Original Article
Increased expression of long non-coding RNA PVT1 correlates with clinical progression and poor prognosis in bladder cancer

Yu Cui, Longfei Liu, Jinbo Chen, Chao Li, Xu Cheng, Xiongbing Zu

Department of Urology, Xiangya Hospital, Central South University, Changsha 410000, Hunan, China

Abstract: Several studies have showed that lncRNA PVT1 contributes to cancer progression. However, the relationship between PVT1 and bladder cancer remains uncertain. The aim of this study was to investigate the expression of PVT1 in bladder cancer tissues and further explore its clinical significance. The expression levels of PVT1 were determined using RT-qPCR in a total of 146 patients with bladder cancer. Furthermore, the expression levels of PVT1 were determined in 4 bladder cancer cell lines (RT4, RT112, 5637 and T24) and one normal human uroepithelial cell line (CRL-9520). The association between PVT1 expression and overall survival of bladder cancer patients was evaluated by Kaplan-Meier analysis and log-rank tests. To identify the risk factors associated with overall survival of bladder cancer patients, several factors were evaluated by Cox univariate and multivariate analyses. The results from RT-PCR showed that the expression of PVT1 was not only increased in bladder cancer tissues relative to that in paired adjacent non-tumor tissues, but also upregulated in four bladder cancer cell lines relative to that in a normal human uroepithelial cell line. High PVT1 expression was associated with advanced histological grade, higher tumor stage and positive lymph-node metastasis. Kaplan-Meier survival analysis indicated that high PVT1 expression levels in bladder cancer are significantly associated with worse overall survival. Multivariate analysis showed that high PVT1 expression was an independent prognostic indicator for overall survival of bladder cancer patients. Our study indicated that PVT1 may be considered as a novel prognostic marker and therapeutic target of bladder cancer.

Keywords: LncRNA, PVT1, bladder cancer, biomarker

Introduction

Bladder cancer is the most common malignancy of the urinary tract. In the United States, 74,000 new cases of the bladder cancer were diagnosed and 16,000 deaths were attributable to bladder cancer in 2015 [1]. About 70% of the patients with bladder cancer are noninvasive urothelial carcinoma, and the rest are muscle-invasive disease [2]. Transurethral resection of bladder tumour (TURBT) remains the most common treatment for patients with non-invasive urothelial carcinoma, with a 5-year survival rate around 70%. However, the recurrence rates after TURBT within 12 months are 50%-70% and 15%-30% patients will develop muscle-invasive disease [3]. Therefore, it is essential to detected effective diagnostic biomarkers and potential targets for therapy.

Long non-coding RNAs (lncRNA) are RNA molecules that longer than 200 nucleotides and are not translated into a protein [4]. Many lncRNAs have been identified and recognized as critical regulators of various biological processes. LncRNAs are found in the nucleus or cytosol, and many lncRNAs are tissue specifically expressed [5-7]. Abnormal expression of lncRNAs has been shown to be associated with many cancers.

lncRNA PVT1 was downstream of MYC and localized in 8q24. PVT1 was found to be up-regulated in a series of human cancers. Yang et al found that PVT1 is up-regulated and associated with aggressive progression and poor prognosis in non-small cell lung cancer [8]. Ding et al indicated that PVT1 was increased in gastric cancer and higher PVT1 expression was correlated with lymph node invasion of gastric cancer [9]. However, the relationship between PVT1 and bladder cancer remains uncertain. The aim of the present study was to investigate
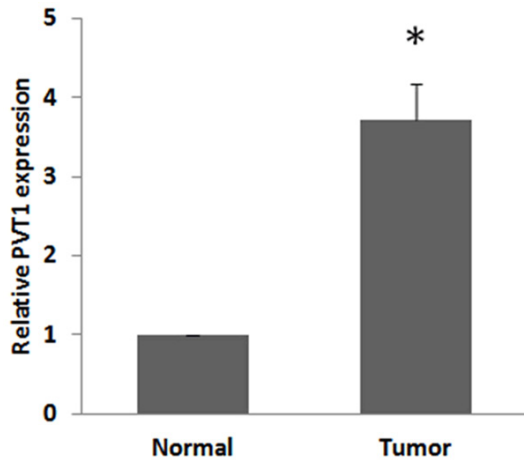
Figure 1. Expression of PVT1 is increased in bladder cancer tissues compared with paired adjacent non-tumor tissues through RT-PCR.

the expression of PVT1 in bladder cancer tissues and further explore its clinical significance in bladder cancer.

