

The Prognostic Significance of Proteasome 26S Subunit, Non-ATPase (PSMD) Genes for Bladder Urothelial Carcinoma Patients

Cancer Informatics
Volume 20: 1–10
© The Author(s) 2021
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/11769351211067692



AbdulFattah Salah Fararjeh¹, Ali Al-Khader^{2,3}, Malak Al-Saleem¹ and Rinad Abu Qauod¹

¹Department of Medical Laboratory Analysis, Faculty of Science, Al-Balqa Applied University, Al-Salt, Jordan. ²Department of Pathology and Forensic Medicine, Faculty of Medicine, Al-Balqa Applied University, Al-Salt, Jordan. ³Department of Pathology, Al-Hussein Salt Hospital, Al-Salt, Jordan.

ABSTRACT: Proteasome a highly sophisticated systems that alter protein structure and function. Proteasome 26S Subunit, Non-ATPase (PSMD) genes have been implicated in several types of malignancies. This is the first study to look at how proteasomal subunits are expressed in patients with bladder urothelial carcinoma (BLCA). BLCA was used to evaluate the predictive value of PSMD genes (PSMD1 to PSMD12) in relation to clinicopathological characteristics. PSMD genes' expression patterns at the mRNA level were analyzed using a variety of bioinformatics methods, including gene expression profile integrative analysis (GEPIA), Oncomine, TCGA, and Gene expression Omnibus (GEO) databases. The GEPIA and TCGA dataset survival plot functions were used to assess the prognostic significance of PSMD genes. PSMD2, PSMD3, PSMD4, PSMD8, and PSMD11 genes were significantly overexpressed in BLCA compared with normal bladder tissues. PSMD2 and PSMD8 were significantly overexpressed in BLCA more than other types of cancer. High level of PSMD2 and PSMD8 predicted shorter overall (OS) and progression free survival (PFS) in BLCA patients. High level of PSMD2 was significantly associated with elder age ($P < .001$), female gender ($P = .014$), tumor grade ($P < .001$), and metastasis ($P = .003$). PSMD2 has been shown to be an independent predictor for OS in BLCA patients based on univariate and multivariate analysis ($P < .001$). Overall, according to this study, PSMD2 and PSMD8 could be served as a prognostic biomarker for BLCA patients.

KEYWORDS: Bladder cancer, PSMD, TCGA, prognosis, overall survival

RECEIVED: July 29, 2021. **ACCEPTED:** November 16, 2021.

TYPE: Original Research

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: AbdulFattah Salah Fararjeh, Department of Medical Laboratory Analysis, Faculty of Science, Al-Balqa Applied University, P.O. Box 206, Al-Salt 19117, Jordan. Email: Fararjehas@bau.edu.jo

Introduction

Bladder cancer (BLCA) is the ninth most common genitourinary tract malignancy in the general population, and the fourth among males, with an expected 573 378 new cases and 200 000 deaths in 2020.¹ Because men are the primary consumers of cigarettes, smoking ranks as the top major cause of bladder cancer.² Further studies aimed to explain the association between BLCA and several other risk factors such as age, sex, genetics, race, alcohol, occupational carcinogenic exposure, and dietary factors.^{3,4}

BLCA is classified as non-muscle invasive bladder cancer (NMIBC) or muscle invasive bladder cancer (MIBC).⁵ NMIBC represents the most shape, almost 75% and usually is treated through transurethral resection. NMIBC indicates a high relapse rate, and patients need strict examination through cystoscopy and urine cytology, which imparts great pressure to the affected person and prices for the health care gadget.^{6,7} MIBC is a heterogeneous, aggressive illness which is associated with a 5-years survival rate, almost 60% for patients with localized illness and less than 10% for patients with remote metastases. It is characterized by means of genomic instability with high mutation rate.⁸ Each subtype has its novel pathological and molecular features, which can be used to predict the response to treatment⁹⁻¹¹

A single successful treatment strategy for all MIBC patients has been challenging to achieve. Thus, individualized therapy is

recommended. However, radical cystectomy alongside pelvic lymph node dissection is deemed the gold standard treatment for MIBC. A 2009 meta-analysis has supported that neo-adjuvant chemotherapy preceding the treatment is the optimal curative strategy for MIBC.¹²

Kamat et al¹³ investigated the synergetic inhibitory effect of proteasomal inhibitors of Bortezomib and Gemcitabine as a therapeutic option for bladder cancer through strong suppression of tumor cell proliferation, which facilitates tumor growth inhibition.

The proteasome (26S proteasome) is a 2.5 MDa hollow cylinder-shaped multi-protein structure that consists of a core particle (20S proteasome) and a regulatory particle (RP or 19S proteasome) on 1 or both sides. Six ATPases and 3 additional peptides without ATPase activity make up the 19S base sub-complex. To carry out the proteasome function, various subunits of RP have specialized activities. For example, PSMD4, Rpn13, and PSMD10, the 3 subunits of the base sub complex, having ubiquitin recognition domains that allow them to identify poly-ubiquitin chains.^{14,15}

The ubiquitin-proteasome system (UPS) is a protein turnover pathway that operates in various cellular processes to degrade or modify about 85% of cellular proteins in order to sustain adequate cell processes in eukaryotic cells.¹⁶⁻¹⁸ UPS consists of



ubiquitin (Ub) a small β -grasp fold tag protein that functions in post-translational modification of protein, ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), ubiquitin-ligase enzymes (E3), 26S proteasome, Deubiquitinase enzymes (DUBs), and target proteins which act in ubiquitination and in other important signaling pathways that take part in different physiological processes including apoptosis, angiogenesis as well as antigen presentation and DNA damage control. Malfunction or altered expression of UPS may lead to accumulation of proteins,¹⁹ and is correlated with various human diseases including malignancies.²⁰⁻²²

The Proteasome 26S Subunit, Non-ATPase (PSMD) gene family, which includes the PSMD1 to PSMD14, regulate ubiquitinated protein breakdown in circulation as well as tumorigenesis. Overexpression of PSMD4 has been linked to poor survival in individuals with breast cancer,^{23,24} while it may inhibit neoplastic invasiveness in colorectal cancer.²⁴ PSMD4 expression is related with a considerable increase in well-differentiated tumors in hepatocellular carcinoma (HCC).²³ A 2019 study revealed a novel clinical prognostic association of Proteasome 26S Subunit, non-ATPase 3 (PSMD3) expression pattern in breast cancer cell lines that identified PSMD3 as a stabilizing protein of human epidermal growth factor receptor 2 (HER2).²⁰ A more recent study observed high expression of PSMD6, PSMD9, PSMD11, and PSMD14 in pancreatic cancer²⁵ which have paved the way for further studies to investigate PSMDs as a strong diagnostic and prognostic biomarker in BLCA. The aim of this study is to perform a web-based analysis of PSMD genes' expression levels in BLCA in regard to different clinicopathological parameters.

