case of SMARCD1, we did not observe significant associations with Gleason score, BCR, positive surgical margins, pre-operative PSA levels, progression to metastatic disease, and progression-free survival (Table 2; Figure 2). The correlation between SMARCD1 expression and T stage did not quite reach statistical significance ($p = 0.051$) (Table 2). However, we found that high SMARCD1 staining intensities (>20% positive cells) were clearly more frequent in T4 tumors compared to less advanced disease stages (T2: 9.8%, $n = 112$; T3: 10.9%, $n = 44$; T4: 40%, $n = 10$; data not shown).

While SMARCD3 protein levels were not associated with T stage, Gleason score, BCR, positive surgical margins, or progression-free survival, correlation analysis revealed an inverse association with pre-operative PSA levels ($p = 0.011$) (Table 2). Moreover, SMARCD3 protein abundance was associated with progression to metastasis ($p = 0.01$) (Table 2); 57.1% of patients, who developed metastases after RP exhibited low SMARCD3 levels (<5% positive cells), while, in turn, only 13.1% of patients who did not progress to to metastatic disease exhibited low staining intensities (data not shown). We further observed an association between SMARCD1 and SMARCD3 abundance ($p < 0.001$) (Table 2).

4 | DISCUSSION

Numerous studies have demonstrated that chromatin-remodeling SWI/SNF complexes are involved in the pathogenesis of PCa and suggested both tumor suppressor and oncogenic activities.[6,20–25] To shed more light on SWI/SNF functions in PCa, we previously studied the mutually exclusive accessory subunits SMARCD1, SMARCD2, and SMARCD3 that are incorporated in all defined SWI/SNF subtypes and are thought to confer specificity to a given complex.[12,13] Conducting in vitro studies, we showed that the SMARCD family members represent crucial factors for the maintenance of cellular morphology and accurate cytokinesis.[11] We further demonstrated that the SMARCD proteins are involved in the regulation of hormone-dependent AR-target genes, but can also act as antagonizers of AR-signaling.[11] Furthermore, SMARCD1, SMARCD2 and SMARCD3 were found to be involved in the regulation of AR-driven target genes under androgen-depleted conditions.[11]

To study the SMARCD proteins in a clinical context and to assess their potential role as molecular biomarkers, we measured SMARCD1, SMARCD2, and SMARCD3 protein levels in malignant samples and matching healthy tissue of non-metastatic PCa patients,

TABLE 2 Associations of SMARCD1 and SMARCD3 protein levels with clinical parameters.

		T stage	Disease state	Gleason score	Surgical margins	BCR*	Metastasis*	pre-op PSA (ng/ml)	SMARCD1 categ.**	SMARCD3 categ.**	SMARCD1 pos. cells (%)***	SMARCD3 pos. cells (%)***
T stage	χ2	X	**168.0**	**33.854**	**48.472**	**13.196**	0.436		9.417	3.435		
	p value	X	**>0.001**	**>0.001**	**>0.001**	**0.001**	0.804		0.051	0.488		
Disease state	χ2	**168.0**	X	**32.08**	**46.56**	**12.312**	0.074		2.69	2.967		
	p value	**>0.001**	X	**>0.001**	**>0.001**	**>0.001**	0.786		0.261	0.227		
Gleason score	χ2	**33.854**	**32.08**	X	**16.027**	**13.774**	7.443		5.295	1.336		
	p value	**>0.001**	**>0.001**	X	**0.001**	**0.003**	0.059		0.507	0.97		
Surgical margins	χ2	**48.472**	**46.56**	**16.027**	X	**10.655**	0.008		1.429	2.006		
	p value	**>0.001**	**>0.001**	**0.001**	X	**0.001**	0.93		0.49	0.367		
BCR*	χ2	**13.196**	**12.312**	**13.774**	**10.655**	X	**21.848**		2.801	0.791		
	p value	**0.001**	**>0.001**	**0.003**	**0.001**	X	**>0.001**		0.246	0.673		
Metastasis*	χ2	0.436	0.074	7.443	0.008	**21.848**	X		3.636	**9.159**		
	p value	0.804	0.786	0.059	0.93	**>0.001**	X		0.162	**0.01**		
Pre-op PSA (ng/ml)	Pearson's *r*							X			−0.003	**−0.203**
	p value							X			0.966	**0.011**
SMARCD1** categ.	χ2	9.417	2.69	5.295	1.429	2.801	3.636		X			
	p value	0.051	0.261	0.507	0.49	0.246	0.162		X			
SMARCD3** categ.	χ2	3.435	2.967	1.336	2.006	0.791	**9.159**			X		
	p value	0.488	0.227	0.97	0.367	0.673	**0.01**			X		
SMARCD1*** pos. cells (%)	Pearson's *r*							−0.003			X	**0.316**
	p value							0.966			X	**>0.001**
SMARCD3*** pos. cells (%)	Pearson's *r*							**−0.203**			**0.316**	X
	p value							**0.011**			**>0.001**	X

Note: Correlations amongst various clinical parameters and/or SMARCD1/3 protein levels in tumor tissues of non-metastatic patients ($n = 168$) were assessed. Associations between nominal and/or ordinal parameters were evaluated applying Chi-squared tests. Correlations between metric parameters were assessed performing Pearson correlation analyses. Significant associations are marked in bold letters.

