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The protein arginine methyltransferases (PRMTs) PRMT1 and CARM1 as candidate epigenetic drivers in prostate cancer progression

Ioanna Maria Grypari, MD[a], Souzana Logotheti, Msc[a], Vasiliki Zolota, MD, PhD[a], Patricia Troncoso, MD[b], Eleni Efstathiou, MD, PhD[c], Vasiliki Bravou, MD, PhD[d], Maria Melachrinou, MD, PhD[a], Christopher Logothetis, MD, PhD[c], Vasiliki Tzelepi, MD, PhD[a,*]

Abstract

Epigenetic changes are implicated in prostate cancer (PCa) progression and resistance to therapy. Arginine residue methylation is an understudied histone post-translational modification that is increasingly associated with cancer progression and is catalyzed by enzymes called protein arginine methyltransferases (PRMTs). The molecular consequences of aberrant expression of PRMTs in PCa and the relationship between PRMTs and PCa progression are largely unknown. Using immunohistochemistry, we examined the expression of PRMT1 and CARM1, two of the best-studied PRMTs, in 288 patients across the spectrum of PCa and correlated them with markers of androgen receptor (AR) signaling, and milestones of carcinogenesis. Our findings indicate that PRMT1 and CARM1 are upregulated early in PCa progression, and that CARM1 is further upregulated after therapy. In addition, a correlation of CARM1 with AR post-translational modifications was noted in the setting of therapy resistance, highlighting CARM1 as one of the adaptation mechanisms of PCa cells in an androgen-depleted environment. Finally, CARM1 correlated with markers of cell cycle regulation, and both CARM1 and PRMT1 correlated with markers of epithelial-to-mesenchymal transition signaling. Taken together these findings indicate that an epigenetic network drives PCa progression through enhancement of milestone pathways including AR signaling, the cell cycle, and epithelial-to-mesenchymal transition.

Abbreviations: AR = androgen receptor, CRPC = castrate-resistant prostate cancer, EMT = epithelial-to-mesenchymal transition, NECA = neuroendocrine carcinoma, pAR = phosphorylated AR, PCa = prostate cancer, PRMTs = protein arginine methyltransferases, PSA = Prostate specific antigen.

Keywords: androgen signaling, arginine methylation, CARM1, epigenetics, epithelial-to-mesenchymal transition, PRMT1, prostate cancer, therapy resistance

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The datasets generated during and/or analyzed during the present study are not publicly available, but are available from the corresponding author on reasonable request.

[a] Department of Pathology, School of Medicine, University of Patras, Patras, Greece, [b] Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX, [c] Department of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, [d] Department of Anatomy, Histology and Embryology, School of Medicine, University of Patras, Patras, Greece.

[*] Correspondence: Vasiliki Tzelepi, Department of Pathology, School of Medicine, University of Patras, Patras, Greece (e-mail: btzelepi@upatras.gr).

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1. Introduction

The genetic alterations characteristic of metastatic treatment-refractory prostate cancer (PCa) are underrepresented in early PCa.[1] The emergence of epigenetically driven alterations may drive the progression of the disease and may account for the paucity of mutations observed in early PCa. This reasoning is supported by the evidence that epigenetic changes are implicated in PCa progression and resistance to therapy.[2,3] In line with this view, we and others have demonstrated that DNA methylation is implicated in PCa progression.[4,5] The underlying mechanism(s) are under investigation and have yet to be fully elucidated.

Several lines of evidence suggest that the aberrant gene expression in PCa cannot be fully explained by DNA methylation aberrations. The absence of androgen receptor (AR) expression in AR-negative neuroendocrine PCa is attributed to enrichment in silencing histone modifications[6,7] rather than DNA methylation. The potential relevance of this observation is supported by the fact that pharmacologic inhibition of histone silencing results in AR re-expression.[6] Combinations of DNA- and histone-targeting agents are more effective than either drug alone in experimental systems.[8,9] These observations support the notion that aberrations in DNA methylation exert their influence on gene expression in PCa through interaction with a network of epigenetic drivers.

Histone acetylation and lysine methylation are the major histone modifications controlling gene transcription. Recently,

however, the focus has shifted toward arginine residue methylation, an understudied histone post-translational modification that is increasingly associated with cancer progression.[10] Arginine methylation is catalyzed by enzymes called protein arginine methyltransferases (PRMTs).[10] Both arginine depletion strategies[11] and PRMT inhibitors[12] are being tested in clinical trials as therapeutic strategies against cancer.

Apart from its role in epigenetic signaling, arginine methylation has been shown to regulate consensus milestones of cancer progression including the cell cycle, stem cell function, and escape from immune surveillance.[10] Numerous cellular proteins have been shown to serve as substrates for PRMTs,[13] including AR[14] and arginine methylation of a specific protein can facilitate or disrupt interactions with other proteins,[10] thereby regulating the relevant pathway.

Previous studies have shown differential expression of specific PRMT family members that interact with androgen signaling in PCa compared to non-neoplastic tissue.[14–16] However, the relationship between PRMTs and PCa progression or the molecular consequences of PRMT aberrations in PCa are largely unknown.