Material and Methods

Expression profile of PSMD genes in BLCA using GEPIA

In GEPIA, (<http://gepia.cancer-pku.cn/index.html>), there are more than 10000 tumor samples and around 9000 normal samples in an interactive web-based program that gives RNA sequencing data based on TCGA and GTEx. Differential expression analysis (DIY), profiling graphing, analysis the correlation between genes, overall survival analysis, similar gene detection, and principle component analysis are just a some of the interactive and customizable features available in GEPIA. For survival analysis, GEPIA use the Log-rank test, often known as the Mantel-Cox test. The prognostic significance of PSMD genes were evaluated using the GEPIA survival plot function; patients' PSMD genes mRNA levels were divided into 2 groups, high and low, based on median PSMD mRNA expression levels, with no follow-up restrictions.

Expression profile of PSMD in BLCA using Oncomine and TCGA dataset

Oncomine, a cancer microarray dataset-providing server that grants access to about 800 datasets from numerous study

cohorts(<http://www.oncomine.org>), used to analyzed the mRNA levels of PSMD genes in tumor versus normal bladder tissues in several studies. TCGA-BC cohort by UCSC (University of California at Santa Cruz, CA, USA) Cancer Genomics Browser (<https://xenabrowser.net/>) assisted the collection of BLCA patients data.

Gene expression Omnibus (GEO) dataset

We used GEO datasets website (<https://www.ncbi.nlm.nih.gov/gds>) looking for studies on non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC). A 3 validated GEO microarray datasets, (GSE163899) includes 32 patients with NMIBC and (GSE145137) dataset from patients with NMIBC progressed to MIBC and (GSE149582) which analyzed the gene expression signature between de novo MIBC and progressive MIBC, were downloaded from GEO database (<https://www.ncbi.nlm.nih.gov/geo/>).

Statistical analysis

SPSS software was used to analyze the BLCA data (SPSS, Inc., Chicago, IL, USA). One-way ANOVA, Student *t*-tests and chi-squared tests were used to analyze the expression level of PSMD gene in tumor versus normal BLCA tissues in addition to association of the PSMD genes with BLCA clinicopathological features. The clinical prognostic importance in BC was investigated using Cox-regression analysis in both univariate and multivariate studies. The log-rank test was used to compare survival curves plotted using Kaplan-Meier analysis. * $P < .05$, ** $P < .001$ were used to indicate statistical significance.

Results

Expression level of PSMD genes in bladder urothelial carcinoma

We investigated the expression level of PSMDs in normal versus tumor tissues in bladder urethral carcinoma (BLCA) using gene expression profile integrative analysis (GEPIA) based on Box blot analysis. We found that all the analyzed PSMD genes were upregulated in tumor tissues more than normal tissues except PSMD5. Exclusively, PSMD2, PSMD3, PSMD4, PSMD8, and PSMD11 genes were significantly overexpressed at mRNA level in tumor tissues compared with normal bladder tissues (Figure 1). When compared to other proteasomal subunits with less than 7.0 Transcription Per Million (TPM) PSMD2, PSMD4, and PSMD8 showed the greatest expression in tumor cells (7.6 TPM), (7.9 TPM), and (7.4 TPM), respectively.

Prognostic significance of PSMD genes in bladder urothelial carcinoma

Because PSMD2 and PSMD8 showed the highest expression level in BLCA tumor tissues and high level of both

