

Upregulation of lncRNA plasmacytoma variant translocation 1 predicts poor prognosis in patients with muscle-invasive bladder cancer

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Abstract

lncRNA plasmacytoma variant translocation 1 (PVT1) has been recognized as an oncogenic lncRNA, which participates in the migration and invasion of many kinds of cancer cells and the development of cancers. In the present study, we explored its clinical significance and prognostic value in muscle invasive bladder cancer (MIBC).

A total of 98 MIBC patients' samples were collected, who had undergone radical cystectomy from the March 2013 to December 2018. The associations between PVT1 expression and clinical data were calculated using the Chi²-test. Overall survival curves were determined by the Kaplan–Meier technique and contrasted via log-rank test. We utilized univariate and multivariate Cox proportional hazard models to examine the HR and 95% CI.

The expression levels of PVT1 were significantly higher in MIBC tissues than that in normal bladder tissues ($P < .001$). PVT1 expression was significantly correlated with tumor grade ($P = .009$), margin ($P = .002$), T stage ($P = .02$), and lymph node metastasis ($P < .001$). MIBC patients with high PVT1 expression level had shorter overall survival than those with low PVT1 expression level (log-rank test, $P = .004$). Multivariate Cox regression analysis showed that PVT1 expression level (HR = 2.381, 95% CI: 1.821–7.012, $P = .014$) was an independent factor in predicting the overall survival of MIBC patients.

In summary, increased PVT1 expression in MIBC patients is correlated with a higher MIBC stage and is significantly associated with poor prognosis for MIBC patients, which may provide new insights into new therapeutic strategy and postoperative intervention against bladder cancer.

Abbreviations: EMT = epithelial-mesenchymal transition, lncRNA = long noncoding RNAs, MIBC = muscle invasive bladder cancer, PVT1 = lncRNA plasmacytoma variant translocation 1, UICC = Union for International Cancer Control.

Keywords: bladder cancer, long noncoding RNAs, muscle invasive bladder cancer, prognosis, lncRNA plasmacytoma variant translocation 1

1. Introduction

Bladder cancer ranks 9th in cancer incidence and 13th in cancer mortality worldwide.^[1,2] It is the most common urogenital malignant tumor, and is classified into 2 distinct categories: non-muscle invasive bladder cancer (NMIBC) and muscle invasive

bladder cancer (MIBC) according to the 8th edition of the TNM classification published by the Union for International Cancer Control (UICC).^[3,4] Despite numerous advances in surgery, and adjuvant or neoadjuvant chemoradiotherapy, the recurrence rate of bladder cancer is still high and the prognosis is still poor. Approximately 30% of patients are diagnosed with MIBC at the time of their initial presentation, and they have a less favorable prognosis with a 5-year survival rate of <50%.^[5,6] Therefore, identifying novel prognostic biomarkers is urgently required to guide adjuvant therapy after surgery and to improve clinical outcome of patients with bladder cancer.

Long noncoding RNAs (lncRNAs) are a group of noncoding RNAs longer than 200 nucleotides which participate in numerous biological and physiological processes, including cell development, survival, invasion, metastasis, differentiation, and apoptosis.^[7,8] Accumulating evidence has revealed that aberrantly expressed lncRNAs plays crucial roles in cancer occurrence, development, and metastasis.^[9,10]

lncRNAs plasmacytoma variant translocation 1 (PVT1), which located in the 8q24 chromosomal region, is an important carcinogenic lncRNA.^[11] PVT1 can promote the progression of various types of cancer, including prostate cancer, lung cancer, glioma, hepatocellular carcinoma (HCC), cervical cancer, esophageal adenocarcinoma, nasopharyngeal carcinoma, ovarian cancer, colorectal cancer (CRC), and renal cell carcinoma (RCC).^[12–23] The role of PVT1 in bladder cancer has also been investigated. Yu et al^[24] found that PVT1 levels were higher in bladder cancer tissues and PVT1 regulated VEGFC through

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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inhibiting miR-128 in bladder cancer cells. Tian et al^[25] found that PVT1 was over-expressed in bladder cancer cells, and it down-regulated miR-31 to enhance CDK1 expression and facilitated bladder cancer cells proliferation, migration, and invasion. In the present study, we examined the lncRNA PVT1 expression levels of bladder cancer and adjacent normal bladder tissues and explored its clinical significance and prognostic value in bladder cancer.