We examined the expression of two members of the PRMT family, PRMT1 and CARM1 (PRMT4), across the spectrum of PCa progression. In addition, we correlated their expression with markers of androgen signaling, the cell cycle, and epithelial-to-mesenchymal transition (EMT) to screen for a potential role in specific milestones of PCa progression.

2. Materials and methods

2.1. Patients

Radical prostatectomy specimens from 288 patients with PCa, including 211 cases from the Genitourinary Tissue Bank of MD Anderson Cancer Center, Houston, USA (material transfer agreement #MT2017-17807) and 77 cases from the archives of the Department of Pathology of the University Hospital of Patras, Greece (approval by the Ethics and Research Committee of the University Hospital of Patras, #10-30/10/2015), were included in the study. The study was conducted in accordance with the Declaration of Helsinki.

Forty-nine patients had hormone-naïve low-grade PCa (prognostic grade group 1-2), 143 patients had hormone-naïve high-grade PCa (prognostic grade group 3-5), 52 patients had hormonally treated (3–12 months) high-grade PCa, and 44 patients had castrate-resistant prostate cancer (CRPC). With the exception of 5 patients, tumors from the treated cases showed morphologic effects of previous therapy (ie, vacuolated cytoplasm, shrunken pyknotic nuclei, inconspicuous nucleoli, the distorted architecture of the glandular component with compressed lumina or single cells aligned in cords, tiny clusters, chains, or solid sheets) and as a result no Gleason Score was applied to them. Among the CRPC patients, 4 had pure small cell carcinoma, 6 had mixed neuroendocrine carcinoma and adenocarcinoma, and the rest had pure adenocarcinoma. All CRPC cases represented primary locally advanced PCa that had received androgen ablation preoperatively, developed castration resistance and underwent salvage cystoprostatectomy to control local symptoms. All adenocarcinoma cases were acinar type. The pathologic characteristics of the patients are shown in Table 1. In 64 of the hormone-naïve lymph node metastatic cases, tissue from lymph node metastases was also available. Adjacent non-neoplastic prostatic tissue was also examined in 95 hormone-naïve cases (41 cases with low-grade and 54 cases with high-grade PCa).

Table 1

Pathologic characteristics of the patients.

	Low grade, N = 49	High grade, N = 143	Treated, N = 52	CRPC, N = 44
PGG				
1	38			
2	11			
3		47		
4		17	1	
5		79	4	
NA			47	44
pT				
pT2	36	15	17	
pT3	13	128	35	11
pT4				33
pN				
N0	29	58	40	17
pN1	4	80	9	23
pNx	16	5	3	4
Age (mean)	60	67	51	67

CRPC = castrate-resistant prostate cancer.

2.2. Tissue microarray construction

Six tissue microarrays were constructed from the radical prostatectomy specimens as previously reported.[4] Areas that represented the different patterns of high-grade prostate carcinoma (ie, fused glands, poorly formed glands, cribriform formations, and intraductal carcinoma) were sampled, as well as both the neuroendocrine carcinoma and the adenocarcinoma in mixed tumors.

2.3. Immunohistochemistry

Immunohistochemistry was performed as previously described.[4] Antigen retrieval was performed at 600W in a microwave for 20 minutes. Endogenous peroxidase blocking was performed by incubating the slides in a 3% H_2O_2 solution for 15 minutes. Envision (Dako, Carpentaria, CA, USA) was used as the detection system. Sections were counterstained with Harris' acidified hematoxylin. All cases, including the lymph node metastatic foci, were stained with antibodies against PRMT1 and CARM1. In an effort to dissect the specific roles of PRMT1 and CARM1, primary tumors were also stained with antibodies against markers of cell cycle signaling (p53, Cyclin D1, ki67), EMT (ZEB1, TWIST1), androgen receptor signaling (AR, phosphorylated AR [pAR], Prostate specific antigen [PSA], NKX3.1), and the neuroendocrine marker chromogranin. For technical reasons, TWIST1 and ZEB1 staining was only performed in high-grade hormone-naïve cases. Table 2 lists the source and dilution for each of the antibodies used.

2.4. Evaluation of immunohistochemical stains

The whole stained slides for PRMT1, CARM1, ZEB1, and TWIST1 were scanned with Pannoramic DESK Scanner (3DHISTECH Ltd., Hungary) and viewed with Pannoramic Viewer 1.15.4 (3DHISTECH Ltd). The immunohistochemically

Table 2

Dilution and source of the antibodies used.

Antigen	Dilution	Source
AR	1:50	Dako, Carpentaria, CA, USA
CARM1	1:750	Novus Biologicalis. Littleton, Co, USA
Chromogranin A	1:200	Dako
Cyclin D1	prediluted	Thermo Scientific, Rockford, IL, USA
ki67	1:50	Dako
NKX3.1	1:500	Athena Enzyme Systems, Baltimore, MD, USA
PRMT1	1:750	Novus Biologicalis
PSA	1:2	Ventana Medical Systems, Inc. Tucson, AZ, USA
pAR (Ser 213/210)	1:10	Imgenex Corp., San Diego, CA, USA
p53	1:1000	Dako
Rb	1:30	Calbiochem-EMD Chemicals, Inc. Gibbstown, NJ, USA,
TWIST1	1:400	Merck KGaA, Darmstadt, Germany
ZEB1	1:250	Sigma Aldrich, Saint Luis, MI, USA