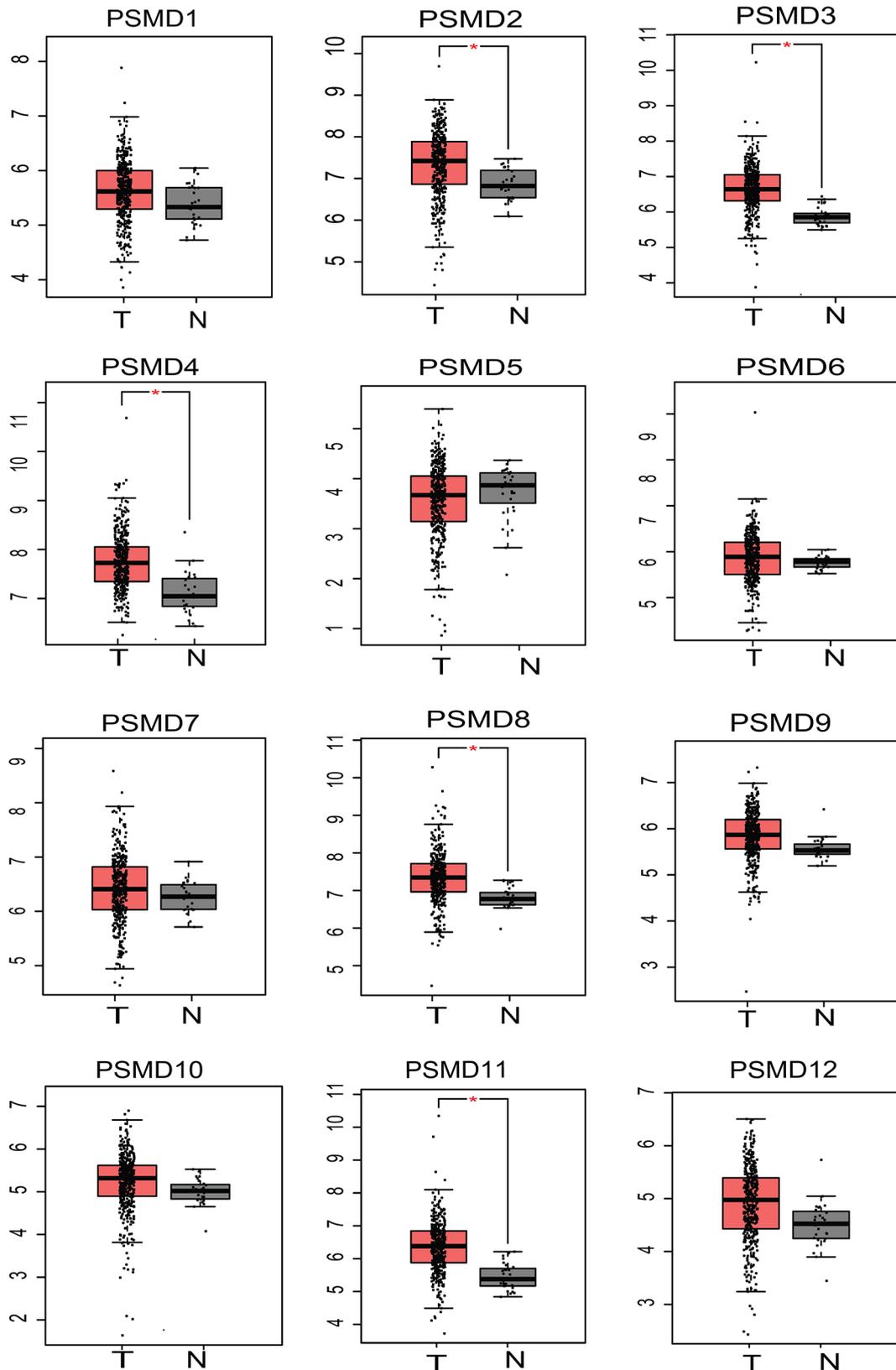


Figure 1. Expression of PSMD genes family in BLCA tumor and normal tissues. Box plots show PSMD1, PSMD2, PSMD3, PSMD4, PSMD5, PSMD6, PSMD7, PSMD8, PSMD9, PSMD10, PSMD11, PSMD12 levels in transcription per million (TPM) in Y axis. Abbreviation: PSMD, proteasome 26S subunit, non-ATPase, N; Normal, T; Tumor. The method for differential analysis was one-way ANOVA, with disease condition (tumor or normal) as the variable for differential expression calculation; $P < .05$ was considered to be significant.

genes predicts bad overall survival in BLCA, differential expression of PSMD2 and PSMD8 was subsequently evaluated in several types of cancer. Interestingly, both genes;

PSMD2 and PSMD8, were significantly overexpressed in all type of tumor tissues as shown in Bladder urothelial carcinoma (BLCA) tumor, Colon adenocarcinoma (COAD),

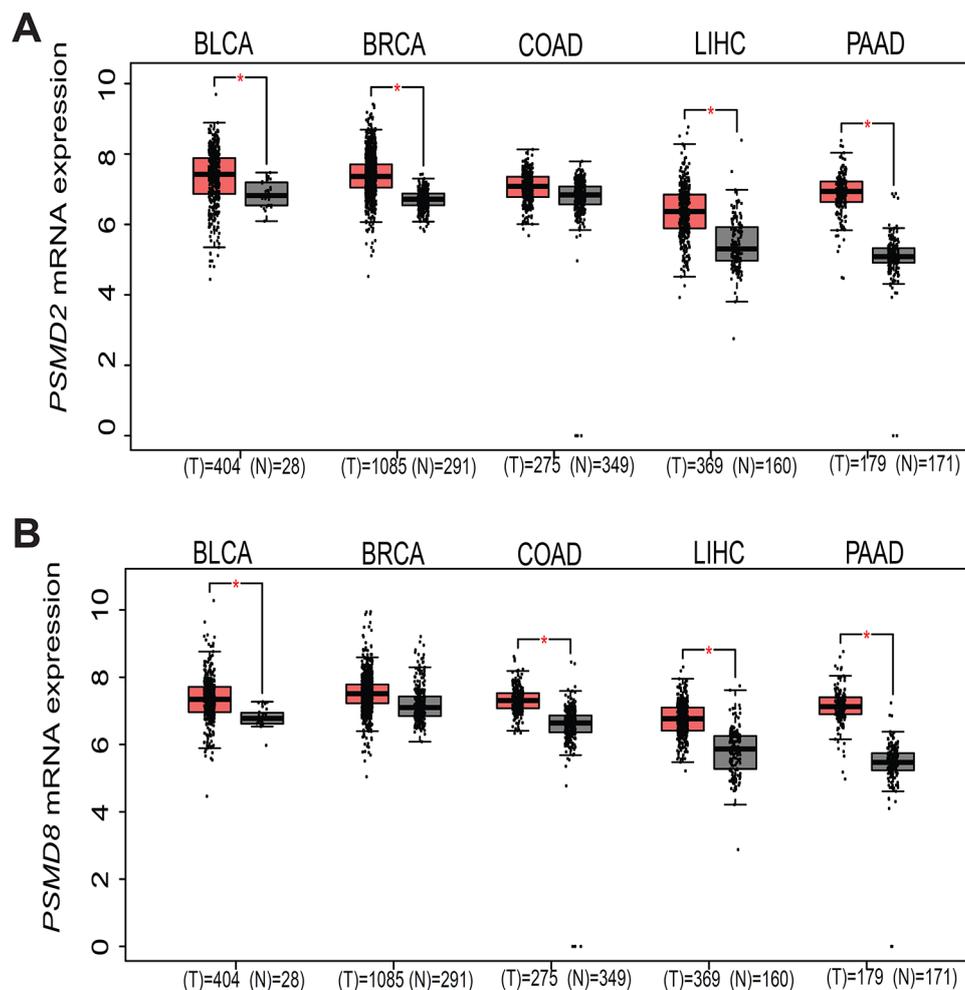


Figure 2. Comparison between mRNA expression of (A) PSMD2 and (B) PSMD8 in Tumor (T) versus Normal (N) tissues in several type of cancers; BLCA; bladder carcinoma, BRCA; breast carcinoma, COAD; colon adenocarcinoma, LIHC; Liver hepatocellular carcinoma, PAAD, pancreatic adenocarcinoma. The method for differential analysis was one-way ANOVA, with disease condition (tumor or normal) as the variable for differential expression calculation; $P < .05$ was considered to be significant.

Liver hepatocellular carcinoma (LIHC), and Pancreatic adenocarcinoma (PAAD) (Figure 2). Particularly, BLCA was shown to have the highest level of PSMD2 7.9 Transcription Per Million (TPM) and PSMD8 7.7 TPM in comparison to other type of cancers.

The prognostic significance of PSMD genes were evaluated using GEPIA survival analysis plotter. The overall survival curves showed that high mRNA level of PSMD2 ($P = .03$), PSMD8 ($P = .052$), and PSMD9 ($p = .032$) was associated with worse overall survival in BLCA (Figure 3). However, no significant results have been found in low versus high level of PSMD1, PSMD3, PSMD4, PSMD6, PSMD7, PSMD10, and PSMD11.