2. Materials and methods

A total of 98 MIBC patients' samples were collected, who had undergone radical cystectomy in the Department of Urology, Affiliated Hospital of Qingdao University, Qingdao, from the March 2013 to December 2018. Participants were chosen based on the inclusion criteria: patients were at least 18 years of age; there was a verified histopathological diagnosis (muscle-invasive disease, pT2-T4, N0-N3, M0), and complete follow-up data available. Exclusion criteria: bladder cancer with distant metastasis; patients with non-muscular invasive bladder cancer; patient underwent neoadjuvant chemotherapy before surgery. All the patients included in this study were underwent laparoscopic or open radical cystectomy and standard bilateral lymphadenectomy. Tumor staging was performed according to the standard TNM staging guidelines of the UICC and tumor grading was performed according to the 2004 World Health Organization (WHO) grading system. Tumor histology was reviewed by 2 urologists. The present study was approved by the Affiliated Hospital of Qingdao University. The clinical research informed consent was signed by each patient prior to surgery. Patient characteristics are shown in Table 1.

2.1. RNA isolation and qRT-PCR

Total RNA was extracted using Trizol (Ambion, Austin, TX) according to manufacturer's instructions. RNA was reverse-

transcribed into complementary DNA (cDNA) by using a Promega M-MLV Kit. Quantitative real-time PCR (qPCR) was performed using SYBR Select Master Mix (Applied Biosystems, CA) on the Applied Biosystems 7500 Fast platform (Applied Biosystems). The PCR conditions were as follows: 30 seconds at 95°C, followed by 40 cycles of 10 seconds at 95°C, and 30 seconds at 60°C.

The relative expression was normalized using GAPDH as an internal reference gene. Fold changes were calculated using the formula $2^{-\Delta\Delta C_t}$. All qRT-PCR reactions were performed 3 times independently. The primer sequences used for qRT-PCR as follows: PVT1, forward 5'-AAAACGGCAGCAGGAAATGT-3', and reverse 5'-GGAGTCATGGGTGTCAGACA-3'; GAPDH, forward 5'-AGAAGGCTGGGGCTCATTTG-3', and reverse 5'-AGGGCCATCCACAGTCTTC-3-3'.

2.2. Statistical analysis

Student's *t* test was used to compare differences between two groups. The associations between the PVT1 expression and clinical data were calculated using the Chi²-test. Overall survival curves were determined by the Kaplan–Meier technique and contrasted via log-rank test. We utilized univariate and multivariate Cox proportional hazard models to examine the HR and 95% CI. Statistical analyses were performed with the SPSS 21.0 software package (SPSS Inc., Chicago, IL) and GraphPad Prism 7 software (GraphPad Software, Inc.). All *P* values were two-sided with *P* values < .05 indicating statistical significance.

3. Results

3.1. The expression of PVT1 in the MIBC tissues and the adjacent normal bladder tissues

We profiled PVT1 expression in the 98 pairs of MIBC tissues and normal bladder tissues by qRT-PCR. The expression levels of PVT1 were significantly higher in MIBC tissues than that in normal bladder tissues (*P* < .001, shown in Fig. 1). The median PVT1 expression level of all MIBC tissues was utilized to divide MIBC patients into 2 groups. Fifty patients were assigned to the high-expression group, and the remaining 48 patients were assigned to the low-expression group.

3.2. Correlation between PVT1 expression and clinicopathological variables of patients with MIBC

We then investigated the correlation of PVT1 expression with clinicopathological variables of the MIBC patients. As shown in Table 1, PVT1 expression was correlated with tumor grade (*P* = .009), margin (*P* = .002), T stage (*P* = .02), and lymph node metastasis (*P* < .001). However, PVT1 expression was not significantly associated with other clinicopathological factors of patients, including age, sex, and type of urinary diversion (all *P* > .05).

3.3. PVT1 up-regulation associates with poor prognosis of MIBC patients

To evaluate the prognostic value of PVT1 expression in MIBC, survival curves were constructed by Kaplan–Meier method and compared by the log-rank test. As shown in Figure 2, MIBC patients with high PVT1 expression level had shorter overall survival than those with low PVT1 expression level (log-rank test,

Table 1

Association of lncRNA PVT1 expression with clinicopathological features of muscle-invasive bladder cancer.