Methods

Patient collection

One hundred and forty-six bladder cancer tissues and corresponding adjacent normal tissues were obtained from the Xiangya Hospital between 2005 and 2010. Patients were excluded from our study if they had been treated with radiotherapy or chemotherapy before surgery. The tissues were immediately frozen in liquid nitrogen following excision and stored at -80°C prior to the extraction of total RNA. Clinical and pathological data were recorded for each patient. The study was approved by the Ethics Review Board of Xiangya Hospital, Central South University (Changsha, China). The informed consent was obtained from all patients. The patients were followed up at outpatient every 2-3 months during the first 2 years, and every 6 months thereafter. The median followed up time was 32 months.

Cell culture

The human bladder cancer cell lines (RT4, RT112, 5637 and T24) and normal human uro-epithelial cell line (CRL-9520) were obtained from American Type Culture Collection. Cells were grown in DMEM medium or RPMI1640 supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. All cells were cultured in a humidified 5%

CO_2 incubator at 37°C. All cell lines have been passaged for fewer than 6 months.

Quantitative real-time PCR

Total RNA from specimens and cell lines was extracted using Trizol reagent (Invitrogen, CA). RNAs were reverse transcribed to cDNA using the reverse transcription kit. The real-time PCR was performed by using SYBR-green PCR Master Mix in a Fast Real-time PCR 7500 System (Applied Biosystems) as described in the manufacturer's instructions. GAPDH was used as an internal control. The PCR primers for PVT1 or GAPDH were designed as follows: PVT1 (Up: 5'-CAGTGGTCTGGGGAATAACG-3'; Lo: 5'-AGTCGGGGTCTTACATTCCA-3'), GAPDH (Up: 5'-TGACTTCAACAGCGACACCCA-3'; Lo: 5'-CACC-CTGTTGCTGTAGCCAAA-3').

Statistical analysis

SPSS17.0 software was applied for statistical analysis. Statistical tests used were t test and chisquare test. Survival analysis was performed from the date of surgery to the time of death using the Kaplan-Meier method, and the log-rank test was used to compare the survival in the different groups. A Cox proportional hazard method was also used to analyze the multivariate survival. The level of statistical significance was set at $P<0.05$.

Results

LncRNA PVT1 is up-regulated in bladder cancer tissues and cell lines

The expression levels of PVT1 were determined using RT-qPCR in a total of 146 patients with bladder cancer. The PVT1 expression was significantly upregulated in bladder cancer specimens compared to corresponding adjacent non-tumor tissues (**Figure 1**). Furthermore, the expression levels of PVT1 were determined in 4 bladder cancer cell lines (RT4, RT112, 5637 and T24) and one normal human uroepithelial cell line (CRL-9520). All the 4 bladder cancer cell lines expressed a higher level of PVT1 than the normal human uroepithelial cell line (**Figure 2**).

LncRNA PVT1 up-regulation was associated with the poor prognosis in bladder cancer

According to the mean value (3.64) of relative PVT1 expression, the bladder cancer patients
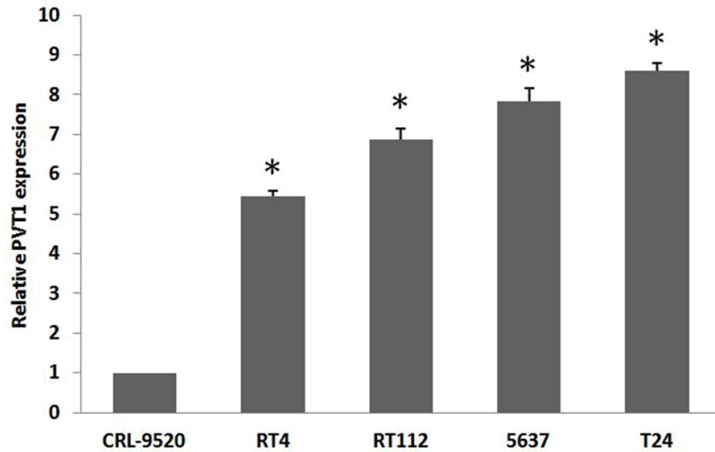
Figure 2. Higher expression levels of PVT1 were detected in bladder cancer cell lines than normal human uroepithelial cell line.