To confirm these results, we downloaded RNA seq data from 3 different microarray datasets based on transcriptome analysis to identify the prognostic role of these genes in NMIBC and MIBC. The first study (GSE163899) was performed on 32 patients who were divided into 3 groups; as shown in the heat map (Figure 4A), the expression level of

PSMD1, PSMD5, PSMD6, PSMD7, PSMD9, PSMD10, PSMD11, and PSMD12 were low in the 3 groups; no relapse $n = 15$ (G1), recurrence $n = 9$ (G2), and during the progression $n = 8$ (G3) of the disease. However, PSMD2, PSMD3, PSMD4, PSMD8 have had higher level in the NMIBC progression phase (Figure 4A).

Moreover, the second microarray dataset GSE145137 using mRNA sequencing data from patients with non-muscle invasive bladder cancer. The RNA seq data analysis was performed at 3 different intervals: at time of diagnosis T1-NMIBC, after progressing to MIBC after second transurethral resection T2 and the third time as recurrent and chemotherapy-resistant. As shown in (Figure 4B), high levels of PSMD2, PSMD8, and PSMD4 were shown in the T2 and in the third group which may be associated with the development of MIBC.

Finally, we downloaded gene expression profile from GSE149582 dataset, 26 samples from the discovery phase were included, the patients were grouped into de novo

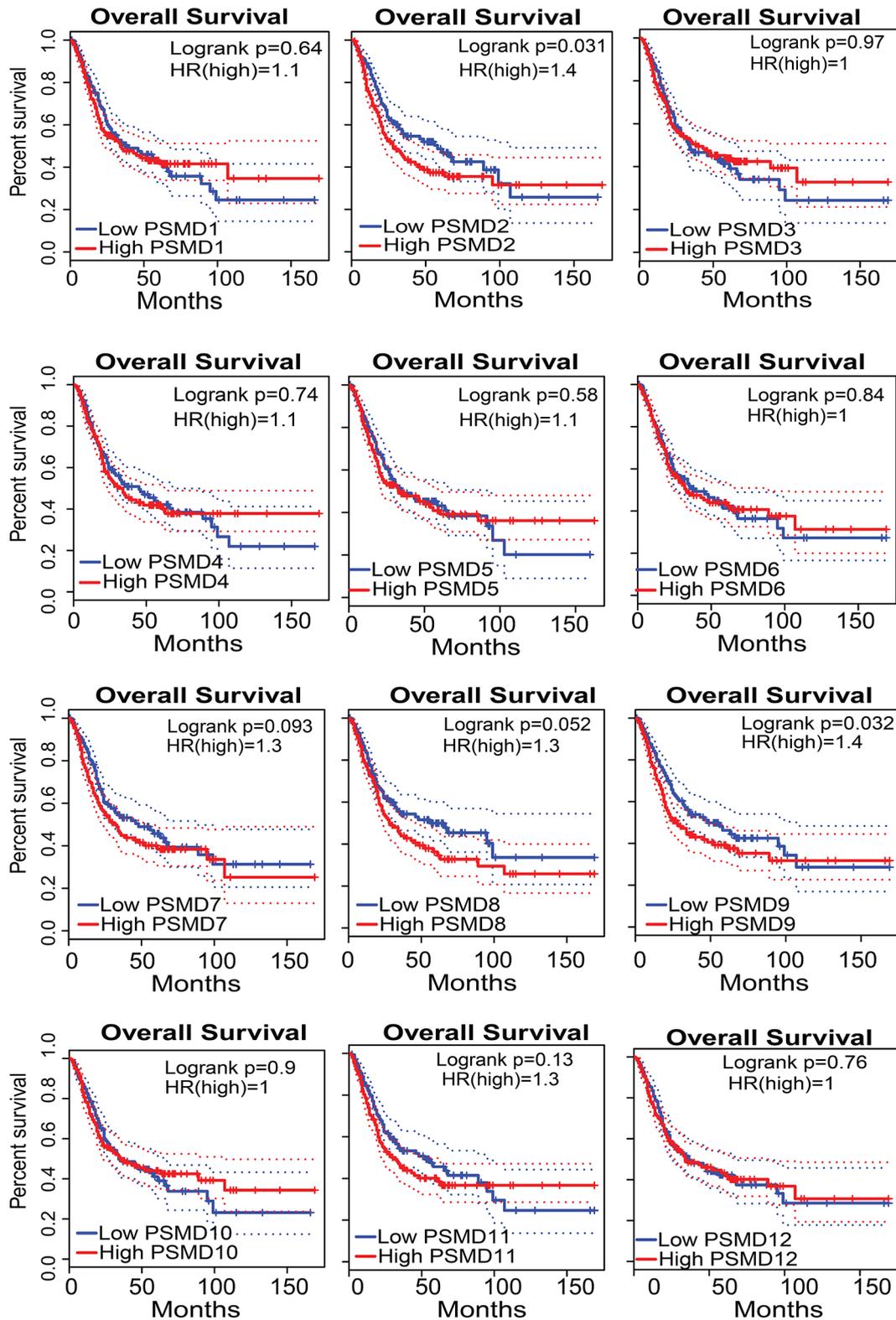


Figure 3. Overall survival curves of BLCA patients. The Kaplan-Meier curves show the comparison in overall survival between PSMD genes expression at mRNA level based on each gene's mean expression, expression above the mean is considered high, and expression below the mean is considered low. PSMD1, PSMD2, PSMD3, PSMD4, PSMD5, PSMD6, PSMD7, PSMD8, PSMD9, PSMD10, PSMD11, PSMD12. PSMD, proteasome 26S subunit, non-ATPase. The statistical method for survival analysis was log rank test. $P < .05$ was considered to be significant.

MIBC and progression MIBC. Based on the microarray results, we found that PSMD2 was high in de novo MIBC compared with non-muscle invasive bladder cancer who

progressed to MIBC (Figure 4C), no significant results were found in PSMD8, PSMD4 or PSMD11 between the 2 groups.