Variables	Cases (n)	lncRNA PVT1 expression		<i>P</i> value
		High (n=50)	Low (n=48)	
Age, y				
<60	31	14	17	.516
≥60	67	36	31	
Gender				
Male	62	34	28	.403
Female	36	16	20	
Type of urinary diversion				
Ileal conduct	67	33	34	.667
Ureterocutaneostomy	31	17	14	
Margin				
Positive	22	18	4	.002
Negative	76	32	44	
Tumor grade				
Low	52	15	37	.009
High	46	35	11	
T stage				
T2/T3	73	32	41	.02
T4	25	18	7	
Lymph node metastasis				
N0/N1	27	3	24	<.001
N2/N3	71	47	24	

lncRNA = long noncoding RNAs; PVT1 = lncRNA plasmacytoma variant translocation 1.

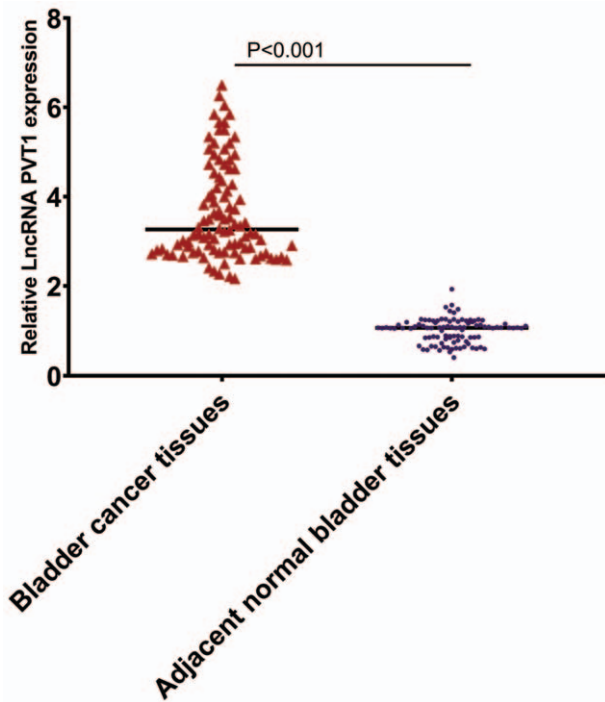


Figure 1. The expression levels of PVT1 were significantly higher in MIBC tissues than that in normal bladder tissues ($P < .001$). MIBC=muscle invasive bladder cancer; PVT1=lncRNA plasmacytoma variant translocation 1.

$P = .004$). To determine the possibility of PVT1 as an independent risk factor for poor prognosis, both clinicopathological factors and the level of PVT1 expression were evaluated by multivariate

Table 2

Cox regression model for multivariate analysis of prognostic factors for muscle-invasive bladder cancer.

Variable	HR	95% CI	P-value
Age	1.283	0.883–3.197	.101
Gender	0.829	0.627–2.191	.338
Type of urinary diversion	0.772	0.283–1.283	.192
Margin	2.102	1.372–7.293	.041
Tumor grade	2.995	1.846–9.286	.009
T stage	1.921	1.002–5.192	.039
Lymph node metastasis	2.611	1.472–10.539	.008
lncRNA PVT1 expression level	2.381	1.821–7.012	.014

CI=confidence interval; HR=hazard ratio; lncRNA=long noncoding RNAs; PVT1=lncRNA plasmacytoma variant translocation 1.

Cox regression analysis. Results showed that PVT1 expression level (HR=2.381, 95% CI: 1.821–7.012, $P = .014$) was an independent factor in predicting the overall survival of MIBC patients (shown in Table 2).

4. Discussion

The main clinical feature of bladder cancer is easy to recurrence after transurethral resection of bladder tumors (TURBTs) or partial cystectomy.^[26] About 40% of the patients with bladder cancer experience multiple recurrences, which has a significant impact on their life quality and survival. Postoperative bladder instillation chemotherapy or BCG instillation is the most important means to prevent recurrence of bladder cancer after surgery.^[6,27] Current prognostic strategies, such as tumor grade, stage, size, and number of foci, have restricted utility for

