

Aberrant expression of long noncoding RNA *PVT1* and its diagnostic and prognostic significance in patients with gastric cancer

C. L. YUAN^{1,2,*}, H. LI^{3,*}, L. ZHU^{4,*}, Z. LIU², J. ZHOU^{5,*}, Y. SHU^{1,*}

¹Department of Oncology, the First Affiliated Hospital of Nanjing Medical University, No. 300 Guangzhou Road, Nanjing 210029, Jiangsu Province, People's Republic of China; ²Department of Oncology, The First People's Hospital of Lianyungang, Lianyungang 222002, Jiangsu Province, P.R. China; ³Department of Pathology, the Affiliated Hospital of Nanjing University of TCM, Nanjing, Jiangsu, P.R. China; ⁴Department of clinical laboratory, the First People's Hospital of Lianyungang, Lianyungang, Jiangsu, P.R. China; ⁵Department of Molecular Cell Biology and Toxicology, School of Public Health, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210029, Jiangsu Province, People's Republic of China

*Correspondence author: jwzhou@njmu.edu.cn, yongqian_shu@163.com

*Contributed equally to this work.

Received August 25, 2015 / Accepted November 26, 2015

Emerging evidences indicate that dysregulated long noncoding RNAs (lncRNAs) are implicated in cancer tumorigenesis and progression and might be used as diagnosis and prognosis biomarker, or potential therapeutic targets. LncRNA *PVT1* has been reported to be upregulated in diverse human cancers; however, its clinical significance in gastric cancer (GC) remains elusive. This study was to evaluate the expression of *PVT1* in GC and further explore its clinical significance. Previous microarray datasets were analyzed to conduct a preliminary screening for candidate lncRNAs of gastric cancer biomarkers in human gastric cancer tissues. Expression levels of *PVT1* in 111 pairs of gastric cancer and adjacent normal tissues, gastric cancer cell lines and gastric cancer juices compared to their corresponding controls were detected by real-time quantitative RT-PCR assay. A receiver operating characteristic (ROC) curve and Kaplan–Meier analysis were constructed to evaluate the diagnostic and prognostic values. Univariate and multivariate analysis were performed using the Cox proportional hazard analysis. *PVT1* expression was remarkably increased in gastric cancer tissues and cell lines compared with that in the normal control, and its up-regulation was significantly correlated to invasion depth ($P < 0.001$), advanced TNM stage ($P = 0.002$) and regional lymph nodes metastasis ($P < 0.001$) in gastric cancer. *PVT1* levels were robust in differentiating gastric cancer tissues from controls [area under the curve (AUC) = 0.728; 95 % confidence interval (CI) = 0.665–0.786, $p < 0.01$]. Kaplan–Meier analysis demonstrated that increased *PVT1* expression contributed to poor overall survival ($P < 0.01$) and disease-free survival ($P < 0.01$) of patients. A multivariate survival analysis also indicated that *PVT1* could be an independent prognostic marker. The levels of *PVT1* in gastric juice from gastric patients were significantly higher than those from normal subjects ($P = 0.03$). *PVT1* might serve as a promising biomarker for early detection and prognosis prediction of gastric cancer.

Key words: long noncoding RNA, PVT1, gastric cancer, clinical relevance

Gastric cancer is the fourth most common malignancy in the world and is the second most frequent cause of cancer-related deaths worldwide, with particularly high incidence in East Asian [1, 2]. Although gastric endoscopy have contributed to this reduction by enabling early diagnosis gastric cancer, most patients are diagnosed in the advanced stage and have poor prognosis [3]. The clinical stage, based on the TNM classification system, at the time of diagnosis is currently the most important prognostic factor, and the molecular mechanism involved in progression and metastasis of gastric cancer remains unclear [4]. Thus, novel findings on diagnosis and

prognosis factors for gastric cancer would be of great clinical relevance.

Recent advances in whole-genome sequencing technology have contribute to the detection of a new type of regulation gene, long noncoding RNAs (lncRNAs), which are more than 200 bases in length and unable to be translated into proteins. Emerging evidence suggests that lncRNAs may play critical roles in cellular development, differentiation, and many other biological processes. Many studies have revealed that the deregulated expression of lncRNAs plays a functional role in a variety of disease states [5, 6]. Recently, the functions of some

of the lncRNAs have been reported as regulators in different tumors [7-9]. For example, *HOTAIR*, which is one of the few well-studied lncRNAs, plays a significant role in tumor progression by regulation of oncogene or tumor suppressor gene expression through binding to PRC2 [7]. In addition, metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) facilitated renal cell carcinoma aggressive through Ezh2 and interacts with miR-205 [10]. Recently, multiple lines of evidences link dysregulation of lncRNAs to gastric cancer, such as *HOTAIR*, *MALAT1*, maternally expressed gene 3 (*MEG3*) and growth arrest-specific 5 (*GAS5*) [11-13]. Functional lncRNAs can be used for cancer diagnosis and prognosis, and serve as potential therapeutic targets; thus, lncRNAs can be considered as a new diagnostic and therapeutic gold mine in cancer [14].