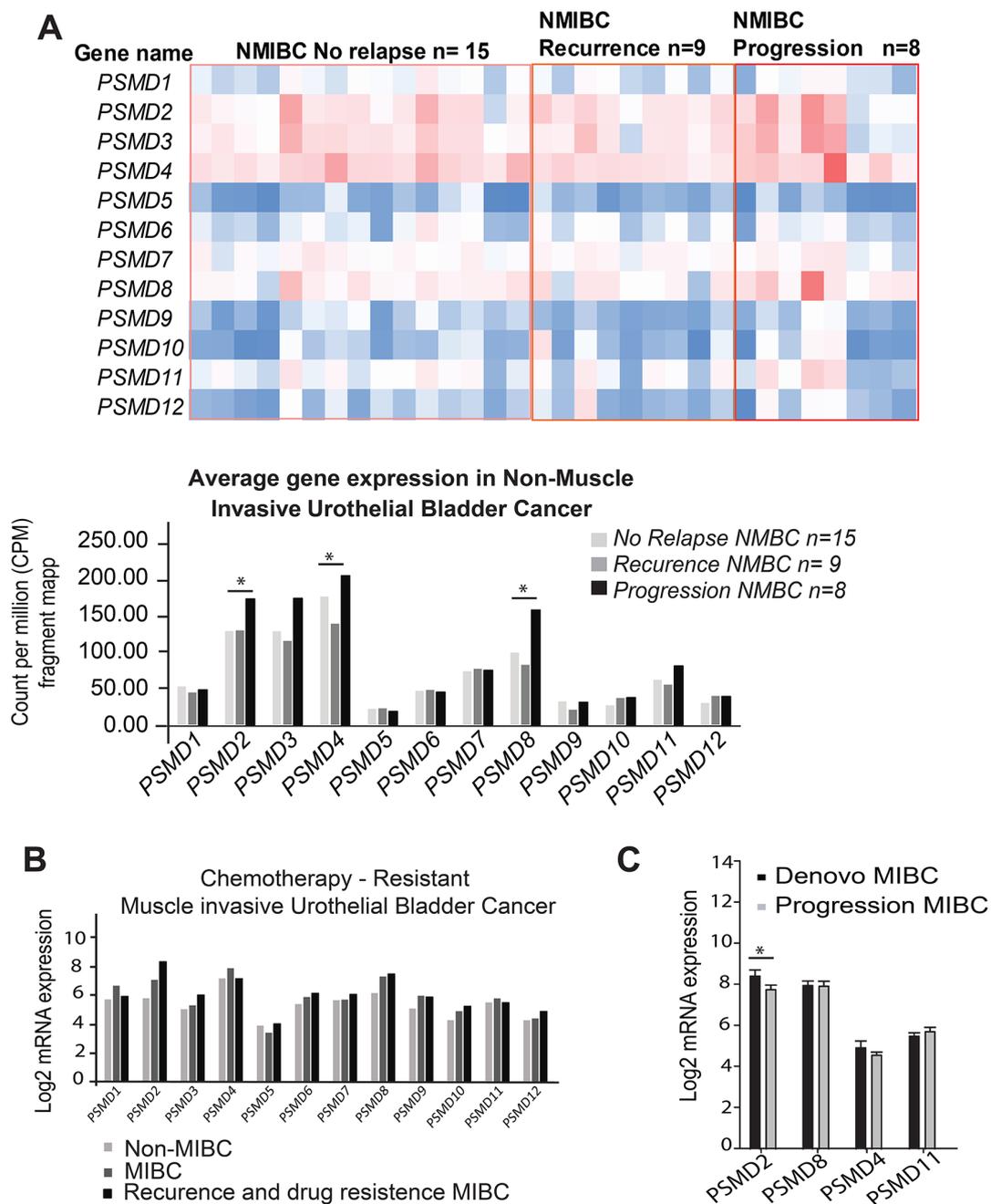


Figure 4. Prognostic role of PSMD genes in NMIBC and MIBC. (A) Heatmap analysis of PSMD genes in non- muscle invasive bladder cancer (NMIBC); no relapse group 1 (n=15), recurrence group 2 (n=9), and progression group 3 (n=8). The data were downloaded from GEO dataset (GSE163899). (B) Differential expression of PSMD genes in a patient at different time interval; T1 patient at diagnosis with NMIBC, T2 patient after developing MIBC and at recurrence and metastasis during chemotherapy (gemcitabine/paclitaxel and sequential paclitaxel) the data was downloaded from GEO (GSE145137) dataset. (C) PSMD gene expression signature between de novo MIBC and progressive MIBC n=12 de novo and n=14 progressive MIBC. The data was downloaded from GEO dataset was (GSE149582). Student *t* test has been used for statistical analysis. $P < .05$ was considered to be significant.

Oncomine database for PSMD2 and PSMD8 analysis

Using several bladder cancer datasets in Oncomine database, we analyzed the expression level of PSMD2 and PSMD8 in several bladder carcinoma subtypes such as; infiltrating bladder urothelial carcinoma and superficial bladder cancer. As shown in (Figure 5A), PSMD2 and PSMD8 mRNA expression were high in most of the study cohorts in both subtypes; infiltrating bladder urothelial carcinoma and superficial bladder cancer.

These results indicated that PSMD2 and PSMD8 could be associated with muscle invasive bladder carcinoma.

Association of PSMD2 and PSMD8 with BLCA clinicopathological features

Next, the correlation between PSMD2, PSMD8 and bladder cancer clinicopathological features has been analyzed from BLCA-TCGA records (Table 1). High level of PSMD2 was significantly associated with older age ($P < .001$), female