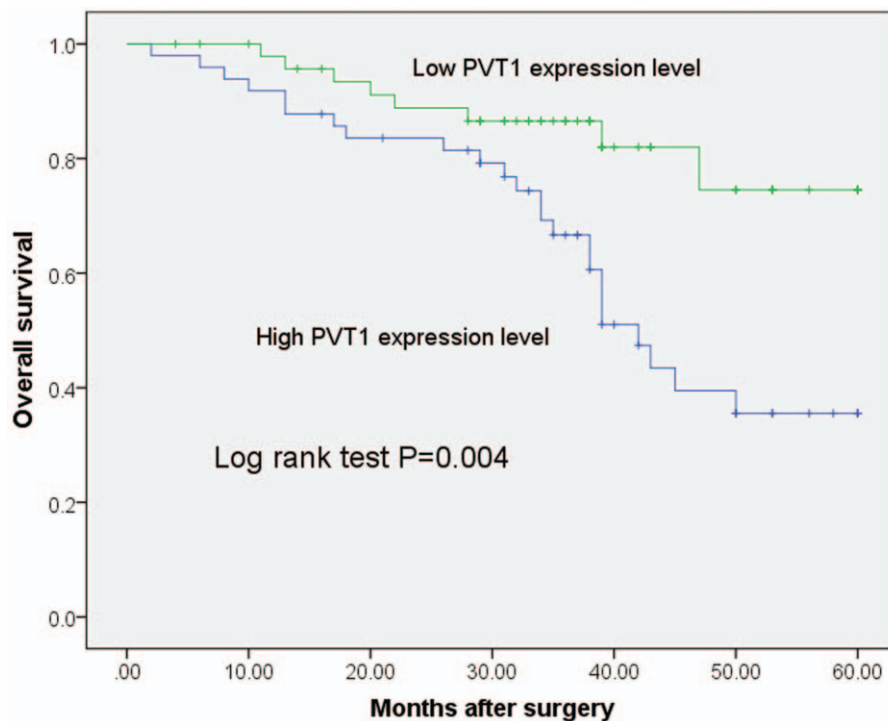


Figure 2. MIBC patients with high PVT1 expression level had shorter overall survival than those with low PVT1 expression level (log-rank test, $P = .004$). MIBC = muscle invasive bladder cancer; PVT1=lncRNA plasmacytoma variant translocation 1.

clinicians, given that they do not specifically reflect the clinical outcomes of bladder cancer patients.^[3,28] Therefore, novel prognostic markers and effective treatment strategies are essential to improve outcomes for patients with bladder cancer.

Long noncoding RNAs play an important role in various biological and pathological processes frequently implicated in cancer, including proliferation, apoptosis, cell cycle progression, migration, and invasion.^[8,29] PVT1 is one of these lncRNAs which promote the pathophysiology of various cancers. For example, Ding et al^[18] showed that PVT1 was the most amplified gene in ovarian cancer patients, and it was highly correlated with poor survival outcomes. Knockdown of PVT1 caused decreased cell viability, metabolic activity, and smaller proportion of S-phase cells. PVT1 directly bound to miR-140 and acted as a microRNA sponge, while transcription of PVT1 was regulated by the transcription factor FOXO4. In conclusion, viability, metabolism, and cell cycle of ovarian cancer are regulated by the FOXO4/PVT1/miR-140 signaling pathway. Chang et al^[15] found that PVT1 was upregulated in cervical cancer cells. PVT1 could bind directly with miR-140-5p, and Smad3 was a downstream target of miR-140-5p. Inhibition of PVT1 could enhance expression of miR-140-5p, inhibit the expression of Smad3, significantly inhibit the proliferation, migration, invasion in cervical cancer cells, revealing that PVT1 could promote the proliferation and metastasis via increasing the Smad3 expression by sponging miR-140-5p, which might be a promising prognostic and therapeutic target for cervical cancer. Xu et al^[16] found that PVT1 expression was up-regulated in esophageal adenocarcinoma (EAC) compared with paired Barrett esophagus (BE), and normal esophageal tissues. High expression of PVT1 was associated with poor differentiation, lymph node metastases, and shorter survival. Effective knockdown of PVT1 in EAC cells resulted in decreased cell proliferation, invasion, colony formation, tumor sphere formation, and reduced proportion of ALDH1A1 cells. Lv et al^[13] found that PVT1 was upregulated in glioma tissues relative to controls. Its level was higher in glioma patients with advanced stage or accompanied by metastasis. The glioma patients with a high level of PVT1 suffered a worse prognosis. The overexpression of PVT1 accelerated proliferative and migratory abilities of U87 and LN229 cells. Furthermore, PVT1 accelerates the proliferative and migratory abilities of glioma via downregulating UPF1. Yang et al^[22] found that PVT1 levels were markedly up-regulated in the corresponding HCC tissues. Importantly, they found that PVT1 could facilitate cell autophagy in HCC cells. Then, they confirmed that the effect of PVT1 promoting autophagy was dependent on regulating ATG3 expression. Ren et al^[20] found that PVT1 was upregulated in RCC tissues compared with the adjacent normal tissues. PVT1 expression was closely correlated with TNM stage, Fuhrman grade, lymph node metastasis, and tumor size. Kaplan–Meier analysis revealed that high expression of PVT1 was significantly associated with poor overall survival. In accordance, overexpression of PVT1 was observed in RCC cells comto HK-2 cell. Silencing of PVT1 significantly repressed cell viability, induced apoptosis, and inhibited cell migration and invasion in vitro. Furthermore, bioinformatic analysis and luciferase reporter assay confirmed that miR-16-5p was a target of PVT1. Silencing of miR-16-5p mostly reversed the regulatory effects on RCC cells induced by downregulation of PVT1. These results indicated that targeting PVT1 might represent a rational therapeutic strategy for RCC.