The human *PVT1* gene (also known as *PVT1* oncogene) is a long intergenic noncoding RNA (lincRNA) homologous to the mouse plasmacytoma variant translocation gene (*PVT1*), is located on chromosome 8q24.21, which does not encode for protein but encodes for a 1.7 kb noncoding RNA. Currently, several studies have shown that *PVT1* is overexpressed in tumors and functions as an oncogene gene, including colorectal cancer [15], serous ovarian and breast cancers [16], and has been associated with reduced survival duration in patients. Recent studies have also showed that *PVT1* is upregulated in human gastric carcinomas [17]. However, the overall clinical role of *PVT1* in GC has not yet been well characterized.

In this study, we uncovered that *PVT1* expression was upregulated in gastric cancer tissues and cell lines. High expression of *PVT1* was associated with clinicopathological characteristics and poor prognosis in gastric cancer patients. We also determined its prognostic role in GC, which might dramatically improve the therapeutic strategy of GC.

Materials and methods

Computational analysis. One human microarray datasets (GSE51575) were downloaded from GEO database and background adjusted using Robust Multichip Average. GATE Explorer was used to process microarrays on a local computer for gene expressions of lncRNAs [18]. This GATE Explorer provides a series of R packages, designed to be used with BioConductor tools, that allow to apply in a simple way the probe mapping data included in GATE Explorer. A type of files called ncRNA Mapper were also obtained from GATE Explorer, which include the probes that do not map to any coding region but that were mapped to a database for non-coding RNA of human and mouse derived from RNAdb [19]. A customized R scripts was used to perform a microarray expression calculation according to the re-mapping data (file ncrnamapperhgu133plus2cdf_3.0) obtained from public database NCBI.

Cell lines. Human gastric adenocarcinoma cancer cell lines SGC7901, BGC823, MGC803, AGS and MKN45 and the normal gastric epithelium cell line (GES1) were obtained from the Chinese Academy of Sciences Committee on Type Culture

Collection cell bank (Shanghai, China). MGC803, AGS and BGC823 cells were cultured in RPMI 1640; MKN45, GES-1 and SGC7901 cells were cultured in DMEM (GIBCO-BRL) medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C in 5% CO₂.

Tissue samples and clinical data collection. In this study, we analyzed 111 patients who underwent resection of the primary gastric cancer at the First Affiliated Hospital of Nanjing Medical University and Yangzhou No.1 People's hospital. The study was approved by the Ethics Committee on Human Research of the First Affiliated Hospital of Nanjing Medical University and Yangzhou No.1 People's hospital, and written informed consent was obtained from all patients. The clinicopathological characteristics of the gastric cancer patients are summarized in Table 2. All patients with gastric cancer have been followed up at intervals of 1–2 months until September 2014, and the median follow-up period was 36 months (range, 20–48 months). Follow-up studies included physical examination, laboratory analysis, and computed tomography if necessary. OS was defined as the interval between the dates of surgery and death. DFS was defined as the interval between the dates of surgery and recurrence; if recurrence was not diagnosed, patients were censored on the date of death or the last follow-up.

Gastric juices were collected from 56 subjects, including 33 patients with gastric cancer (mean age, 60.3 ± 9.8 years) and 23 cases with normal mucosa or minimal gastritis (mean age, 55.6 ± 11.5 years) between July 2012 and November 2013 in the Endoscopy Center of the First Affiliated Hospital of Nanjing Medical University. For each case, the diagnosis was confirmed by endoscopic examination followed by pathological diagnosis of biopsies. Cases with normal mucosa or minimal gastritis, which had no family history of gastric cancer, were treated as the controls. Gastric juice specimens were centrifuged at 2,000×g for 30 min at 4 °C to remove cell fragments and mucus. Then, the pH was measured using a glass electrode pH meter. Finally, the supernatants were stored at –80 °C until use. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University. All specimens were handled and made anonymous following the ethical and legal standards.

RNA preparation and quantitative real-time PCR. Total RNAs were extracted from tumorous and adjacent normal tissues or cultured cells using Trizol reagent (Invitrogen) following the manufacturer's protocol. For gastric juice RNA extraction, 750 µl Trizol LS reagent (Invitrogen) was mixed with 250 µl gastric juice. After vortex mixing for 30 s and then standing for 5 min, 200 µl chloroform was added. The Trizol–chloroform mixture was vortex-mixed for 15 s and then centrifuged at 12,000×g for 15 min at 4 °C. The upper aqueous phase was transferred to a fresh tube. Finally, RNA was extracted following the manufacturer's instructions. Total RNA was quantified using a SmartSpec Plus spectrophotometer (Bio-Rad, Hercules, CA, USA). The A260/A280 ratio was used to evaluate RNA purity. RT and qPCR kits (Takara, Dalian,