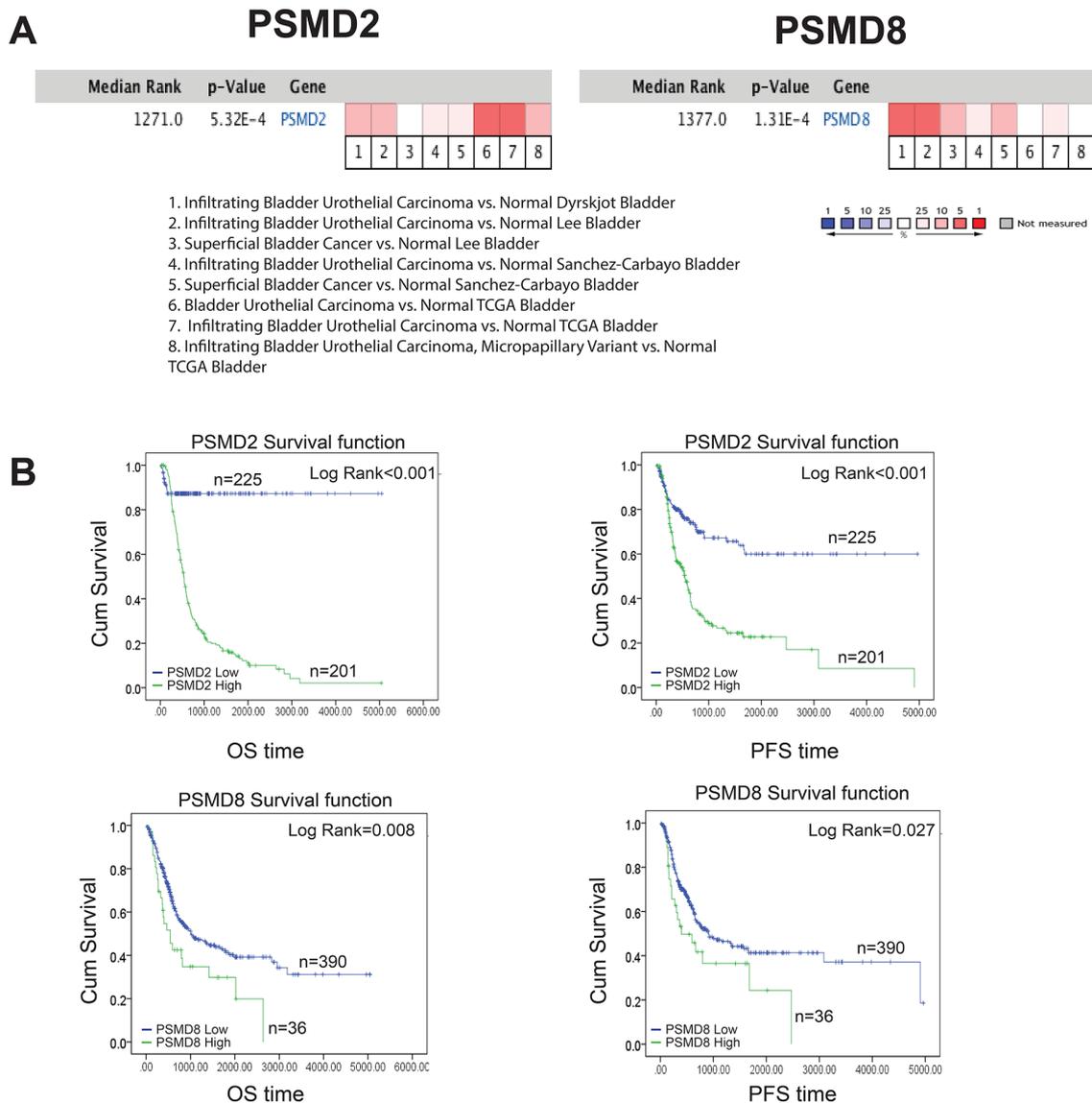


Figure 5. (A) PSMD2 and PSMD8 expression in different study cohort in non-invasive and invasive bladder urothelial carcinoma using Oncomine database. (B) Survival curves overall survival (OS) and progression free survival (PFS) for PSMD2 and PSMD8 in bladder carcinoma using TCGA database. The statistical method for survival analysis was log rank test. $P < .05$ was considered to be significant.

gender ($P = .014$), tumor grade ($P < .001$), and metastasis ($P = .003$). No significant association had been found with clinical stage or lymph node metastasis or Lymphovascular invasion. On the other hand, high level of PSMD8 was significantly associated with older age at initial diagnosis ($P < .01$) and with tumor extent ($P = .025$), no clear correlations have been found with other clinical parameters.

Univariate survival analysis showed that PSMD2, PSMD8, and tumor grade were predictors for overall survival; PSMD2 hazard ratio (HR) = 7.689, 95% confident interval (CI) = (5.143-11.494) ($P < .001$). PSMD8 HR = 1.746, 95% CI = (1.147-2.659) ($P = .009$). Tumor grade, HR = 7.292, 95% CI = (1.21-52.078) ($P = .048$). However, Multivariate analysis showed that only PSMD2 was predictive for OS in BLCA patients, PSMD2 HR = 8.041, 95% confident interval CI = (4.715-13.713) ($P < .001$) (Table 2).

Consistent with survival curves from GEPIA websites, Kaplan Meier survival analysis from TCGA database showed that high level of PSMD2 and PSMD8 had worse overall survival (OS) and Progression Free Interval (PFI) (Figure 5B). These results clearly indicated that PSMD2 and PSMD8 are important predictors for Clinical consequences in BLCA and might have critical roles for BLCA tumorigenesis.

Discussion

The high BLCA recurrence rate and requirement for invasive diagnostic and tracking techniques, along with cystoscopy, makes bladder urothelial carcinoma one of the costliest human cancers from early evaluation to death. While the 2 main techniques; cystoscopy and urine cytology are considered as the gold standard for preliminary BLCA diagnosis and monitoring, there is still a need to discover novel, non-invasive specific

Table 1. Correlation between PSMD2 and PSMD8 mRNA level and clinicopathological parameters of bladder urothelial carcinoma.

PARAMETERS	PSMD2 LOW (N)	PSMD2 HIGH (N)	P-VALUE	PSMD8 LOW (N)	PSMD8 HIGH (N)	P-VALUE
Gender						
Female	51	67	.014*	110	8	.443
Male	174	134		280	28	
Age						
<60	106	0	<.001**	105	1	.01**
>61	119	201		285	35	
Tumor extent						
T1	2	2	.050*	4	0	.025*
T2	71	53		32	92	
T3	94	110		68	136	
T4	40	22		18	44	
Grade						
1	20	203	<.001**	10	11	.101
2	1	199		123	279	
Node						
N0	123	115	.247	81	166	.454
N1	21	28		11	38	
N2	41	39		24	56	
N3	28				16	.28
Stage						
			16			
I	2	0	.229	2	0	.143
II	78	56		41	93	
III	72	74		49	97	
IV	72	72		40	102	
Metastasis						
No	126	80	.003*	74	132	.163
Yes	98	119		59	158	
Lymphovascular invasion						
No	63	69	.515	121	11	.936
Yes	83	78		148	13	

Chi-square test was used to analyze the correlation between PSMD2 or PSMD8 with BLCA clinicopathological features. High; expression above the mean, and Low; expression below the mean. * $P < .05$, ** $P < .001$. The bold is indicated for the significant results.

biomarkers for the better diagnosis and prediction for tissues after cystectomy or transurethral resection (TURBT).

PSMD genes have been implicated in several types of malignancies. Recently, we identified PSMD3, a 19S regulatory subunit, as a prognostic and therapeutic marker for HER2 positive breast cancer.²⁰ A new recent study had been recognized several

proteasomal subunits as prognostic biomarkers for pancreatic ductal adenocarcinoma.²⁵ To the best of our knowledge, the current study is the first for analyzing the expression level of proteasomal subunit genes in bladder urothelial carcinoma.

In the current study, we used several bioinformatics websites to investigate the expression level of PSMD genes in bladder

Table 2. Univariate and multivariate analysis of OS in TCGA-BLCA patients according to PSMD2 and PSMD8 mRNA level.