The role of PVT1 in bladder cancer has also been investigated. Yu et al^[24] found that PVT1 levels were higher in bladder cancer

tissues and PVT1 regulated VEGFC through inhibiting miR-128 in bladder cancer cells. Tian et al^[25] found that PVT1 was overexpressed in bladder cancer cells, and it downregulated miR-31 to enhance CDK1 expression and facilitated bladder cancer cells proliferation, migration, and invasion. In the present study, we profiled PVT1 expression in the 98 pairs of MIBC tissues and normal bladder tissues by qRT-PCR. The expression levels of PVT1 were significantly higher in MIBC tissues than that in normal bladder tissues. We then investigated the correlation of PVT1 expression with clinicopathological variables of the MIBC patients. PVT1 expression was significantly correlated with tumor grade, margin, T stage, and lymph node metastasis. However, PVT1 expression was not significantly associated with other clinicopathological factors of patients, including age, sex, and type of urinary diversion. To evaluate the prognostic value of PVT1 expression in MIBC, survival curves were constructed by Kaplan–Meier method and compared by the log-rank test. MIBC patients with high PVT1 expression level had shorter overall survival than those with low PVT1 expression level. To determine the possibility of PVT1 as an independent risk factor for poor prognosis, both clinicopathological factors and the level of PVT1 expression were evaluated by multivariate Cox regression analysis. Results showed that PVT1 expression level was an independent factor in predicting the overall survival of MIBC patients. There are several limitations in our research. First of all, we only investigated the clinical significance of PVT1 in bladder cancer. In the future, we need to study the specific mechanism of its role in bladder cancer. Secondly, we only investigated its clinical significance in Asian population, further investigation should be performed in other race.

In summary, increased PVT1 expression in MIBC patients is correlated with a higher MIBC stage and is significantly associated with poor prognosis for MIBC patients, which may provide new insights into new therapeutic strategy and postoperative intervention against bladder cancer.

Author contributions

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References

- [1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019;69:7–34.
- [2] Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: a global overview and recent trends. *Eur Urol* 2017;71:96–108.
- [3] Kaufman DS, Shipley WU, Feldman AS. Bladder cancer. *Lancet* 2009;374:239–49.
- [4] Robertson AG, Kim J, Al-Ahmadie H, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. *Cell* 2017;171:540.e25–56.e25.
- [5] Grossman HB, Natale RB, Tangen CM, et al. Neoadjuvant chemotherapy plus cystectomy compared with cystectomy alone for locally advanced bladder cancer. *N Engl J Med* 2003;349:859–66.
- [6] Park JC, Citrin DE, Agarwal PK, et al. Multimodal management of muscle-invasive bladder cancer. *Curr Probl Cancer* 2014;38:80–108.
- [7] Guttman M, Rinn JL. Modular regulatory principles of large non-coding RNAs. *Nature* 2012;482:339–46.
- [8] Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet* 2009;10:155–9.
- [9] Chan JJ, Tay Y. Noncoding RNA:RNA regulatory networks in cancer. *Int J Mol Sci* 2018;19.