AR = androgen receptor, PSA = prostate-specific antigen, pAR = phosphorylated AR at ser 213/210.

stained slides were scored by 2 pathologists (IMG and VT). The rest of the slides were scanned and viewed using the Bliss imaging system with WebSlide Browser 4 (both from Bacus Laboratories, Inc., Lombard, IL, USA). Each core was scored separately by dividing the number of positive epithelial cells by the total number of epithelial cells to define the percentage of positive cells in increments of 10 (ie, 0, 10, and 20 etc). At least 100 cells were evaluated in each core. The intensity of staining was scored as 1+, 2+, and 3+. The percentage of positive cells was then multiplied by the intensity of staining and a final score ranging from 0 to 300 was calculated. The mean expression of all cores per case was then evaluated. Nuclear and cytoplasmic staining were separately evaluated when present.

2.5. Statistical analysis

Patient characteristics and biomarker expression data were summarized with descriptive statistics and exploratory data analysis. Categorical data were described using contingency tables. Continuously scaled measures were summarized with descriptive statistical measures (eg, mean with standard deviation [SD]). One-way analysis of variance was used to compare the expression of biomarkers between patient groups. Paired sample *t* test was used for paired comparisons. Pearson's correlation was used to correlate between expressions of biomarkers. All reported *P* values are two-sided at a significance level of 0.05. To adjust for multiple comparisons, a Bonferroni correction was used. Analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp. Armonk, NY).

3. Results

3.1. PRMT1 is overexpressed in PCa

PRMT1 showed both nuclear and cytoplasmic staining. The mean values and standard deviation of PRMT1 expression in the different groups of cases are shown in Figure 1 and Table 3. Statistical analysis revealed an increase of PRMT1 expression (nuclear and cytoplasmic) in neoplastic cells compared to normal cells ($P < .001$ for both comparisons). No difference was noted among the other stages of prostate cancer progression or between primary and lymph node metastatic foci (Fig. 2).

3.2. CARM1 is overexpressed in PCa and correlates with cancer progression and androgen ablation

Nuclear and cytoplasmic staining was observed for CARM1. The mean values and standard deviation of CARM1 expression are shown in Fig. 1 and Table 3. Statistical analysis showed that similar to PRMT1, nuclear and cytoplasmic CARM1 expression was higher in neoplastic cells compared to non-neoplastic parenchyma ($P < .001$ for both comparisons). In addition, elevated nuclear CARM1 expression was noted from low-grade to high-grade ($P < .001$) and treated cases ($P = .036$) (Fig. 3). Lymph node metastatic foci showed higher levels of CARM1 expression in both the nucleus ($P < .001$) and the cytoplasm ($P < .001$) compared to their primary tumors (Fig. 3).

3.3. There is a significant correlation between PRMT1 and CARM1 expression in PCa

Nuclear expression of PRMT1 significantly correlated with nuclear CARM1 expression in both the primary neoplasms (Table 4) and lymph node metastases (data not shown), but not in non-neoplastic epithelial cells (Table 4). Subgroup analysis showed that correlation in primary tumors was significant in high-grade, treated, and CRPC cases, but not in low-grade carcinomas. Regarding cytoplasmic expression, PRMT1 and CARM1 showed a significant correlation only in CRPC. These findings further reinforce the idea that PRMTs are implicated in the progression of PCa. Even though CARM1 appears to be more significant, some form of redundancy and/or interplay may exist and requires further investigation at the molecular level. The Pearson's correlation coefficient (*r*) and the significance (*P* value) of the correlations between the markers are presented in Table 4.

3.4. PRMT1 and CARM1 expression in PCa correlates with androgen signaling, cell cycle, and EMT regulators

Androgen signaling: Nuclear CARM1 correlated with nuclear AR, nuclear pAR, cytoplasmic pAR, and NKX3.1 expression in neoplastic cells, but not in non-neoplastic cells. Interestingly, it was nuclear PRMT1 expression that correlated with nuclear AR, pAR, and NKX3.1 expression in non-neoplastic cells. Thus, a change from PRMT1 to CARM1 crosstalk with androgen signaling is noted in the shift from non-neoplastic to neoplastic cells.

Subgroup analysis showed that the correlation of nuclear CARM1 expression with markers of AR signaling (NKX3.1, AR, and pAR) was significant for low-grade and high-grade tumors, but not for treated tumors. Interestingly in CRPC, nuclear CARM1 correlated with nuclear and cytoplasmic pAR, but not with AR expression, and was inversely correlated with PSA expression.

Cytoplasmic CARM1 expression correlated with nuclear AR, nuclear pAR, and cytoplasmic pAR only in low-grade neoplasms, and not in high-grade or treated tumors. In CRPC, cytoplasmic CARM1, similar to nuclear CARM1, correlated with nuclear and cytoplasmic pAR expression, but not AR expression.

Based on these findings, we hypothesize that CARM1 is associated with AR expression and AR post-translational modifications in untreated tumors through either a nuclear-(histone?) or cytoplasmic-(non-histone) mediated mechanism, whereas in CRPC a shift toward CARM1/pAR crosstalk is noted. However, associations do not imply a causative effect, and further studies are warranted to validate this hypothesis.