PARAMETERS	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS	
	P-VALUE	HR RATIO (95% CL)	P-VALUE	HR RATIO (95% CL)
PSMD2 (high vs low)	<.001**	7.689 (5.143-11.494)	<.001**	8.041 (4.715-13.713)
PSMD8 (high vs low)	.009*	1.746 (1.147-2.659)	.681	1.118 (0.657-1.900)
Stage (III and IV vs I and II)	.580	1.093 (0.797-1.500)	.383	1.490 (0.609-3.646)
Tumor extent (I and II vs III and IV)	.920	1.017 (0.734-1.408)	.568	0.788 (0.348-1.784)
Grade (high vs low)	.048*	7.292 (1.21-52.078)	.482	2.042 (0.279-14.930)
Node (Positive vs negative)	.289	1.073 (0.942-1.221)	.992	1.001 (0.841-1.191)
Lymphovascular invasion	.130	0.774 (0.555-1.078)	.109	0.748 (0.524-1.067)
Metastasis (yes vs no)	.896	1.000 (0.999-1.001)	.720	1.000 (0.999-1.001)

CI, confidence interval; Cox regression analysis, hazard ratio (95% confidence interval). High; expression above the mean, and Low; expression below the mean. * $P < .05$, ** $P < .001$. The bold is indicated for the significant results.

urothelial carcinoma such as GEPIA, Oncomine, TCGA, and GEO databases. The mRNA expression levels for PSMD genes were obtained from several cohort studies which were performed based on RNA seq analysis. Most of PSMD genes were upregulated in BLCA in contrast to regular normal tissues, notably PSMD2 and PSMD8 were the most expressed genes in tumor tissues. Interestingly, several studies demonstrated the upregulation of PSMD2 in solid tumors such as lung adenocarcinoma, breast cancer, gastric cancer and in hepatocellular carcinoma cell line, HepG2 cell line^{21,22,26,27} which indicated the important role of PSMD2 in tumorigenesis. However, the exact role of PSMD8 in cancer is still elusive. We found a lower level of PSMD5 in tumor tissues compared to normal bladder cells, which is consistent with a prior study that found PSMD5 mRNA and protein levels were downregulated in colorectal tumors. PSMD5 silencing promotes the formation of 26S proteasomes. PSMD5 interaction with proteasome subunits is, in other words, transitory.²⁸

Based on both GEPIA and TCGA databases, high level of PSMD2 and PSMD8 predicted shorter overall survival and progression free survival, which indicated the unfavorable prognostic role of PSMD2 and PSMD8 in BLCA. High level of PSMD2 was also associated with age at diagnosis, gender, grade, and metastasis status. PSMD2 has been shown to be an independent predictor for OS in BLCA patients based on univariate and multivariate analysis ($P < .001$).

When the PSMD2 mRNA level was high, we found that 32% of the patients were male and only 16% of the patients were female. There was no substantial difference in the relationship between PSMD8 and gender. It has also long been known that BLCA incidence is approximately 4 times higher in males than in females in the US.²⁹ In accordance with the gender disparity in BLCA risk, the mortality of this sickness inside the US is likewise greater than 3-fold higher in men than in women.³⁰ Furthermore, high level of PSMD2 was

associated with patients elder than 60 years' old which account for around 47% of the patients. Further studies are recommended to test the usefulness of PSMD2 as a biomarker to diagnosis BLCA in men and for people exceed 60 years old.

Consistently, PSMD2 was previously examined in breast cancer included in 797 ubiquitin proteasomal system (UPS) genes. PSMD2, was identified as oncogene and high level of PSMD2 had predicted worse overall survival. PSMD2 was also shown to be associated with age, lymph node metastasis and TNM stage.²¹ A genome-wide association study inside a highly metastatic lung cancer cell line identified genes related to UPS including PSMD2 as a metastatic signature gene that was associated with worse prognosis in lung and breast cancers.³¹

PSMD2 has been shown to be involved in cell cycle and to increase cell proliferation, loss of PSMD2 function leads to the inhibition of cell proliferation in BC and HepG2 cell lines.²² However, it is still not clear based on in vitro or in vivo if there is a role of PSMD2 in metastasis.

Most bladder cancers are non-muscle invasive at initial diagnosis. However, they are characterized by high recurrence rate reaching around 75%³² and around 25% in MIBC. MIBC is highly aggressive and the 5-years overall survival is less than 15% in patients with no treatment history. In Spite of its favorable survival, NMIBC recurs frequently and may finally develop to muscle-invasive bladder cancer (MIBC), which requires repeated cystoscopic resection or may require radial cystectomy.³³ Therefore, identifying novel prognostic biomarkers can be the initial step for accurate prognosis prediction and treatment. This study showed that PSMD2 and PSMD8 are low expressed in NMIBC. However, they seem to be associated with progressive NMIBC but not recurrence. Moreover, PSMD2 and PSMD8 showed high level of expression in MIBC and recurrent MIBC in association with lung metastasis and chemotherapy resistance based on GEO study GSE149582³⁴ in comparison with the non-MIBC.

These data could provide a novel prognostic differences between NMIBC and MIBC, further studies are needed to validate the role of PSMD2 or/and PSMD8 in progression steps from NMIBC to MIBC or in de novo MIBC. This research is of an investigative character, we used the RNA-Seq versus Microarray datasets to validate the expression and prognostic values of PSMD2 and PSMD8 in NMIBC and MIBC. In order to translate these finding to clinical settings, PSMD2 expression level and function in vitro, in vivo and in large cohort samples from BLCA patients are recommended.

Conclusion

We found that high level of PSMD2 and PSMD8 associated with worse overall survival and progression free survival, which indicated the unfavorable prognostic role in patients with BLCA. Furthermore, high level of PSMD2 was correlated with age at diagnosis, gender, grade, and metastasis status. PSMD2 found to be an independent predictor for OS in BLCA patients. Interestingly, the mRNA level of PSMD2 and PSMD8 was detected at high level in muscle invasive bladder cancer patients and low level in non-invasive bladder cancer patients, suggesting that they may play a role in disease development.

Author Contributions

Data collection, data analysis, results interpretations, paper writing, AF. Data collection and results interpretations, AA. Data collection and paper writing, MAS. Data collection and paper writing, RAQ.