Table 1. Correlations between PVT1 expression and clinicopathological characteristics in bladder cancer

Parameters	Group	Number of patients	PVT1 expression High	PVT1 expression Low	P value
Age (years)	≥60	58	32	26	0.31
	<60	88	41	47	
Gender	Female	82	40	42	0.86
	Male	64	33	31	
Tumor size (cm)	≥3	71	31	40	0.19
	<3	75	42	33	
Tumor number	Single	95	49	46	0.72
	Multiple	51	24	27	
Histological grade	G1	52	19	33	0.02
	G2 G3	94	54	40	
Tumor stage	Ta, T1	68	27	41	0.03
	T2, T3, T4	78	46	32	
Lymph-node metastasis	No	119	52	67	<0.01
	Yes	27	21	6	

were divided into high (n=73) and low (n=73) expression groups. The correlation between the expression level of PVT1 and the clinicopathological characteristics were evaluated. High PVT1 expression was associated with advanced histological grade, higher tumor stage and positive lymph-node metastasis. However, PVT1 expression was irrelevant with age, gender, tumor size and tumor number. These data indicate that high expression levels of PVT1 in bladder cancer may be associated with disease progression (**Table 1**).

The association between PVT1 expression and overall survival of bladder cancer patients was evaluated by Kaplan-Meier analysis and log-rank tests. Kaplan-Meier survival analysis indicated that high PVT1 expression levels in bladder cancer are significantly associated with worse overall survival. These results demonstrated that LncRNA PVT1 may play an important role in the progression of bladder cancer (**Figure 3**).

PVT1 predicts overall survival of bladder cancer patients

To identify the risk factors associated with overall survival of bladder cancer patients, several factors were evaluated by Cox univariate and multivariate analyses. Univariate analysis demonstrated that tumor stage, histological grade, lymph-node metastasis, and pvt1 expression were significantly associated with overall survival of bladder cancer patients. Multivariate analysis showed that higher tumor stage, positive lymph-node metastasis, and high PVT1 expression were independent prognostic indicators for overall survival of bladder cancer patients (**Table 2**).

Discussion

Numerous research has been confirmed the complexity of human genomes transcription, only 2% of transcripts encode proteins [5, 10-12]. In recent years, various studies have certified that the noncoding-RNAs are playing an important role in the post-transcriptional regulation. While microRNAs have been widely investigated, long noncoding RNAs still a fresh, fascinating field to be explored.

Studies have identified many tumor related lncRNAs in recent years. However, only a few of bladder cancer related lncRNAs have been characterized. Zhao et al demonstrated that the lncRNA SPRY4-IT1 was positively correlated with histological grade, tumor stage, and lymph node metastasis and its expression is an independent prognostic factor of overall survival in bladder cancer [13]. Li et al revealed that
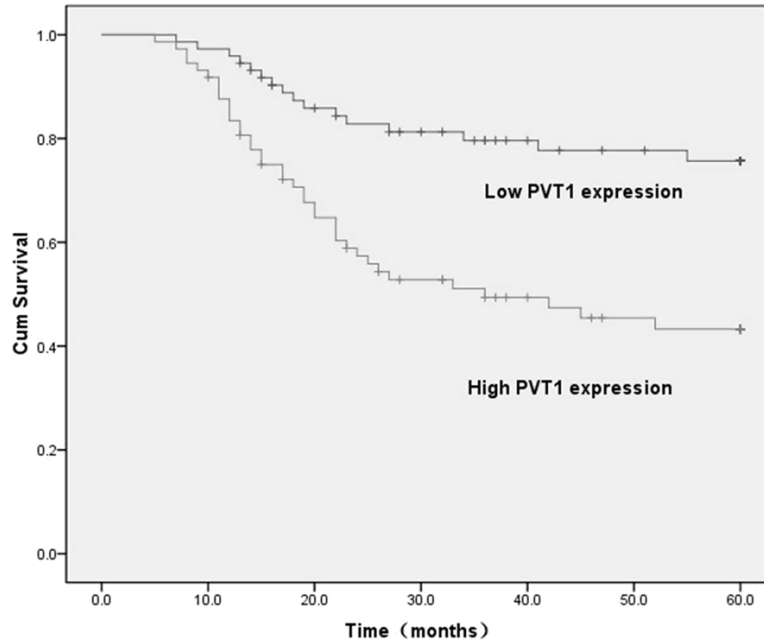
Figure 3. Kaplan-Meier curves revealed an association of higher PVT1 levels with a short overall survival. (P<0.01).