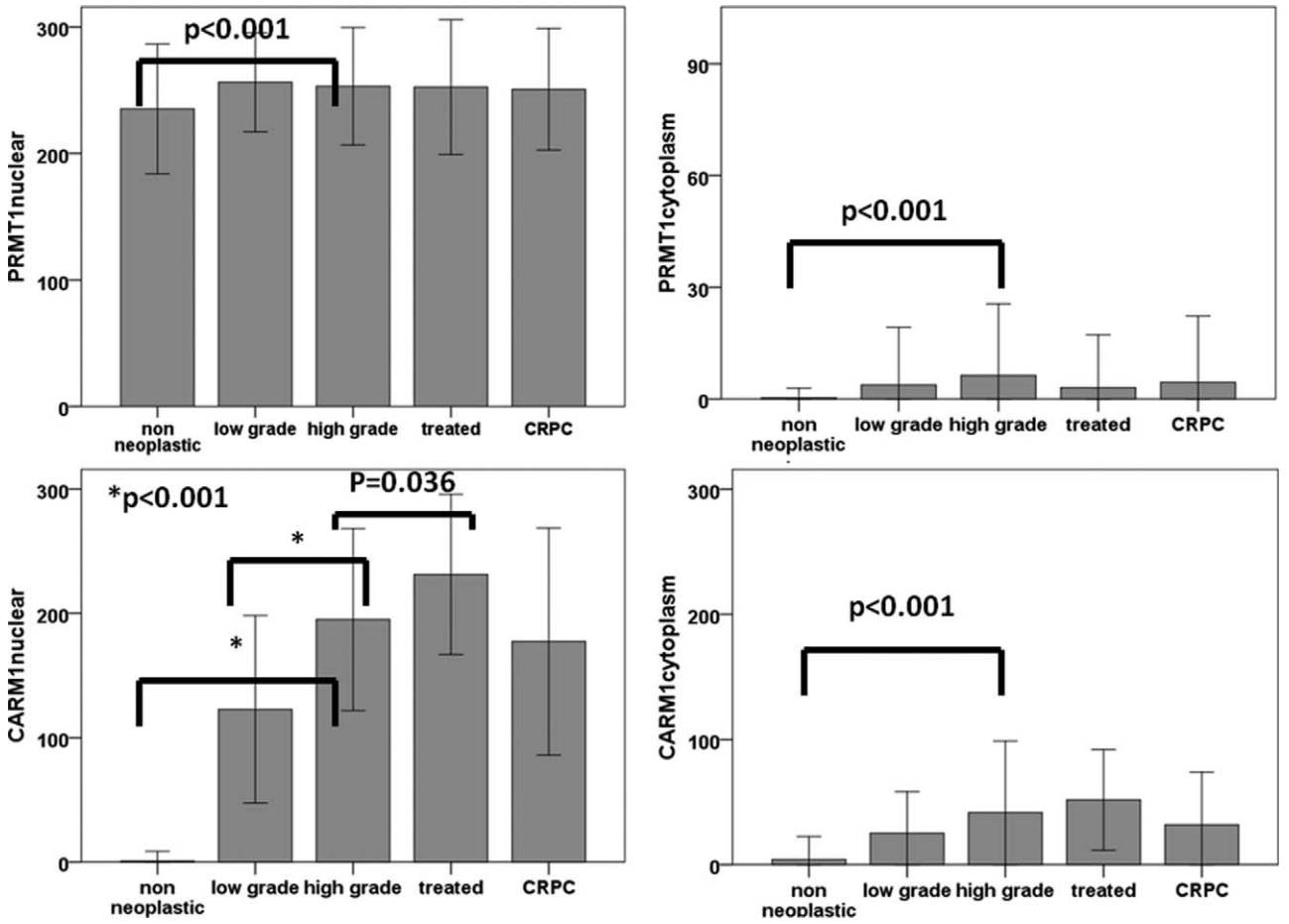

Figure 1. Graphical representation of the nuclear and cytoplasmic expression of PRMT1 and CARM1 across the spectrum of PCa progression. PCa = prostate cancer, PRMTs = protein arginine methyltransferases.

Table 3

Mean expression levels of PRMT1 and CARM1 in carcinomas, non-neoplastic peripheral zone tissue, and lymph node metastasis.

	Low grade		High grade		Treated		CRPC		NECA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CANCER										
PRMT1nu	256	39	254	38	253	53	252	47	247	45
PRMT1cyt	4	15	10	21	3	14	6	20	0	0
CARM1nuc	123	75	208	61	231	65	169	97	207	55
CARM1cyt	28	33	54	67	52	40	37	43	1	35
Non-neoplastic										
PRMT1nu	223	53	247	36						
PRMT1cyt	0	0	0	3						
CARM1nu	0	0	2	12						
CARM1cyt	2	12	7	25						
Lymph node										
PRMT1nu			269	53						
PRMT1cyt			12	31						
CARM1nu			245	68						
CARM1cyt			74	38						

cyt = cytoplasm, NECA = neuroendocrine carcinoma, nu = nuclear, PRMTs = protein arginine methyltransferases.