- [10] Kondo Y, Shinjo K, Katsushima K. Long non-coding RNAs as an epigenetic regulator in human cancers. *Cancer Sci* 2017;108:1927–33.
- [11] Tseng YY, Moriarity BS, Gong W, et al. PVT1 dependence in cancer with MYC copy-number increase. *Nature* 2014;512:82–6.
- [12] Pal G, Huaman J, Levine F, et al. Long Noncoding RNA from PVT1 Exon 9 is overexpressed in prostate cancer and induces malignant transformation and castration resistance in prostate epithelial cells. *Genes (Basel)* 2019;10.
- [13] Lv ZH, Wang ZY, Li ZY. LncRNA PVT1 aggravates the progression of glioma via downregulating UPF1. *Eur Rev Med Pharmacol Sci* 2019;23:8956–63.
- [14] Xiong X, Yuan J, Zhang N, et al. Silencing of lncRNA PVT1 by miR-214 inhibits the oncogenic GDF15 signaling and suppresses hepatocarcinogenesis. *Biochem Biophys Res Commun* 2019;521:478–84.
- [15] Chang QQ, Chen CY, Chen Z, et al. LncRNA PVT1 promotes proliferation and invasion through enhancing Smad3 expression by sponging miR-140-5p in cervical cancer. *Radiol Oncol* 2019;53:443–52.
- [16] Xu Y, Li Y, Jin J, et al. LncRNA PVT1 up-regulation is a poor prognosticator and serves as a therapeutic target in esophageal adenocarcinoma. *Mol Cancer* 2019;18:141.
- [17] Wang Y, Chen W, Lian J, et al. The lncRNA PVT1 regulates nasopharyngeal carcinoma cell proliferation via activating the KAT2A acetyltransferase and stabilizing HIF-1 α . *Cell Death Differ* 2019;27:695–710.
- [18] Ding Y, Fang Q, Li Y, et al. Amplification of lncRNA PVT1 promotes ovarian cancer proliferation by binding to miR-140. *Mamm Genome* 2019;30:217–25.
- [19] Shang AQ, Wang WW, Yang YB, et al. Knockdown of long noncoding RNA PVT1 suppresses cell proliferation and invasion of colorectal cancer via upregulation of microRNA-214-3p. *Am J Physiol Gastrointest Liver Physiol* 2019;317:G222–32.
- [20] Ren Y, Huang W, Weng G, et al. LncRNA PVT1 promotes proliferation, invasion and epithelial-mesenchymal transition of renal cell carcinoma cells through downregulation of miR-16-5p. *Onco Targets Ther* 2019;12:2563–75.
- [21] Fang J, Huang J. Clinical significance of the expression of long non-coding RNA PVT1 in glioma. *Cancer Biomark* 2019;24: 509–13.
- [22] Yang L, Peng X, Jin H, et al. Long non-coding RNA PVT1 promotes autophagy as ceRNA to target ATG3 by sponging microRNA-365 in hepatocellular carcinoma. *Gene* 2019;697:94–102.
- [23] Zhang Y, Yang G, Luo Y. Long non-coding RNA PVT1 promotes glioma cell proliferation and invasion by targeting miR-200a. *Exp Ther Med* 2019;17:1337–45.
- [24] Yu C, Longfei L, Long W, et al. LncRNA PVT1 regulates VEGFC through inhibiting miR-128 in bladder cancer cells. *J Cell Physiol* 2019;234:1346–53.
- [25] Tian Z, Cao S, Li C, et al. LncRNA PVT1 regulates growth, migration, and invasion of bladder cancer by miR-31/CDK1. *J Cell Physiol* 2019;234:4799–811.
- [26] 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;74:784–95.
- [27] Bosschieter J, Nieuwenhuijzen JA, Vis AN, et al. An immediate, single intravesical instillation of mitomycin C is of benefit in patients with non-muscle-invasive bladder cancer irrespective of prognostic risk groups. *Urol Oncol* 2018;36:400.e7–14.
- [28] Abufaraj M, Gust K, Moschini M, et al. Management of muscle invasive, locally advanced and metastatic urothelial carcinoma of the bladder: a literature review with emphasis on the role of surgery. *Transl Androl Urol* 2016;5:735–44.
- [29] Prensner JR, Chinnaiyan AM. The emergence of lncRNAs in cancer biology. *Cancer Discov* 2011;1:391–407.