*Follow-up ≥ 10 years.

**SMARCD1 or SMARCD3 staining intensities in jointly analyzed cores of a given patient: low (<5% positive cells); medium (5–20% positive cells); high (>20% positive cells).

***Percentage of SMARCD1 or SMARCD3 positive cells in jointly analyzed cores of a given patient.

FIGURE 2 SMARCD1 and SMARCD3 levels do not correlate with progression-free survival. Correlations of (A) T stage, (B) Gleason score (C) positive surgical margins, (D) progression to metastatic disease, (E) SMARCD1 protein levels, and (F) SMARCD3 protein levels with progression-free survival were assessed using the Kaplan–Meier method. To evaluate the overall statistical significance levels, Log-Rank (Mantel–Cox) tests were performed. [Color figure can be viewed at wileyonlinelibrary.com]

as well as in cores representing castration-resistant primary tumors and lymph node metastases. Performing IHC, we found significantly higher SMARCD1 levels in malignant samples compared to benign tissues, being the highest in castration-resistant tumors and particularly in lymph node metastases. We further observed that the protein was more abundant in T4 tumors compared to less advanced diseases stages (i.e., T2 and T3 stage). These observations implicate that SMARCD1 may be involved in carcinogenic processes, what is in congruence with our previous findings.[11] SMARCD1 represents a target of the miR-99 family of microRNAs.[26] The miR-99 family was previously shown to be downregulated in prostate tumors, resulting in hyperactivity of AR, which in turn may contribute to androgen-independence.[26] This finding suggests that SMARCD1 may play a role in the progression to an androgen-refractory state.[26] This hypothesis was not supported by our approach; even though we observed higher SMARCD1 levels in castration-resistant tumors than in androgen-dependent malignant samples, the difference was not statistically significant. However, this may be explained by the small number of androgen-refractory samples included in our study.

Apart from the relatively low number of patients, the failure to assess a potential clinical significance of SMARCD2 is a major limitation of our approach. Since no significant differences in protein abundance between benign prostate tissues, non-metastatic primary tumors, lymph node metastases, and/or castration-resistant samples were observed, the protein had to be excluded from further analyses. The specificity of our antibodies was verified by Western botting and this finding may, thus, implicate that SMARCD2 is indeed equally expressed across all tissue types. However, given the fact that we also found comparable expression levels in kidney tissue that was included as control, we rather suppose that the staining was non-specific due to technical issues of our IHC protocol.

Assessing SMARCD3, we found significantly higher protein levels in tumor samples and especially lymph node metastases compared to healthy tissue. Analyzing TCGA data, we previously showed that SMARCD3 is altered in 6% and 9% of patients suffering from non-metastatic and metastatic PCa, respectively; the vast majority of alterations accounted for mRNA upregulation and gene amplifications.[11] We further found increased mRNA and protein levels of SMARCD3 in PCa cell lines derived from lymph node, bone or brain metastases (i.e., LnCAP, C4-2, PC3, and DU145) compared to the benign prostate cell line RWPE1.[11] Thus, our observation is in congruence with previous findings.

Correlating SMARCD3 levels in tumor samples of non-metastatic PCa patients with clinical parameters, we found a significant association of low protein abundance with high preoperative PSA levels. Previous studies revealed that *SMARCD3* is an androgen-dependent gene that is downregulated in response to AR signaling, while *KLK3*, the gene encoding for PSA, represents a direct, positively regulated AR-target.[11,27] The fact that SMARCD3 and PSA exhibit opposed protein levels is in accordance with these findings. Low SMARCD3 levels in primary tumors were also found to be associated with progression to a metastatic disease state. Given the androgen-responsiveness of *SMARCD3*, one might speculate that the gene is downregulated in tumors with high AR activity that are prone to an aggressive biologic and clinical behavior. This suggestion is supported by several studies that demonstrated an association of high AR protein levels in primary tumors with unfavorable prognosis.[28–30]