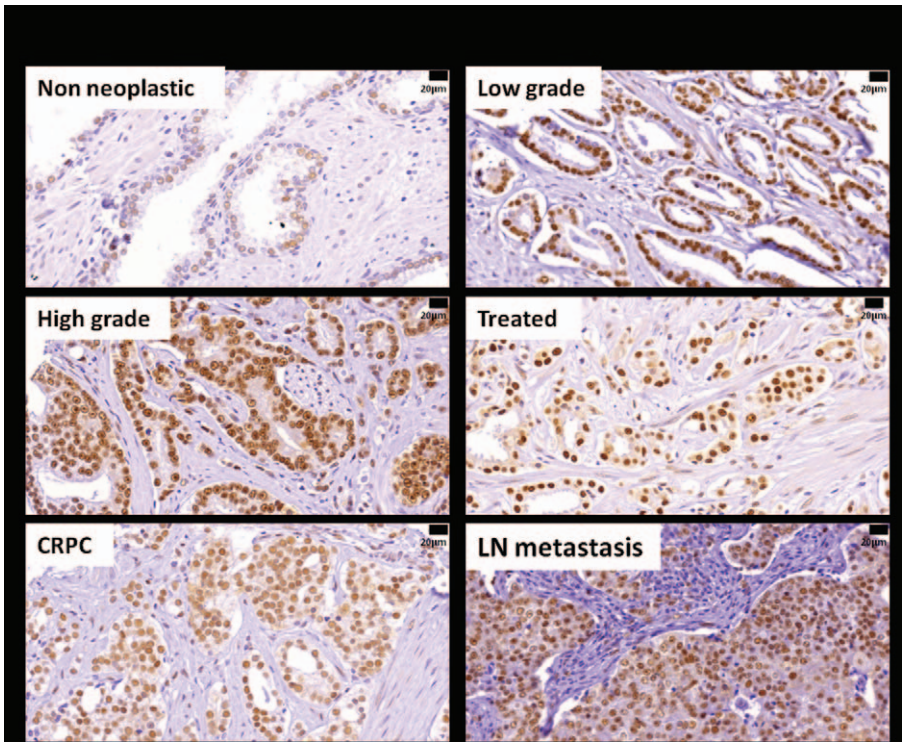

Figure 2. PRMT1 expression is moderate in non-neoplastic cells, whereas increased expression is seen in the neoplastic cells of hormone naïve low and high-grade cases. No difference is noted in the later stages of PCa progression (original magnification ×400). PCa = prostate cancer, PRMTs = protein arginine methyltransferases.

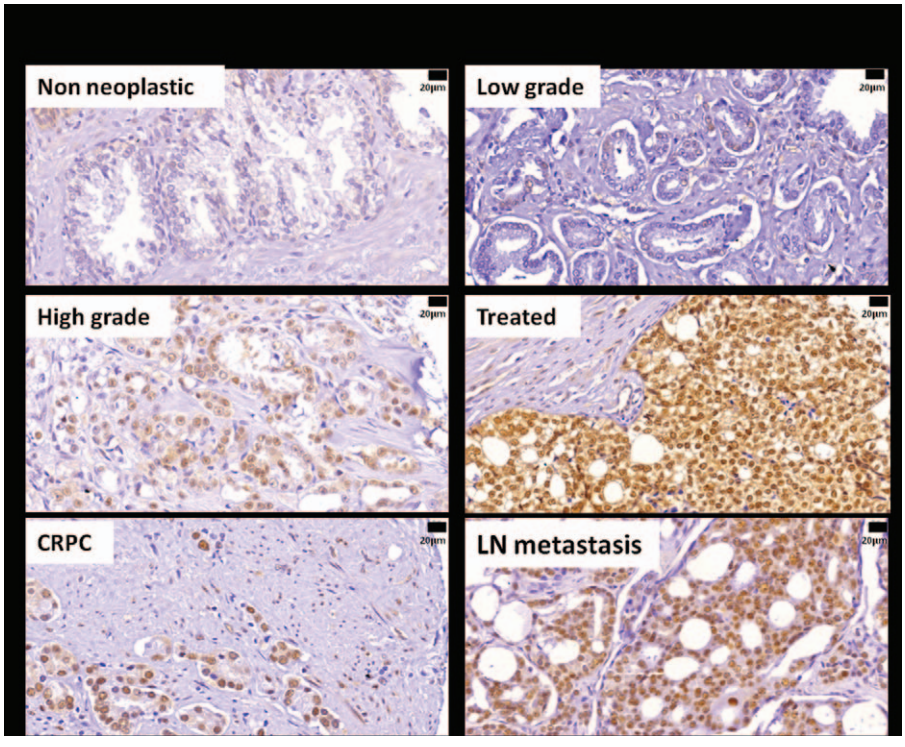

Figure 3. CARM1 expression increases from non-neoplastic to neoplastic cells and from low grade, to high grade to treated cases (original magnification ×400). PCa = prostate cancer, PRMTs = protein arginine methyltransferases.

Table 4

Correlations between the expression of PRMT1, CARM1, and markers of AR signaling, cell cycle, and EMT.