Table 2. Prognostic factors in Cox proportional hazards model

Variable for overall survival	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
Age (years)	1.20	0.70-2.05	0.49			
Gender	1.08	0.63-1.84	0.77			
Tumor size (cm)	1.04	0.61-1.78	0.87			
Tumor number	1.18	1.69-2.02	0.52			
Histological grade	1.89	1.04-3.43	0.03	1.44	0.78-2.66	0.24
Tumor stage	3.47	1.85-6.48	<0.01	2.58	1.34-4.95	<0.01
Lymph-node metastasis	3.90	2.13-7.16	<0.01	2.09	1.09-4.01	0.02
PVT1 expression	3.00	1.67-5.38	<0.01	2.00	1.06-3.79	0.03

PVT1 increases MYC protein levels in 8q24-gain cancers, and they inferred that PVT1 may be a more accessible and less deleterious target than MYC itself for curtailing MYC-driven cancers. Several studies have showed that PVT1 contributes to cancer progression. PVT1 was found significantly upregulated in non-small cell lung cancer, hepatocellular cancer and gastric cancer tissues, and increased PVT1 expression was correlated with poor cancer prognosis and lymph node invasion [8, 9, 17].

In this study, we investigated the clinical significance of PVT1 in bladder cancer patients. The present study identified that PVT1 was overrexpression in bladder cancer and increased expression of PVT1 was associated with advanced histological grade, higher tumor stage and positive lymph-node metastasis. This indicated that PVT1 may play a role in bladder cancer progression and is a predictive marker of metastasis. In addition, high PVT1 expression levels was related to worse overall survival and could be an independent prognostic factor for overall survival of bladder cancer patients, this suggesting its potential therapeutic value.

lncRNA GHET1 was upregulated in bladder cancer tissues and its over-expression correlated with advanced tumor, lymph node status, and poor survival [14]. However, to our knowledge, no studies have investigated the clinical significance of PVT1 in bladder cancer.

PVT1 lies in chromosome 8q24, which is a recognized cancer risk locus. Guan et al identified that inhibition of PVT1 expression induced an apoptotic response in breast and ovarian cancer cell lines [15]. Takahashi et al also demonstrated that the TGF-β signalling pathway and apoptotic signals were significantly activated after PVT1 knockdown in colorectal cancers [16]. The well-known MYC oncogene is also lies in 8q24. Tseng et al demonstrated lncRNA

Conclusion

In conclusion, we demonstrated that PVT1 is highly expressed in bladder cancer. PVT1 overexpression was associated with advanced histological grade, higher tumor stage and positive lymph-node metastasis. In addition, PVT1 served as an independent prognostic factor for overall survival of bladder cancer patients. Our study indicated that PVT1 may be considered as a novel prognostic marker and therapeutic target of bladder cancer.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Xiongbing Zu, Department of Urology, Xiangya Hospital, Central South University, Changsha 410000, Hunan, China. E-mail: xiongbingzuxy@163.com

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.

[2] Kirkali Z, Chan T, Manoharan M, Algaba F, Busch C, Cheng L, Kiemeney L, Kriegmair M, Montironi R, Murphy WM, Sesterhenn IA, Tachibana M, Weider J. Bladder cancer: epidemiology, staging and grading, and diagnosis. Urology 2005; 66: 4-34.

[3] Adiyat KT, Katkoori D, Soloway CT, De los Santos R, Manoharan M, Soloway MS. "Complete transurethral resection of bladder tumor": are the guidelines being followed? Urology 2010; 75: 365-367.

[4] Esteller M. Non-coding RNAs in human disease. Nat Rev Genet 2011; 12: 861-874.