At first glance, it seems contradictory that SMARCD3 levels are elevated in malignant tissues, but exhibit a negative correlation with clinical variables associated with aggressive disease. However, our previous study showed that SMARCD3 exhibits highly specific and/or divergent functions in various cell lines and hormonal environments. For instance, we demonstrated that knockdown of *SMARCD3* resulted in a significant decrease of cell viability in androgen-responsive LnCAP cells, but not in the androgen-refractory derivative cell line C4-2.[11] We also found that the protein regulates distinct sets of target genes in LnCAP cells in the presence of physiologic levels of androgens or under hormone-deprived conditions.[11] PCa is a highly heterogeneous disease that can be classified in at least seven subtypes with variable degrees of aggressiveness.[31] Thus, even though*SMARCD3* seems to be upregulated in many prostate tumors, low protein levels may represent a characteristic feature of a certain PCa subclass with high malignant potential.

Although our findings are in congruence with previous in vitro studies in PCa cell lines, we cannot state with certainty that the increase of SMARCD3 abundance in malignant tissues is caused by protein upregulation in tumor cells. Since the software used for the analysis of the TMA is not able to identify malignant cells, we cannot neglect the possibility that changed SMARCD3 levels are rather caused by alterations of the tumor microenvironment (TME). It is widely accepted that the TME plays a crucial role in the pathogenesis and progression of prostate tumors, and that it has a strong impact on the prognosis of PCa patients.[32–34] Consequently, the identification of TME-associated prognostic genes may contribute to improved clinical management.[33,34]

Taken together, our approach suggests that *SMARCD3* may represent a novel prognostic marker in clinically non-metastatic PCa. However, in order to verify this finding, studies in an independent cohort comprising a higher number of patients will be required. To translate our findings into the clinics, it will further be necessary to clarify whether tumor cells or components of the TME exhibit changes in SMARCD3 expression.

In conclusion, our approach showed congruently with the literature, that the SMARCD family members play a relevant role in prostate tumorigenesis that is worth to be investigated in more detail.

CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Iris E. Ertl http://orcid.org/0000-0002-7564-9694
Pawel Rajwa http://orcid.org/0000-0003-4073-6584

REFERENCES

1. Hargreaves DC, Crabtree GR. ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. *Cell Res*. 2011;21(3):396-420.
2. Euskirchen GM, Auerbach RK, Davidov E, et al. Diverse roles and interactions of the SWI/SNF chromatin remodeling complex revealed using global approaches. *PLoS Genet*. 2011;7(3):e1002008.
3. Euskirchen G, Auerbach RK, Snyder M. SWI/SNF chromatin-remodeling factors: multiscale analyses and diverse functions. *J Biol Chem*. 2012;287(37):30897-30905.
4. Kadoch C, Hargreaves DC, Hodges C, et al. Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. *Nat Genet*. 2013;45(6):592-601.
5. Shain AH, Pollack JR. The spectrum of SWI/SNF mutations, ubiquitous in human cancers. *PLoS ONE*. 2013;8(1):e55119.
6. Lee RS, Roberts CWM. Linking the SWI/SNF complex to prostate cancer. *Nat Genet*. 2013;45(11):1268-1269.
7. Sun A, Tawfik O, Gayed B, et al. Aberrant expression of SWI/SNF catalytic subunits BRG1/BRM is associated with tumor development and increased invasiveness in prostate cancers. *Prostate*. 2007;67(2):203-213.
8. Heebøll S, Borre M, Ottosen PD, et al. SMARCC1 expression is upregulated in prostate cancer and positively correlated with tumour recurrence and dedifferentiation. *Histol Histopathol*. 2008;23(9):1069-1076.
9. Balasubramaniam S, Comstock CES, Ertel A, et al. Aberrant BAF57 signaling facilitates prometastatic phenotypes. *Clin Cancer Res*. 2013;19(10):2657-2667.
10. Hansen RL, Heeboll S, Ottosen PD, Dyrskjøt L, Borre M. Smarcc1 expression: a significant predictor of disease-specific survival in patients with clinically localized prostate cancer treated with no intention to cure. *Scand J Urol Nephrol*. 2011;45(2):91-96.
11. Ertl IE, Brettner R, Kronabitter H, et al. The SMARCD family of SWI/SNF accessory proteins is involved in the transcriptional regulation