			CARM1 nu	CARM1 cyt	AR	pAR nu	pAR cyt	PSA	NKX3.1	ki67	RB	cyclin D1	p53	ZEB1	TWIST1
Non neoplastic	PRMT1 nu	*r*	0.139	0.032	**0.412**	**0.388**	**0.388**	**0.449**	**0.449**	0.202	**0.458**	0.333	−0.101		
		p	0.223	0.777	**0.000**	**0.001**	**0.001**	**0.000**	**0.000**	0.087	**0.000**	0.040	0.395		
	PRMT1 cyt	*r*	1.000	0.007	−0.144	−0.107	−0.107	0.072	−0.096	0.030	−0.049	−0.110	−0.158		
		p	0.000	0.951	0.225	0.366	0.366	0.576	0.419	0.980	0.679	0.927	0.183		
	CARM1 nu	*r*	1.000	0.070	−0.144	−0.107	−0.107	0.146	−0.096	0.003	−0.049	−0.110	0.158		
		p		0.951	0.225	0.366	0.366	0.259	0.419	0.980	0.679	0.927	0.183		
	CARM1 cyt	*r*	0.070	1.000	0.274	0.108	0.108	0.111	0.101	−0.100	0.031	0.115	−0.069		
		p	0.951		0.019	0.365	0.365	0.391	0.396	0.401	0.792	0.334	0.560		
Low grade	PRMT1 nu	*r*	0.269	0.226	0.252	0.094	0.067	−0.135	0.229	0.166	0.241	0.258	0.330		
		p	0.129	0.206	0.157	0.603	0.710	0.455	0.200	0.356	0.177	0.147	0.061		
	PRMT1 cyt	*r*	−0.205	−0.212	0.018	0.187	0.227	0.096	0.122	0.103	0.068	−0.001	−0.008		
		p	0.252	0.237	0.919	0.296	0.204	0.596	0.497	0.567	0.705	0.994	0.963		
	CARM1 nu	*r*	1.000	**0.851**	**0.462**	**0.526**	**0.345**	−0.283	**0.507**	**0.518**	**0.556**	**0.351**	**0.488**		
		p		**0.000**	**0.007**	**0.002**	**0.049**	0.110	**0.003**	**0.002**	**0.001**	**0.045**	**0.004**		
	CARM1 cyt	*r*	**0.851**	1.000	**0.508**	**0.550**	**0.432**	−0.231	0.434	0.308	**0.518**	0.242	**0.432**		
		p	**0.000**		**0.003**	**0.001**	**0.012**	0.195	0.012	0.082	**0.002**	0.175	**0.012**		
High grade	PRMT1 nu	*r*	**0.648**	**0.346**	**0.463**	**0.356**	0.072	−0.098	**0.487**	−0.091	**0.498**	0.083	0.047	**0.581**	**0.550**
		p	**0.000**	**0.006**	**0.000**	**0.004**	0.576	0.448	**0.000**	0.483	**0.000**	0.521	0.719	**0.000**	**0.000**
	PRMT1 cyt	*r*	0.098	**0.303**	0.124	0.215	0.129	−0.005	0.283	−0.126	0.146	0.105	0.260		
		p	0.450	**0.016**	0.338	0.094	0.317	0.968	0.026	0.330	0.259	0.417	0.041		
	CARM1 nu	*r*	1.000	**0.520**	**0.450**	**0.454**	0.111	−0.132	**0.425**	0.067	**0.542**	0.125	0.095	**0.694**	0.205
		p		**0.000**	**0.000**	**0.000**	0.391	0.306	**0.001**	0.603	**0.000**	0.332	0.464	**0.000**	0.146
	CARM1 cyt	*r*	**0.520**	1.000	0.282	0.241	0.204	−0.106	0.414	0.159	0.215	0.126	**0.342**		
		p	**0.000**		0.026	0.060	0.112	0.413	0.274	0.218	0.093	0.330	**0.006**		
Treated	PRMT1 nu	*r*	**0.397**	**0.334**	−0.031	−0.039	−0.194	0.075	0.243	0.065	0.284	**0.308**	0.005		
		p	**0.004**	**0.017**	0.828	0.787	0.173	0.601	0.086	0.648	0.043	**0.028**	0.975		
	PRMT1 cyt	*r*	−0.249	−0.068	−0.062	0.132	−0.109	−0.039	0.161	0.120	0.117	0.114	0.081		
		p	0.075	0.634	0.661	0.352	0.444	0.783	0.256	0.397	0.410	0.423	0.570		
	CARM1 nu	*r*	1.000	**0.687**	0.156	0.140	−0.052	−0.256	0.330	−0.022	0.288	0.213	0.104		
		p		**0.000**	0.268	0.322	0.716	0.067	0.017	0.877	0.038	0.129	0.463		
	CARM1 cyt	*r*	**0.687**	1.000	0.159	0.199	0.161	0.073	0.203	0.060	0.201	0.208	0.028		
		p	**0.000**		0.261	0.158	0.255	0.608	0.149	0.671	0.153	0.140	0.842		
CRPC	PRMT1 nu	*r*	**0.399**	0.271	0.254	0.236	0.047	−0.036	0.121	0.112	0.316	0.309	−0.214		
		p	**0.011**	0.090	0.114	0.143	0.772	0.827	0.458	0.490	0.047	0.052	0.185		
	PRMT1 cyt	*r*	0.244	**0.494**	0.070	0.201	0.179	0.017	0.085	−0.007	0.176	0.017	−0.153		
		p	0.129	**0.001**	0.667	0.213	0.270	0.915	0.603	0.966	0.277	0.918	0.345		
	CARM1 nu	*r*	1.000	**0.687**	0.307	**0.518**	**0.498**	**−0.328**	0.142	0.257	0.307	**0.325**	−0.083		
		p		**0.000**	0.054	**0.001**	**0.001**	**0.039**	0.381	0.109	0.054	**0.041**	0.610		
	CARM1 cyt	*r*	0.687	1.000	0.206	**0.401**	**0.391**	**−0.166**	0.121	0.157	0.281	**0.329**	−0.144		
		p	0.000		0.202	**0.010**	**0.013**	0.307	0.458	0.332	0.079	**0.038**	0.374		