[5] Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, Oyama R, Ravasi T, Lenhard B, Wells C, Kodzius R, Shimokawa K, Bajic VB, Brenner SE, Batalov S, Forrest AR, Zavolan M, Davis MJ, Wilming LG, Aidinis V, Allen JE, Ambesi-Impiombato A, Apweiler R, Aturaliya RN, Bailey TL, Bansal M, Baxter L, Beisel KW, Bersano T, Bono H, Chalk AM, Chiu KP, Choudhary V, Christoffels A, Clutterbuck DR, Crowe ML, Dalla E, Dalrymple BP, de Bono B, Della Gatta G, di Bernardo D, Down T, Engstrom P, Fagiolini M, Faulkner G, Fletcher CF, Fukushima T, Furuno M, Futaki S, Gariboldi M, Georgii-Hemming P, Gingeras TR, Gojobori T, Green RE, Gustincich S, Harbers M, Hayashi Y, Hensch TK, Hirokawa N, Hill D, Huminiecki L, Iacono M, Ikeo K, Iwama A, Ishikawa T, Jakt M, Kanapin A, Katoh M, Kawasawa Y, Kelso J, Kitamura H, Kitano H, Kollias G, Krishnan SP, Kruger A, Kummerfeld SK, Kurochkin IV, Lareau LF, Lazarevic D, Lipovich L, Liu J, Liuni S, McWilliam S, Madan Babu M, Madera M, Marchionni L, Matsuda H, Matsuzawa S, Miki H, Mignone F, Miyake S, Morris K, Mottagui-Tabar S, Mulder N, Nakano N, Nakauchi H, Ng P, Nilsson R, Nishiguchi S, Nishikawa S, Nori F, Ohara O, Okazaki Y, Orlando V, Pang KC, Pavan WJ, Pavesi G, Pesole G, Petrovsky N, Piazza S, Reed J, Reid JF, Ring BZ, Ringwald M, Rost B, Ruan Y, Salzberg SL, Sandelin A, Schneider C, Schönbach C, Sekiguchi K, Semple CA, Seno S, Sessa L, Sheng Y, Shibata Y, Shimada H, Shimada K, Silva D, Sinclair B, Sperling S, Stupka E, Sugiura K, Sultana R, Takenaka Y, Taki K, Tammoja K, Tan SL, Tang S, Taylor MS, Tegner J, Teichmann SA, Ueda HR, van Nimwegen E, Verardo R, Wei CL, Yagi K, Yamanishi H, Zabarovsky E, Zhu S, Zimmer A, Hide W, Bult C, Grimmond SM, Teasdale RD, Liu ET, Brusic V, Quackenbush J, Wahlestedt C, Mattick JS, Hume DA, Kai C, Sasaki D, Tomaru Y, Fukuda S, Kanamori-Katayama M, Suzuki M, Aoki J, Arakawa T, Iida J, Imamura K, Itoh M, Kato T, Kawaji H, Kawagashira N, Kawashima T, Kojima M, Kondo S, Konno H, Nakano K, Ninomiya N, Nishio T, Okada M, Plessy C, Shibata K, Shiraki T, Suzuki S, Tagami M, Waki K, Watahiki A, Okamura-Oho Y, Suzuki H, Kawai J, Hayashizaki Y; FANTOM Consortium; RIKEN Genome Exploration Research Group and Genome Science Group (Genome Network Project Core Group). The transcriptional landscape of the mammalian genome. Science 2005; 309: 1559-1563.

[6] ENCODE Project Consortium, Birney E, Stamatoyannopoulos JA, Dutta A, Guigó R, Gingeras TR, Margulies EH, Weng Z, Snyder M, Dermitzakis ET, Thurman RE, Kuehn MS, Taylor CM, Neph S, Koch CM, Asthana S, Malhotra A, Adzhubei I, Greenbaum JA, Andrews RM, Flicek P, Boyle PJ, Cao H, Carter NP, Clelland GK, Davis S, Day N, Dhami P, Dillon SC, Dorschner MO, Fiegler H, Giresi PG, Goldy J, Hawrylycz M, Haydock A, Humbert R, James KD, Johnson BE, Johnson EM, Frum TT, Rosenzweig ER, Karnani N, Lee K, Lefebvre GC, Navas PA, Neri F, Parker SC, Sabo PJ, Sandstrom R, Shafer A, Vetrie D, Weaver M, Wilcox S, Yu M, Collins FS, Dekker J, Lieb JD, Tullius TD, Crawford GE, Sunyaev S, Noble WS, Dunham I, Denoeud F, Reymond A, Kapranov P, Rozowsky J, Zheng D, Castelo R, Frankish A, Harrow J, Ghosh S, Sandelin A, Hofacker IL, Baertsch R, Keefe D, Dike S, Cheng J, Hirsch HA, Sekinger EA, Lagarde J, Abril JF, Shahab A, Flamm C, Fried C, Hackermüller J, Hertel J, Lindemeyer M, Missal K, Tanzer A, Washietl S, Korbel J, Emanuelsson O, Pedersen JS, Holroyd N, Taylor R, Swarbreck D, Matthews N, Dickson MC, Thomas DJ, Weirauch MT, Gilbert J, Drenkow J, Bell I, Zhao X, Srinivasan KG, Sung WK, Ooi HS, Chiu KP, Foissac S, Alioto T, Brent M, Pachter L, Tress ML, Valencia A, Choo SW, Choo CY, Ucla C, Manzano C, Wyss C, Cheung E, Clark TG, Brown JB,