ORCID iD

AbdulFattah Salah Fararjeh  <https://orcid.org/0000-0002-2850-7001>

REFERENCES

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68:394-424.
- Janisch F, Shariat SF, Schernhammer E, Rink M, Fajkovic H. The interaction of gender and smoking on bladder cancer risks. *Curr Opin Urol.* 2019;29:249-255.
- Awan KH, Siddiqi K, Patil SH, Hussain QA. Assessing the effect of waterpipe smoking on cancer outcome - a systematic review of current evidence. *Asian Pac J Cancer Prev.* 2017;18:495-502.
- Cumberbatch MGK, Jubber I, Black PC, et al. Epidemiology of bladder cancer: a systematic review and contemporary update of risk factors in 2018. *Eur Urol.* 2018;74:784-795.
- Fonteyne V, Ost P, Bellmunt J, et al. Curative treatment for muscle invasive bladder cancer in elderly patients: a systematic review. *Eur Urol.* 2018;73:40-50.
- Lodewijk I, Dueñas M, Rubio C, et al. Liquid biopsy biomarkers in bladder cancer: a current need for patient diagnosis and monitoring. *Int J Mol Sci.* 2018;19:2514.
- Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. *Nat Rev Cancer.* 2015;15:25-41.
- Kamoun A, de Reynies A, Allory Y, et al. A consensus molecular classification of muscle-invasive bladder cancer. *Eur Urol.* 2020;77:420-433.
- Sanli O, Dobruch J, Knowles MA, et al. Bladder cancer. *Nat Rev Dis Primers.* 2017;3:17022.
- Şanlı Ö, Lotan Y. Alternative therapies in patients with non-muscle invasive bladder cancer. *Turk J Urol.* 2017;43:414-424.
- Minoli M, Kiener M, Thalmann GN, Kruihof-de Julio M, Seiler R. Evolution of urothelial bladder cancer in the context of molecular classifications. *Int J Mol Sci.* 2020;21:5670.
- Del Bene G, Calabrò F, Giannarelli D, et al. Neoadjuvant vs. Adjuvant chemotherapy in muscle invasive bladder cancer (MIBC): analysis from the RISC database. *Front Oncol.* 2018;8:463.
- Kamat AM, Karashima T, Davis DW, et al. The proteasome inhibitor bortezomib synergizes with gemcitabine to block the growth of human 253JB-V bladder tumors in vivo. *Mol Cancer Ther.* 2004;3:279-290.
- Voutsadakis IA. Ubiquitination and the ubiquitin-proteasome system as regulators of transcription and transcription factors in epithelial mesenchymal transition of cancer. *Tumour Biol.* 2012;33:897-910.
- Voutsadakis IA. Proteasome expression and activity in cancer and cancer stem cells. *Tumour Biol.* 2017;39:1010428317692248.
- Gui Y, Guo G, Huang Y, et al. Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. *Nat Genet.* 2011;43:875-878.
- Mukhopadhyay D, Riezman H. Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science.* 2007;315:201-205.
- Sadanandom A, Bailey M, Ewan R, Lee J, Nelis S. The ubiquitin-proteasome system: central modifier of plant signalling. *New Phytol.* 2012;196:13-28.
- Golonko A, Pienkowski T, Swislocka R, Lazny R, Roszko M, Lewandowski W. Another look at phenolic compounds in cancer therapy the effect of polyphenols on ubiquitin-proteasome system. *Eur J Med Chem.* 2019;167:291-311.
- Fararjeh AS, Chen LC, Ho YS, et al. Proteasome 26S subunit, non-ATPase 3 (PSMD3) regulates breast cancer by stabilizing HER2 from degradation. *Cancers.* 2019;11:527.
- Li Y, Huang J, Zeng B, et al. PSMD2 regulates breast cancer cell proliferation and cell cycle progression by modulating p21 and p27 proteasomal degradation. *Cancer Lett.* 2018;430:109-122.
- Tan Y, Jin Y, Wu X, Ren Z. PSMD1 and PSMD2 regulate HepG2 cell proliferation and apoptosis via modulating cellular lipid droplet metabolism. *BMC Mol Biol.* 2019;20:24.
- Fejzo MS, Anderson L, Chen HW, et al. Proteasome ubiquitin receptor PSMD4 is an amplification target in breast cancer and may predict sensitivity to PARPi. *Genes Chromosomes Cancer.* 2017;56:589-597.
- Lin PL, Chang JT, Wu DW, Huang CC, Lee H. Corrigendum to "Cytoplasmic localization of Nrf2 promotes colorectal cancer with more aggressive tumors via upregulation of PSMD4" [Free Radic. Biol. Med. 95 (2016) 121-132]. *Free Radic Biol Med.* 2017;104:380-381.
- Zhou C, Li H, Han X, et al. Prognostic value and molecular mechanisms of proteasome 26S subunit, non-ATPase family genes for pancreatic ductal adenocarcinoma patients after pancreaticoduodenectomy. *J Invest Surg.* 2021;34:1-17.
- Matsuyama Y, Suzuki M, Arima C, et al. Proteasomal non-catalytic subunit PSMD2 as a potential therapeutic target in association with various clinicopathologic features in lung adenocarcinomas. *Mol Carcinog.* 2011;50:301-309.
- Zhang Z, Li H, Zhao Y, et al. Asporin promotes cell proliferation via interacting with PSMD2 in gastric cancer. *Front Biosci.* 2019;24:1178-1189.
- Levin A, Minis A, Lalazar G, Rodriguez J, Steller H. PSMD5 inactivation promotes 26S proteasome assembly during colorectal tumor progression. *Cancer Res.* 2018;78:3458-3468.
- Cook MB, Dawsey SM, Freedman ND, et al. Sex disparities in cancer incidence by period and age. *Cancer Epidemiol Biomarkers Prev.* 2009;18:1174-1182.
- Cook MB, McGlynn KA, Devesa SS, Freedman ND, Anderson WF. Sex disparities in cancer mortality and survival. *Cancer Epidemiol Biomarkers Prev.* 2011;20:1629-1637.
- Tomida S, Yanagisawa K, Koshikawa K, et al. Identification of a metastasis signature and the DLX4 homeobox protein as a regulator of metastasis by combined transcriptome approach. *Oncogene.* 2007;26:4600-4608.
- Sylvester RJ, van der Meijden AP, Oosterlinck W, et al. Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. *Eur Urol.* 2006;49:466-475; discussion 475-477.
- Babjuk M, Burger M, Compérat EM, et al. European Association of Urology Guidelines on non-muscle-invasive bladder cancer (TaT1 and carcinoma in situ) - 2019 update. *Eur Urol.* 2019;76:639-657.
- Carrasco R, Izquierdo L, van der Heijden AG, et al. Differential gene expression profile between progressive and de novo muscle invasive bladder cancer and its prognostic implication. *Sci Rep.* 2021;11:6132.