AR = androgen receptor, cyt = cytoplasmic, nu = nuclear, PRMTs = protein arginine methyltransferases. Significant correlations ($r > 0.3$) are depicted in bold.

Cell cycle: Nuclear CARM1 correlated with Rb expression in low-grade, high-grade and treated tumors but not in CRPC carcinomas. Similarly, nuclear PRMT1 expression correlated with RB expression in non-neoplastic cells and in high-grade, treated, and CRPC tumors. In addition, CARM1 correlated with a cyclin D1 p53 and ki67 expression only in low-grade tumors. Cytoplasmic CARM1 expression correlated with p53 in low and high-grade carcinomas. Taken together these findings indicate a potential crosstalk between CARM1 and the cell cycle and highlight a subgroup of low-grade carcinomas with adverse molecular features (increased expression of CARM1, cyclin D1, ki67, and p53). The clinical implications of this subgroup of low-grade carcinomas require further investigation.

EMT: Significant correlations between ZEB1 and nuclear PRMT1 expression, TWIST1 and nuclear PRMT1 expression, ZEB1 and nuclear CARM1 expression, and ZEB1 and TWIST1 expression were noted. Interestingly, the correlations were significant only in neoplastic cells, whereas non-neoplastic cells did not show any statistically significant correlation between either PRMT1 or CARM1 and EMT markers. Representative images of PRMT1, CARM1, ZEB1, and TWIST1 expression in PCa are shown in Fig. 4. These findings imply that both PRMT1 and CARM1 may be implicated in EMT in PCa.

The Pearson's correlation coefficient (r) and the significance (P value) of the correlations mentioned above are included in Table 4.

4. Discussion

Epigenetic regulation is critically implicated in cancer progression and therapy resistance. The findings of this study are in line with our previous observations regarding the role of DNA methyltransferases in PCa progression.[4] We now show that PRMT1 and CARM1 are upregulated early in PCa progression, and CARM1 is further upregulated after therapy, suggesting it has a role in therapy resistance. These correlative observations support

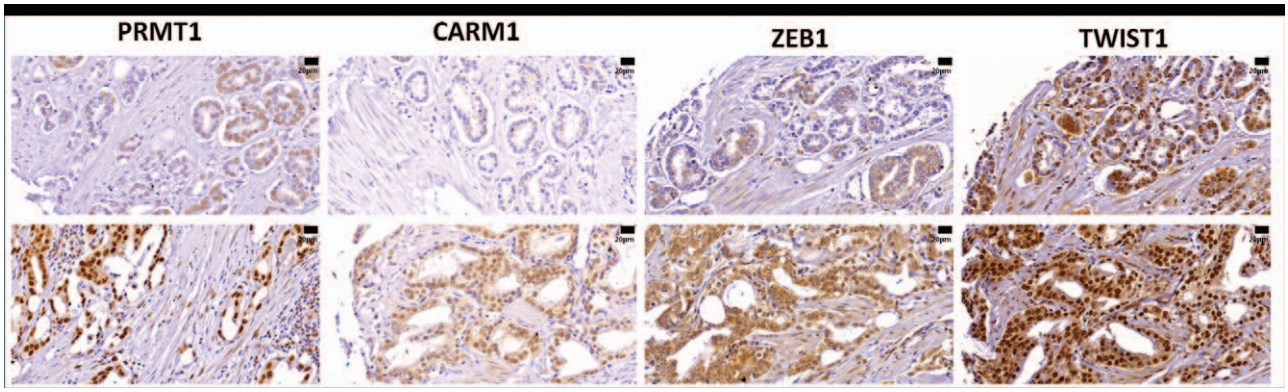

Figure 4. PRMT1, CARM1, ZEB1, and TWIST1 expression correlated with one-another in PCa (original magnification ×400). A case with low expression of all markers is shown in the upper panel and a case with high expression of all markers in the lower panel. PCa = prostate cancer, PRMTs = protein arginine methyltransferases.

the hypothesis that an epigenetic network drives lethal PCa progression. In addition, an interplay between PRMTs and AR signaling, the cell cycle, and EMT was identified, linking PRMTs to specific milestones of PCa progression. Thus, comprehensive characterization and functional understanding of the epigenetic events is critical to understand the precise mechanism of epigenetically driven lethal PCa progression.