Ganesh M, Patel S, Tammana H, Chrast J, Henrichsen CN, Kai C, Kawai J, Nagalakshmi U, Wu J, Lian Z, Lian J, Newburger P, Zhang X, Bickel P, Mattick JS, Carninci P, Hayashizaki Y, Weissman S, Hubbard T, Myers RM, Rogers J, Stadler PF, Lowe TM, Wei CL, Ruan Y, Struhl K, Gerstein M, Antonarakis SE, Fu Y, Green ED, Karaöz U, Siepel A, Taylor J, Liefer LA, Wetterstrand KA, Good PJ, Feingold EA, Guyer MS, Cooper GM, Asimenos G, Dewey CN, Hou M, Nikolaev S, Montoya-Burgos JI, Löytynoja A, Whelan S, Pardi F, Massingham T, Huang H, Zhang NR, Holmes I, Mullikin JC, Ureta-Vidal A, Paten B, Seringhaus M, Church D, Rosenbloom K, Kent WJ, Stone EA; NISC Comparative Sequencing Program; Baylor College of Medicine Human Genome Sequencing Center; Washington University Genome Sequencing Center; Broad Institute; Children's Hospital Oakland Research Institute, Batzoglou S, Goldman N, Hardison RC, Haussler D, Miller W, Sidow A, Trinklein ND, Zhang ZD, Barrera L, Stuart R, King DC, Ameur A, Enroth S, Bieda MC, Kim J, Bhinge AA, Jiang N, Liu J, Yao F, Vega VB, Lee CW, Ng P, Shahab A, Yang A, Moqtaderi Z, Zhu Z, Xu X, Squazzo S, Oberley MJ, Inman D, Singer MA, Richmond TA, Munn KJ, Rada-Iglesias A, Wallerman O, Komorowski J, Fowler JC, Couttet P, Bruce AW, Dovey OM, Ellis PD, Langford CF, Nix DA, Euskirchen G, Hartman S, Urban AE, Kraus P, Van Calcar S, Heintzman N, Kim TH, Wang K, Qu C, Hon G, Luna R, Glass CK, Rosenfeld MG, Aldred SF, Cooper SJ, Halees A, Lin JM, Shulha HP, Zhang X, Xu M, Haidar JN, Yu Y, Ruan Y, Iyer VR, Green RD, Wadelius C, Farnham PJ, Ren B, Harte RA, Hinrichs AS, Trumbower H, Clawson H, Hillman-Jackson J, Zweig AS, Smith K, Thakkapallayil A, Barber G, Kuhn RM, Karolchik D, Armengol L, Bird CP, de Bakker PI, Kern AD, Lopez-Bigas N, Martin JD, Stranger BE, Woodroffe A, Davydov E, Dimas A, Eyras E, Hallgrímsdóttir IB, Huppert J, Zody MC, Abecasis GR, Estivill X, Bouffard GG, Guan X, Hansen NF, Idol JR, Maduro VV, Maskeri B, McDowell JC, Park M, Thomas PJ, Young AC, Blakesley RW, Muzny DM, Sodergren E, Wheeler DA, Worley KC, Jiang H, Weinstock GM, Gibbs RA, Graves T, Fulton R, Mardis ER, Wilson RK, Clamp M, Cuff J, Gnerre S, Jaffe DB, Chang JL, Lindblad-Toh K, Lander ES, Koriabine M, Nefedov M, Osoegawa K, Yoshinaga Y, Zhu B, de Jong PJ. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 2007; 447: 799-816.