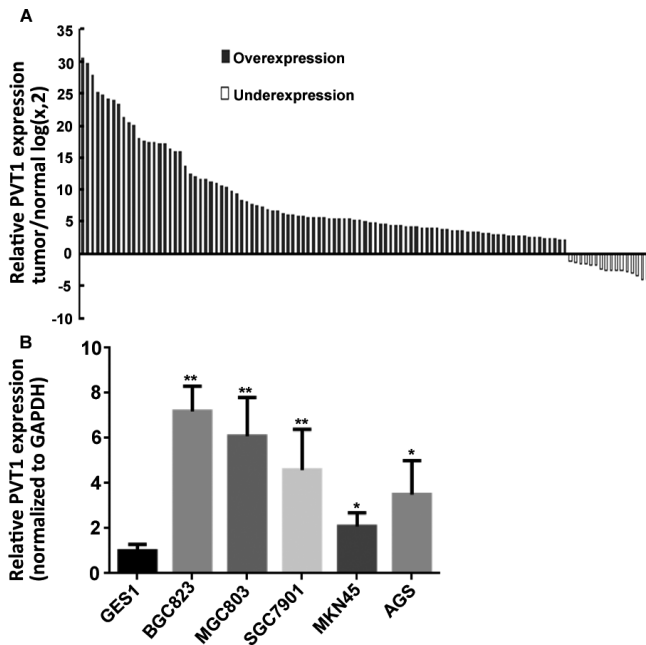


Figure 1. A. Relative expression of *PVT1* in five gastric cancer cell lines (SGC7901, BGC823, MGC803, AGS and MKN45) and human normal gastric epithelial cell line (GES1) analyzed by quantitative RT-PCR (* $p < 0.05$, ** $p < 0.01$). B. Relative expression of *PVT1* in gastric cancer tissues and adjacent normal tissues.

China) were used to evaluate the expression of *PVT1* in tissue samples, cultured cells and gastric juice. The primers used for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and *PVT1* were as follows: 5'-TACAACCACTGCACTACCTG-3' (sense) and 5'-TGTTGCTGTAGCCAAATTCGTT-3' (antisense) for GAPDH. The primer sequences were

Table 1. Fifteen candidate lncRNAs expressed with more than 2.5 fold changes higher compared with corresponding non-tumor tissues

Fold change	P Value	FDR value	GeneName
5.2	<0.001	<0.001	<i>PVT1</i>
4.5	<0.001	<0.001	<i>UCA1</i>
4.8	<0.001	<0.001	<i>H19</i>
4.4	<0.001	0.003269372	<i>HOXA11-AS</i>
4.0	<0.001	<0.001	<i>LOC100131046</i>
3.3	0.006	0.02	<i>BCAR4</i>
3.2	<0.001	<0.001	<i>LINC00152</i>
2.8	0.0055	0.019	<i>ANKRD30BP2</i>
2.6	<0.001	<0.001	<i>KRT16P2</i>
2.5	<0.001	<0.001	<i>HMGB3P1</i>
2.3	<0.001	<0.001	<i>SNHG15</i>
2.2	<0.001	<0.001	<i>SNHG7</i>
2.2	<0.001	<0.001	<i>HOTAIR</i>
2.2	<0.001	<0.001	<i>SPRR2C</i>
2.0	<0.001	<0.001	<i>MAFG-AS1</i>

5'-TGAGAACTGTCCTTACGTGACC-3' (sense) and 5'-AGAGACCAAGACTGGCTCT-3' (antisense) for *PVT1*. Real-time PCR was performed in triplicate, and the relative expression of *PVT1* was calculated using the comparative cycle threshold (CT) ($2^{-\Delta\Delta CT}$) method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous control to normalize the data, and the relative amount in gastric juice of *PVT1* to GAPDH RNA was calculated using the Equation $2^{-\Delta CT}$, where $\Delta CT = (CT_{PVT1} - CT_{GAPDH})$.

Statistical analysis. All statistical analyses were performed using SPSS 20.0 software (IBM, SPSS, Chicago, IL, USA). The significance of the differences between groups was estimated by the Student t-test, χ^2 test, or Wilcoxon test, as appropriate. DFS and OS rates were calculated by the Kaplan–Meier method with the log-rank test applied for comparison. Survival data were evaluated using univariate and multivariate Cox proportional hazards models. Variables with a value of $P < 0.05$ in univariate analysis were used in subsequent multivariate analysis on the basis of Cox regression analyses. Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic value of *PVT1* levels, by plotting sensitivity versus 100 % specificity. Two-sided p-values were calculated, and a probability level of 0.05 was chosen for statistical significance.

Results

Microarray analysis. A total of 125 lncRNAs were altered expressed more than two folds in gastric cancer tissues identified from the microarrays. Twenty-eight of the upregulated lncRNAs had an obvious fold change in the gastric cancer tissues when compared with corresponding non-tumor tissues (Table 1). *PVT1* expression levels in the gastric cancer tissues were the 5.2 times higher than those in the corresponding non-tumor tissues (Table 1). Based on these results and recent discoveries concerning the role of *PVT1* in tumorigenesis, we studied the expression of *PVT1* further in patients with gastric cancer and its correlation with clinical features.

***PVT1* was upregulated in tissues and cell lines.** *PVT1* expression levels were investigated in 111 paired gastric cancer samples and adjacent histologically normal tissues using quantitative polymerase chain reaction (qPCR) assays. *PVT1* expression was significantly higher in tumor tissues compared with adjacent normal tissues ($P < 0.01$; Fig. 1A). qRT-PCR assays were further developed to quantify *PVT1* in gastric cancer cell lines, including MGC803, BGC823, MKN45, SGC7901, AGS and the normal gastric epithelium cell line GES1. A significant high *PVT1* expression was found in SGC7901, BGC823, MGC803, AGS and MKN45 compared to GES-1 (Fig. 1B).