PRMT1 and CARM1 are among the best-studied PRMTs.[17,18] Increased expression of PRMT1 and CARM1 has been found in various tumors and has been associated with an aggressive phenotype and adverse prognosis.[19–22] PRMT1 expression has not been studied in PCa before. We showed that PRMT1 was increased in neoplastic cells, compared to non-neoplastic epithelial cells. The level of increase noted was very low and its biological significance is not certain. In addition, its expression remained stable in the later stages of PCa progression. This may indicate that PRMT1 overexpression is not fundamental for PCa progression. However, a significant correlation of its expression with CARM1 was noted in neoplastic cells, especially in more advanced settings, implying that a form of redundancy or interplay between the two proteins may exist, as has been shown for PRMT1 and PRMT5.[23] In addition, a correlation of PRMT1 expression with the EMT markers ZEB1 and TWIST1 was shown in our study, and pharmacologic inhibition of PRMT1 has been shown to reduce the proliferation of PCa cell lines in androgen-depleted and non-depleted environments.[24] Taken together, the observed correlations strongly support a potential role for PRMT1 in PCa, despite its stable levels during the progression of the disease, and further support for the formation of an epigenetic network that drives PCa progression.

Similar to previous findings,[13,15,16] we have shown that CARM1 is upregulated in neoplastic cells compared to non-neoplastic parenchyma, is further increased in response to androgen ablation, and does not seem to be further enhanced in the later stages of PCa progression. Thus, CARM1 may be implicated in therapy resistance. Indeed, experimental evidence has shown that CARM1 increases the transcriptional activation of steroid nuclear receptors,[25] including AR,[13,16] in PCa cell lines. In line with this, we showed that CARM1 correlated with NKX3.1, AR, and pAR expression in untreated tumors, whereas in CRPC carcinomas CARM1 expression correlated with the expression of pAR phosphorylated at Ser210/213. AR is phosphorylated in various residues by a variety of kinases,[26] and Ser210 AR phosphorylation has been shown to regulate AR transactivation in an androgen-independent manner.[27] Taken together, these findings highlight CARM1 as one of the adaptive mechanisms of PCa cells in an androgen-depleted environment. Thus, targeting the epigenetic network may be of therapeutic value. Further studies are needed to elucidate the exact mechanisms of this phenomenon.

Previous studies have shown that PRMTs are implicated in cell cycle regulation. CARM1 has been shown to phosphorylate Rb in specific residues, acting as a negative regulator of the tumor-suppressing role of Rb and subsequently inducing E2F activation and promoting cell mitosis.[28] The relationship that we found between PRMT1, CARM1, Rb, cyclin D1, p53, and ki67 in our study is in line with the notion that PRMTs have a critical role in cell cycle regulation in PCa. Importantly, the most significant correlations between CARM1 and cell cycle regulators were seen in low-grade tumors. This unexpected observation implies that there is a subset of low-grade tumors with increased proliferation rate and adverse molecular features. Further investigation will be required to determine the significance of this observation.

In addition to their effects in epigenetic signaling, PRMTs have been implicated in EMT, a biologic process that requires cell plasticity to ensure bidirectional transitions between epithelial, mesenchymal, and multiple intermediate (partial EMT) phenotypic states as a response to environmental threats.[29] For example, PRMT1 overexpression increases the transcription of ZEB1 (a key EMT transcription factor) and induces EMT in a breast cancer cell line.[30] In addition, primary amino acid sequencing of TWIST1 (another key EMT transcription factor) has indicated 2 potential PRMT1 binding sites, and with the use of in vitro methylation assays, it has been shown that TWIST1 is a PRMT1 substrate.[22] These data are in agreement with our immunohistochemical findings that TWIST1 and ZEB1 expression is strongly correlated with PRMT1 expression in neoplastic cells. Interestingly, no correlation between EMT markers and PRMT1 was found in non-neoplastic cells, further supporting the idea that PRMT1 is involved in EMT in PCa. The effects of PRMT1 and CARM1 in EMT adds to our confidence that these specific drivers of epigenetic regulation are linked to lethal PCa progression.

The data presented here are in line with our previous findings and provide evidence that multiple epigenetic changes drive PCa progression.[4] This may explain the limited efficacy of delayed targeted therapy because at that time the disease has become too heterogeneous with increased epigenetic instability. Understanding the cascade and interplay of epigenetic changes during PCa progression may facilitate the development of markers to inform secondary prevention strategies that target the relevant pathways.

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Author contributions

Conceptualization: Ioanna Maria Grypari, Vasiliki Zolota, Christopher Logothetis, Vasiliki Tzelepi.
Data curation: Ioanna Maria Grypari, Souzana Logotheti.
Formal analysis: Ioanna Maria Grypari.
Funding acquisition: Maria Melachrinou, Vasiliki Tzelepi.
Investigation: Ioanna Maria Grypari.
Methodology: Ioanna Maria Grypari, Patricia Troncoso, Eleni Efstathiou.
Project administration: Ioanna Maria Grypari.
Resources: Patricia Troncoso, Eleni Efstathiou, Vasiliki Bravou, Maria Melachrinou, Christopher Logothetis, Vasiliki Tzelepi.
Software: Souzana Logotheti.
Validation: Vasiliki Zolota, Vasiliki Tzelepi.
Visualization: Ioanna Maria Grypari, Vasiliki Tzelepi.
Writing – original draft: Ioanna Maria Grypari, Vasiliki Tzelepi.
Writing – review & editing: Christopher Logothetis, Vasiliki Tzelepi.

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