[7]   Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigó R. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 2012; 22: 1775-1789.

[8]   Yang YR, Zang SZ, Zhong CL, Li YX, Zhao SS, Feng XJ. Increased expression of the lncRNA PVT1 promotes tumorigenesis in non-small cell lung cancer. Int J Clin Exp Pathol 2014; 7: 6929-6935.

[9]   Ding C, Yang Z, Lv Z, DU C, Xiao H, Peng C, Cheng S, Xie H, Zhou L, Wu J, Zheng S. Long non-coding RNA PVT1 is associated with tumor progression and predicts recurrence in hepatocellular carcinoma patients. Oncol Lett 2015; 9: 955-963.

[10] Bertone P, Stolc V, Royce TE, Rozowsky JS, Urban AE, Zhu X, Rinn JL, Tongprasit W, Samanta M, Weissman S, Gerstein M, Snyder M. Global identification of human transcribed sequences with genome tiling arrays. Science 2004; 306: 2242-2246.

[11] Kapranov P, St Laurent G, Raz T, Ozsolak F, Reynolds CP, Sorensen PH, Reaman G, Milos P, Arceci RJ, Thompson JF, Triche TJ. The majority of total nuclear-encoded non-ribosomal RNA in a human cell is 'dark matter' un-annotated RNA. BMC Biol 2010; 8: 149.

[12] Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F, Xue C, Marinov GK, Khatun J, Williams BA, Zaleski C, Rozowsky J, Röder M, Kokocinski F, Abdelhamid RF, Alioto T, Antoshechkin I, Baer MT, Bar NS, Batut P, Bell K, Bell I, Chakrabortty S, Chen X, Chrast J, Curado J, Derrien T, Drenkow J, Dumais E, Dumais J, Duttagupta R, Falconnet E, Fastuca M, Fejes-Toth K, Ferreira P, Foissac S, Fullwood MJ, Gao H, Gonzalez D, Gordon A, Gunawardena H, Howald C, Jha S, Johnson R, Kapranov P, King B, Kingswood C, Luo OJ, Park E, Persaud K, Preall JB, Ribeca P, Risk B, Robyr D, Sammeth M, Schaffer L, See LH, Shahab A, Skancke J, Suzuki AM, Takahashi H, Tilgner H, Trout D, Walters N, Wang H, Wrobel J, Yu Y, Ruan X, Hayashizaki Y, Harrow J, Gerstein M, Hubbard T, Reymond A, Antonarakis SE, Hannon G, Giddings MC, Ruan Y, Wold B, Carninci P, Guigó R, Gingeras TR. Landscape of transcription in human cells. Nature 2012; 489: 101-108.

[13] Zhao XL, Zhao ZH, Xu WC, Hou JQ, Du XY. Increased expression of SPRY4-IT1 predicts poor prognosis and promotes tumor growth and metastasis in bladder cancer. Int J Clin Exp Pathol 2015; 8: 1954-1960.

[14] Li LJ, Zhu JL, Bao WS, Chen DK, Huang WW, Weng ZL. Long noncoding RNA GHET1 promotes the development of bladder cancer. Int J Clin Exp Pathol 2014; 7: 7196-7205.

[15] Guan Y, Kuo WL, Stilwell JL, Takano H, Lapuk AV, Fridlyand J, Mao JH, Yu M, Miller MA, Santos JL, Kalloger SE, Carlson JW, Ginzinger DG, Celniker SE, Mills GB, Huntsman DG, Gray JW. Amplification of PVT1 contributes to the pathophysiology of ovarian and breast cancer. Clin Cancer Res 2007; 13: 5745-5755.

[16] Takahashi Y, Sawada G, Kurashige J, Uchi R, Matsumura T, Ueo H, Takano Y, Eguchi H, Sudo T, Sugimachi K, Yamamoto H, Doki Y, Mori M, Mimori K. Amplification of PVT-1 is involved in poor prognosis via apoptosis inhibition in colorectal cancers. Br J Cancer 2014; 110: 164-171.

[17] Ding J, Li D, Gong M, Wang J, Huang X, Wu T, Wang C. Expression and clinical significance of the long non-coding RNA PVT1 in human gastric cancer. Onco Targets Ther 2014; 18: 1625-1630.