***PVT1* expression and clinicopathological factors in gastric cancer.** To assess the correlation of *PVT1* expression with clinicopathological data, *PVT1* expression levels in tumor tissues were categorized as low or high in relation to the median value of relative *PVT1* expression (5.22-fold, tumors/noncancerous). Clinicopathological factors were

analyzed in the high and low *PVT1* expression groups. As shown in Table 1, the high *PVT1* group (n = 55) showed higher invasion depth ($P < 0.001$), advanced TNM stage ($P = 0.002$) and regional lymph nodes metastasis ($P < 0.001$) than the lower *PVT1* expression group (n = 56). However, there was no significant correlation between *PVT1* expression and other clinicopathological features, such as age, gender, tumor

Table 2. Correlation between *PVT1* expression and clinicopathological characteristics of gastric cancer

Clinical parameter	<i>PVT1</i>		Chi-squared test P-value
	High No. cases (n=55)	Low No. cases (n=56)	
Age (years)			0.507
<50	29	26	
>50	26	30	
Gender			0.362
Male	37	33	
Female	18	23	
Location			0.985
Distal	21	21	
Middle	22	22	
Proximal	12	13	
Size			0.768
>5cm	31	30	
<5cm	24	26	
Histologic differentiation			0.058
Well	13	26	
Moderately	25	14	
Poorly	8	8	
Undifferentiated	9	8	
Invasion depth			<0.001*
T1	4	21	
T2	9	23	
T3	25	7	
T4	17	5	
TNM Stages			0.002*
I	4	19	
II	22	23	
III	27	13	
IV	2	1	
Lymphatic metastasis			0.029*
Yes	30	19	
No	25	37	
Regional lymph nodes			<0.001*
PN0	25	37	
PN1	4	10	
PN2	16	4	
PN3	10	5	
Distant metastasis			0.618
Yes	2	1	
No	53	55	

* $P < 0.05$

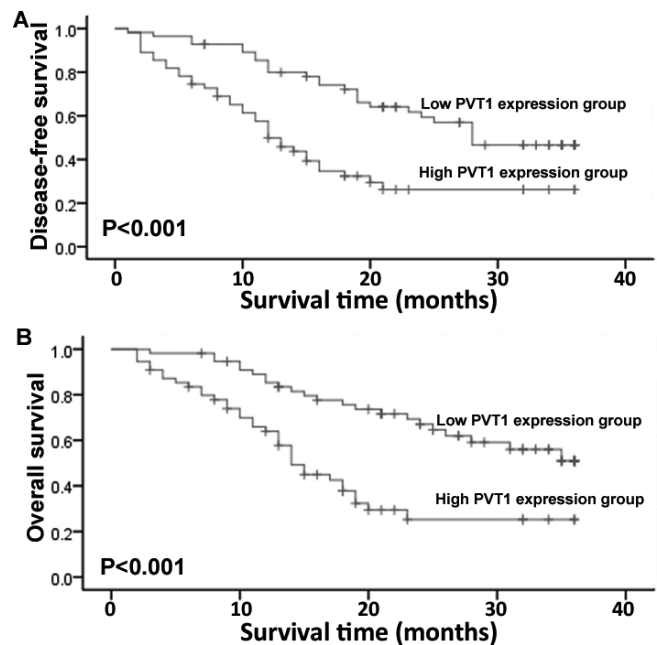


Figure 2. Kaplan–Meier overall survival curves of gastric cancer patients according to the level of *PVT1* expression. A. Disease-free survival of patients with gastric cancer based on *PVT1* expression status ($p < 0.001$, log-rank) B. Overall survival of patients with gastric cancer based on *PVT1* expression status ($p < 0.001$, log-rank).

location, tumor size, histologic grade, and distant metastasis ($P > 0.05$).

High *PVT1* expression is associated with poor prognosis of patients with gastric cancer. Kaplan–Meier analysis and log-rank test were used to evaluate the effects of *PVT1* expression and the clinicopathological characteristics on disease-free survival (DFS) and overall survival (OS). The results showed that patients in the high *PVT1* expression group had a higher recurrence rate (median DFS: 12 months) and much shorter overall survival (median OS: 14 months) than those in the low *PVT1* expression group (median DFS: 26 months; median OS: 28 months; $p < 0.01$ and < 0.01 , respectively; Fig. 2A, 2B). The 3-year DFS and OS were 26.2% and 25.3%, respectively, in the high *PVT1* expression group, and 46.6% and 50.9%, respectively in the low *PVT1* expression group. Univariate analyses of clinical variables considered as potential predictors of survival are shown in Table 2. Further analysis in a multivariate Cox proportional hazards model showed that *PVT1* expression, together with TNM stage, was strongly associated with DFS and OS. *PVT1* expression was an independent prognostic indicator of DFS (hazard ratio [HR] = 2.210; 95% confidence interval [CI], 1.111–4.395; $P = 0.024$) and OS (hazard ratio [HR] = 2.280; 95% confidence interval [CI], 1.054–4.930; $P = 0.036$) in patients with gastric cancer (Table 3).