of androgen receptor-driven genes and plays a role in various essential processes of prostate cancer. *Cells*. 2022;12(1):124.
12. Weissman B, Knudsen KE. Hijacking the chromatin remodeling machinery: impact of SWI/SNF perturbations in cancer. *Cancer Res*. 2009;69(21):8223-8230.
13. Michel BC, D'Avino AR, Cassel SH, et al. A non-canonical SWI/SNF complex is a synthetic lethal target in cancers driven by BAF complex perturbation. *Nat Cell Biol*. 2018;20(12):1410-1420.
14. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2(5):401-404.
15. Gao J, Aksoy BA, Dogrusoz U, et al. u. a. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signaling*. 2013;6(269):pl1.
16. Humphrey PA. Gleason grading and prognostic factors in carcinoma of the prostate. *Mod Pathol*. 2004;17(3):292-306.
17. Kim M, Yoo D, Pyo J, Cho W. Clinicopathological significances of positive surgical resection margin after radical prostatectomy for prostatic cancers: a meta-analysis. *Medicina*. 2022;58(9):1251.
18. Shahabi A, Satkunasivam R, Gill IS, et al. Predictors of time to biochemical recurrence in a radical prostatectomy cohort within the PSA-era. *Can Urol Assoc J*. 2016;10(1-2):17.
19. Ro YK, Lee S, Jeong CW, Hong SK, Byun SS, Lee SE. Biochemical recurrence in Gleason score 7 prostate cancer in Korean men: significance of the primary Gleason grade. *Korean J Urol*. 2012;53(12): 826-829.
20. Prensner JR, Iyer MK, Sahu A, et al. The long noncoding RNA SChLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex. *Nat Genet*. 2013;45(11):1392-1398.
21. Mota STS, Vecchi L, Zóia MAP, et al. New insights into the role of polybromo-1 in prostate cancer. *Int J Mol Sci*. 2019;20(12):2852.
22. Cyrta J, Augspach A, De Filippo MR, et al. Role of specialized composition of SWI/SNF complexes in prostate cancer lineage plasticity. *Nat Commun*. 2020;11(1):5549.
23. Xiao L, Parolia A, Qiao Y, et al. Targeting SWI/SNF ATPases in enhancer-addicted prostate cancer. *Nature*. 2022;601(7893): 434-439.
24. Shen H, Powers N, Saini N, et al. The SWI/SNF ATPase brm is a gatekeeper of proliferative control in prostate cancer. *Cancer Res*. 2008;68(24):10154-10162.
25. Dai Y, Ngo D, Jacob J, Forman LW, Faller DV. Prohibitin and the SWI/SNF ATPase subunit BRG1 are required for effective androgen antagonist-mediated transcriptional repression of androgen receptor-regulated genes. *Carcinogenesis*. 2008;29(9):1725-1733.
26. Sun D, Lee YS, Malhotra A, et al. miR-99 family of microRNAs suppresses the expression of prostate specific antigen and prostate cancer cell proliferation. *Cancer Res*. 2011;71(4):1313-1324.
27. Luke MC, Coffey DS. Human androgen receptor binding to the androgen response element of prostate specific antigen. *J Androl*. 1994;15(1):41-51.
28. Li R, Wheeler T, Dai H, Frolov A, Thompson T, Ayala G. High level of androgen receptor is associated with aggressive clinicopathologic features and decreased biochemical recurrence-free survival in prostate: cancer patients treated with radical prostatectomy. *Am J Surg Pathol*. 2004;28(7):928-934.
29. Henshall SM, Quinn DI, Lee CS, et al. Altered expression of androgen receptor in the malignant epithelium and adjacent stroma is associated with early relapse in prostate cancer. *Cancer Res*. 2001;61(2): 423-427.
30. Hashmi AA, Mudassir G, Irfan M, et al. Prognostic significance of high androgen receptor expression in prostatic acinar adenocarcinoma. *Asian Pac J Cancer Prev*. 2019;20(3):893-896.
31. Arora K, Barbieri CE. Molecular subtypes of prostate cancer. *Curr Oncol Rep*. 2018;20(8):58.
32. Bahmad HF, Jalloul M, Azar J, et al. Tumor microenvironment in prostate cancer: toward identification of novel molecular biomarkers for diagnosis, prognosis, and therapy development. *Front Genet*. 2021;12. doi:10.3389/fgene.2021.652747
33. Zong S, Gao J. Identifying the tumor immune microenvironment-associated prognostic genes for prostate cancer. *Discov Oncol*. 2024;15(1):42.
34. Sun X, Wang L, Li H, et al. Identification of microenvironment related potential biomarkers of biochemical recurrence at 3 years after prostatectomy in prostate adenocarcinoma. *Aging*. 2021;13(12): 16024-16042.

How to cite this article: Ertl IE, Lemberger U, Rajwa P, et al. Low SMARCD3 expression is associated with poor prognosis in patients with prostate cancer. *Prostate*. 2025;85:181-190. doi:10.1002/pros.24815