Observation of the diagnostic value of using *PVT1* as a marker. We observed whether *PVT1* could be used as a gastric cancer marker. We used corresponding adjacent

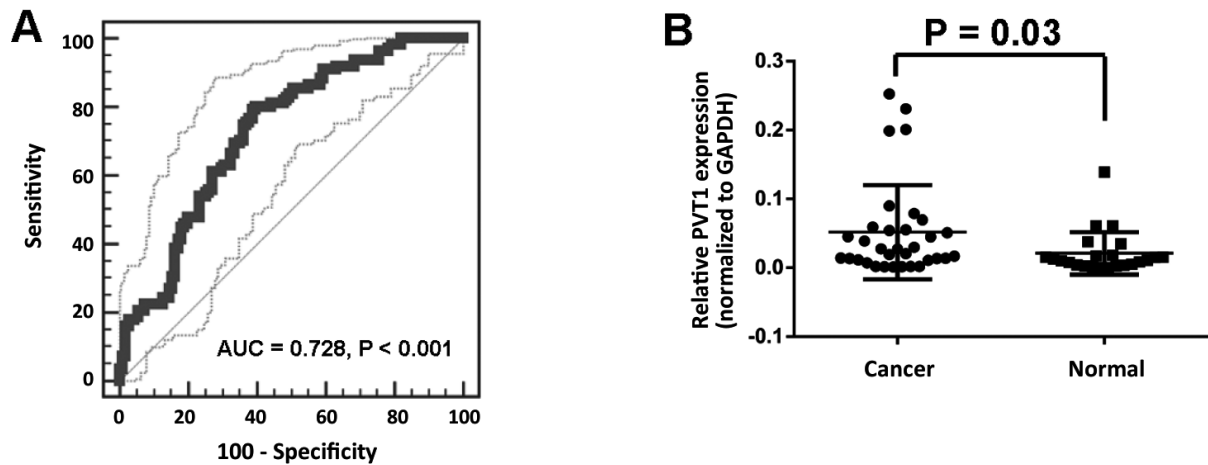


Figure 3. A. Receiver operation characteristics (ROC) curve of using *PVT1* for differentiating gastric cancer tissues from normal tissues. The area under the ROC curve (AUC) is up to 0.728. B. Relative expression of *PVT1* from gastric cancer patients and normal subjects in gastric juice. The relative amount *PVT1* to GAPDH RNA was calculated using the Equation $2^{-\Delta CT}$, where $\Delta CT = (CT_{PVT1} - CT_{GAPDH})$. P values indicated significant differences between specimens determined by t test.

non-tumorous tissues as a control to produce a ROC curve. The cutoff value was 4.46 (ΔCt value). The area under the ROC curve was 0.728; (95% CI = 0.665–0.786, $P < 0.001$; Fig. 3A). The sensitivity and specificity was 0.802 and 0.604, respectively. The Youden index of *PVT1* was 0.406.

Gastric juice is a simple and easy-to-obtain sample which can provide available information in the diagnosis of gastric cancer. To evaluate the diagnostic value of *PVT1*, the levels of gastric juice *PVT1* were detected in gastric juice between

GC patients and normal cases. Interestingly, we found that *PVT1* levels in gastric juice from patients with gastric cancer were significantly higher than those from normal subjects ($p = 0.03$, Fig. 3B).

Discussion

Accumulation evidence suggests that cancer lncRNAs, similar to protein-coding genes, may perform as oncogenic

Table 3. Univariate and multivariate Cox regression analyses *PVT1* for DFS or OS of patients in study cohort (n = 111).

Variables	DFS			OS		
	HR	95% CI	p value	HR	95% CI	p value
Univariate analysis						
Age(<50years vs. >50years)	0.814	0.496-1.335	0.414	0.874	0.519-1.470	0.611
Gender(male vs. female)	0.806	0.479-1.354	0.414	0.765	0.441-1.329	0.342
Location(Distal vs. Middle+ Proximal)	1.100	0.655-1.846	0.719	0.864	0.509-1.467	0.588
tumor size(>5cm vs. <5cm)	0.908	0.554-1.489	0.703	0.936	0.556-1.574	0.802
Histologic differentiation(Well+ Moderately vs. Poorly+ Undifferentiated)	1.479	0.880-2.486	0.140	1.464	0.848-2.526	0.171
Invasion depth(T3+T4 vs. T1+T2)	1.727	1.047-2.849	0.032*	1.949	1.146-3.312	0.014*
TNM stage (III + IV vs. I+II)	2.455	1.478-4.076	0.001*	2.840	1.661-4.857	<0.001*
Lymphatic metastasis(No vs. Yes)	0.613	0.373-1.009	0.054	0.601	0.355-1.016	0.057
Regional lymph nodes(PN2+ PN3 vs. PN0+ PN1)	1.678	0.987-2.854	0.056	2.056	1.185-3.567	0.010*
Distant metastasis(No vs. Yes)	0.338	0.104-1.100	0.072	0.268	0.082-0.877	0.030*
Expression of <i>PVT1</i> (High vs. Low)	2.563	1.532-4.290	<0.001*	1.572	1.210-2.042	0.001*
Multivariate analysis						
TNM stage (I+II vs. III + IV)	2.059	1.217-3.483	0.007*	2.589	1.401-4.784	0.002*
Invasion depth(T3+T4 vs. T1+T2)	0.994	0.517-1.910	0.985	1.274	0.605-2.683	0.524
Regional lymph nodes(PN0+ PN1vs. PN2+ PN3)				0.761	0.371-1.562	0.456
Distant metastasis(No vs. Yes)				0.313	0.088-1.117	0.074
Expression of <i>PVT1</i> (High vs. Low)	2.210	1.111-4.395	0.024*	2.280	1.054-4.930	0.036*

* $P < 0.05$

or tumor-suppressing factors and may be a new class of cancer biomarkers and therapeutic targets [20–22]. *PCGEM1* overexpression and *PRNCR1* have been identified for their involvement in prostate carcinomas [23, 24]. Reportedly, the lncRNA *MALAT-1* has been reported to promote cell motility in non-small cell lung carcinoma [25]. The lncRNA *HOTAIR* induces invasive and metastatic behavior in pancreatic cancer [26]. Recent findings have suggested that many lncRNAs play pivotal roles in GC development. Some lncRNAs, such as *GAS5*, *MEG3*, and *FENDRR*, were reported to regulate proliferation and/or apoptosis and/or metastasis in GC cells [12, 13, 27–29]. These findings suggested that lncRNAs might play a crucial role in carcinogenesis and have a great impact on clinical application.

Current studies revealed an oncogenic role for *PVT1* in the pathophysiology of breast and ovarian cancer by virtue of *PVT1*-mediated inhibition of apoptosis and increasing of proliferation [16]. Recent Nature paper uncovered that *PVT1* controls levels of *MYC* through regulation of the protein stability and they both cooperate to promote cell proliferation in cancer, and the authors analyzed copy number variation data from two large cancer databases (i.e., Progenetix and TCGA) and consistently observed (98% of cases) a co-gain of *MYC* and *PVT1* across a wide variety of cancers including GC with amplified 8q24 region [30]. Numerous studies indicate that *PVT1* may play a key role in tumorigenesis and could contribute to tumor progression and aggressiveness. *PVT1* was found to be upregulated in cancer compared to noncancerous tissues in various cancer tissues including breast [16], ovarian [31], hepatocellular [32], colorectal [15], and lung [30] cancers, and this upregulation was associated with a worse prognosis in a panel of HCC and colorectal patients. Mechanism investigation showed that the upregulated *PVT1* could induce cell cycle genes and activation of the TGF β 1 signaling pathway and a possible feedback loop between *PVT1* and TGF β 1 existed [32]. However, the precise clinical significance in gastric cancer remains less understood.

We first analyzed previous microarray datasets (GSE51575) to detect the differentially expressed lncRNAs between GC and paired non-tumor tissues, and found that *PVT1* was remarkably upregulated in GC tissues. Then, we performed RT-qPCR to investigate whether *PVT1* was altered in 111 pairs of gastric cancer tissues and adjacent normal tissues. Results showed that the expression level of *PVT1* was increased in GC compared with that in adjacent normal specimens. Additionally, *PVT1* expression is markedly increased in gastric cancer cell lines compared with normal gastric epithelium cell line. An ROC curve was constructed for differentiating GC tissues from normal tissues, and the results indicated that the AUC was 0.728. Gastric juice *PVT1* levels in gastric cancer patients were further found to be significantly higher than those of normal subjects. Our results provided evidence that expression level of *PVT1* was up-regulated in human GC. These findings indicated that *PVT1* may play a pivotal role in GC development.

To further determine the clinical significance of *PVT1*, the association between *PVT1* expression and clinicopathological parameters in gastric cancer tissues was analyzed. The results revealed that high *PVT1* expression was more frequently detected in tumors with deeper invasion depth, more lymphatic metastasis and advanced TNM stage. Since the clinicopathological parameters of depth of invasion, regional lymph nodes status and tumor stage represent partially the deterioration and progress of GC, *PVT1* might be involved in the tumorigenesis and progression of gastric cancer. This association is consistent with previous findings in GC, which also found that *PVT1* could promote gastric cancer cell proliferation [33]. Kaplan–Meier analysis and log-rank test were performed to confirm the prognostic value of *PVT1*. Our results showed that patients with a high expression of *PVT1* seemed to have shorter overall and disease-free survival than patients with lower levels. Furthermore, multivariate Cox analysis showed that *PVT1* could serve as an independent prognostic biomarker. Therefore, *PVT1* might serve to identify high-risk individual patients with GC who have higher risk of death and, thus, are good candidates for receiving more aggressive treatment.

In conclusion, we demonstrated that *PVT1* was significantly up-regulated in GC tissues and some GC cell lines. Its level was associated with tumor progression and poor prognosis. Our data also implied that *PVT1* might be capable of distinguishing between cancerous and non-cancerous conditions by examining the expression of *PVT1* in tissues and gastric juices. In addition, the high risk of recurrent GC might be predicted by evaluating *PVT1* expression in GC samples collected. These findings suggested that *PVT1* might be useful as a diagnostic and prognostic biomarker for GC and might be a possible target for diagnosis and gene therapy. However, larger clinical and prospective studies will need to be performed to confirm these preliminary results.

References

- [1] MOORE MA, ATTASARA P, KHUHAPREMA T, LE TN, NGUYEN TH et al. Cancer epidemiology in mainland South-East Asia - past, present and future. *Asian Pac J Cancer Prev* 2010; 11: 67–80.
- [2] JEMAL A, SIEGEL R, XU J, WARD E. Cancer statistics, 2010. *CA Cancer J Clin* 2010; 60: 277–300. <http://dx.doi.org/10.3322/caac.20073>
- [3] SAPARI NS, LOH M, VAITHILINGAM A, SOONG R. Clinical Potential of DNA Methylation in Gastric Cancer: A Meta-Analysis. *Plos One* 2012; 7.
- [4] MILNE AN, CARNEIRO F, O'MORAIN C, OFFERHAUS GJA. Nature meets nurture: molecular genetics of gastric cancer. *Human Genetics* 2009; 126: 615–628. <http://dx.doi.org/10.1007/s00439-009-0722-x>
- [5] MUERS M. RNA Genome-wide views of long non-coding RNAs. *Nature Reviews Genetics* 2011; 12. <http://dx.doi.org/10.1038/nrg3088>

- [6] PONTING CP, OLIVER PL, REIK W. Evolution and Functions of Long Noncoding RNAs. *Cell* 2009; 136: 629–641. <http://dx.doi.org/10.1016/j.cell.2009.02.006>
- [7] RINN JL, KERTESZ M, WANG JK, SQUAZZO SL, XU X et al. Functional demarcation of active and silent chromatin domains in human HOX loci by Noncoding RNAs. *Cell* 2007; 129: 1311–1323. <http://dx.doi.org/10.1016/j.cell.2007.05.022>
- [8] CALIN GA, LIU CG, FERRACIN M, HYSLOP T, SPIZZO R et al. Ultraconserved regions encoding ncRNAs are altered in human leukemias and carcinomas. *Cancer Cell* 2007; 12: 215–229. <http://dx.doi.org/10.1016/j.ccr.2007.07.027>
- [9] LIN R, MAEDA S, LIU C, KARIN M, EDGINGTON TS. A large noncoding RNA is a marker for murine hepatocellular carcinomas and a spectrum of human carcinomas. *Oncogene* 2007; 26: 851–858. <http://dx.doi.org/10.1038/sj.onc.1209846>
- [10] HIRATA H, HINODA Y, SHAHRYARI V, DENG GR, NAKAJIMA K et al. Long Noncoding RNA MALAT1 Promotes Aggressive Renal Cell Carcinoma through Ezh2 and Interacts with miR-205. *Cancer Research* 2015; 75: 1322–1331. <http://dx.doi.org/10.1158/0008-5472.CAN-14-2931>
- [11] OKUGAWA Y, TOIYAMA Y, HUR K, TODEN S, SAIGUSA S et al. Metastasis-associated long non-coding RNA drives gastric cancer development and promotes peritoneal metastasis. *Carcinogenesis* 2014; 35: 2731–2739. <http://dx.doi.org/10.1093/carcin/bgu200>
- [12] SUN M, JIN FY, XIA R, KONG R, LI JH et al. Decreased expression of long noncoding RNA GAS5 indicates a poor prognosis and promotes cell proliferation in gastric cancer. *Bmc Cancer* 2014; 14. <http://dx.doi.org/10.1186/1471-2407-14-319>
- [13] SUN M, XIA R, JIN FY, XU TP, LIU ZJ et al. Downregulated long noncoding RNA MEG3 is associated with poor prognosis and promotes cell proliferation in gastric cancer. *Tumor Biology* 2014; 35: 1065–1073. <http://dx.doi.org/10.1007/s13277-013-1142-z>
- [14] QI P, DU X. The long non-coding RNAs, a new cancer diagnostic and therapeutic gold mine. *Modern Pathology* 2013; 26: 155–165. <http://dx.doi.org/10.1038/modpathol.2012.160>
- [15] TAKAHASHI Y, SAWADA G, KURASHIGE J, UCHI R, MATSUMURA T et al. Amplification of PVT-1 is involved in poor prognosis via apoptosis inhibition in colorectal cancers. *British Journal of Cancer* 2014; 110: 164–171. <http://dx.doi.org/10.1038/bjc.2013.698>
- [16] GUAN YH, KUO WL, STILWELL JL, TAKANO H, LAPUK AV et al. Amplification of PVT1 contributes to the pathophysiology of ovarian and breast cancer. *Clinical Cancer Research* 2007; 13: 5745–5755. <http://dx.doi.org/10.1158/1078-0432.CCR-06-2882>
- [17] DING J, LI D, GONG MZ, WANG JP, HUANG XR et al. Expression and clinical significance of the long non-coding RNA PVT1 in human gastric cancer. *Oncotargets and Therapy* 2014; 7: 1625–1630. <http://dx.doi.org/10.2147/OTT.S68854>
- [18] RISUENO A, FONTANILLO C, DINGER ME, DE LAS RIVAS J. GATEExplorer: Genomic and Transcriptomic Explorer; mapping expression probes to gene loci, transcripts, exons and ncRNAs. *Bmc Bioinformatics* 2010; 11.
- [19] PANG KC, STEPHEN S, DINGER ME, ENGSTROM PG, LENHARD B et al. RNAdb 2.0-an expanded database of mammalian non-coding RNAs. *Nucleic Acids Research* 2007; 35: D178–D182. <http://dx.doi.org/10.1093/nar/gkl926>
- [20] DU Z, FEI T, VERHAAK RGW, SU Z, ZHANG Y, BROWN M et al. Integrative genomic analyses reveal clinically relevant long noncoding RNAs in human cancer. *Nature Structural & Molecular Biology* 2013; 20: 908. <http://dx.doi.org/10.1038/nsmb.2591>
- [21] LI Q, SU ZJ, XU XY, LIU GF, SONG XH et al. AS1DHRS4, a head-to-head natural antisense transcript, silences the DHRS4 gene cluster in cis and trans. *Proceedings of the National Academy of Sciences of the United States of America* 2012; 109: 14110–14115. <http://dx.doi.org/10.1073/pnas.1116597109>
- [22] PASSON DM, LEE M, RACKHAM O, STANLEY WA, SADOWSKA A et al. Structure of the heterodimer of human NONO and paraspeckle protein component 1 and analysis of its role in subnuclear body formation. *Proceedings of the National Academy of Sciences of the United States of America* 2012; 109: 4846–4850. <http://dx.doi.org/10.1073/pnas.1120792109>
- [23] SRIKANTAN V, ZOU Z, PETROVICS G, XU L, AUGUSTUS M et al. PCGEM1, a prostate-specific gene, is overexpressed in prostate cancer. *Proc Natl Acad Sci U S A* 2000; 97: 12216–12221. <http://dx.doi.org/10.1073/pnas.97.22.12216>
- [24] PRENSNER JR, SAHU A, IYER MK, MALIK R, CHANDLER B et al. The lncRNAs PCGEM1 and PRNCR1 are not implicated in castration resistant prostate cancer. *Oncotarget* 2014; 5: 1434–1438. <http://dx.doi.org/10.18632/oncotarget.1846>
- [25] JI P, DIEDERICH S, WANG W, BOING S, METZGER R et al. MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. *Oncogene* 2003; 22: 8031–8041. <http://dx.doi.org/10.1038/sj.onc.1206928>
- [26] KIM K, JUTOORU I, CHADALAPAKA G, JOHNSON G, FRANK J et al. HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. *Oncogene* 2013; 32: 1616–1625. <http://dx.doi.org/10.1038/onc.2012.193>
- [27] XU TP, HUANG MD, XIA R, LIU XX, SUN M et al. Decreased expression of the long non-coding RNA FENDRR is associated with poor prognosis in gastric cancer and FENDRR regulates gastric cancer cell metastasis by affecting fibronectin1 expression. *J Hematol Oncol* 2014; 7: 63. <http://dx.doi.org/10.1186/s13045-014-0063-7>
- [28] SUN M, JIN FY, XIA R, KONG R, LI JH et al. Decreased expression of long noncoding RNA GAS5 indicates a poor prognosis and promotes cell proliferation in gastric cancer. *BMC Cancer* 2014; 14: 319. <http://dx.doi.org/10.1186/1471-2407-14-319>
- [29] SUN M, XIA R, JIN F, XU T, LIU Z et al. Downregulated long noncoding RNA MEG3 is associated with poor prognosis and promotes cell proliferation in gastric cancer. *Tumour Biol* 2014; 35: 1065–1073. <http://dx.doi.org/10.1007/s13277-013-1142-z>
- [30] TSENG YY, MORIARITY BS, GONG WM, AKIYAMA R, TIWARI A et al. PVT1 dependence in cancer with MYC

- copy-number increase. *Nature* 2014; 512: 82. <http://dx.doi.org/10.1038/nature13311>
- [31] HAVERTY PM, HON LS, KAMINKER JS, CHANT J, ZHANG ZM. High-resolution analysis of copy number alterations and associated expression changes in ovarian tumors. *Bmc Medical Genomics* 2009; 2.
- [32] WANG F, YUAN JH, WANG SB, YANG F, YUAN SX et al. Oncofetal Long Noncoding RNA PVT1 Promotes Proliferation and Stem Cell-Like Property of Hepatocellular Carcinoma Cells by Stabilizing NOP2. *Hepatology* 2014; 60: 1278–1290. <http://dx.doi.org/10.1002/hep.27239>
- [33] KONG R, ZHANG EB, YIN DD, YOU LH, XU TP et al. Long noncoding RNA PVT1 indicates a poor prognosis of gastric cancer and promotes cell proliferation through epigenetically regulating p15 and p16. *Molecular Cancer* 2015; 14. <http://dx.doi.org/10.1186/s12943-015-0355-8>