Long noncoding RNA PVT-1 predicts poor patient prognosis in non-small cell lung cancer

Wu-Zhang Wang1,2*, Li Liu1, Shou-Qin Jia2, Hui-Fang Qu2

Departments of 1Interventional Treatment, 2Medical Imaging, Shandong Chest Hospital, Jinan 250013, Shandong, China

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Abstract: Background: Overexpression of PVT1 is a powerful predictor of tumor progression and patient survival in colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, and hepatocellular carcinoma. However, its clinical significance and prognostic value in non-small cell lung cancer (NSCLC) have not been investigated until now. Methods: 145 paired NSCLC tissues and matched adjacent non-tumor tissues were obtained between May 2007 and February 2014. Expression of lncRNA PVT1 was determined by quantitative real-time PCR (qRT-PCR). Overall survival curves were plotted according to the Kaplan-Meier method, and the log-rank test was applied for comparison. The variables were used in multivariate analysis on the basis of the Cox proportional hazards model. Results: lncRNA PVT1 level was significantly up-regulated in NSCLC tissues compared with corresponding adjacent non-tumor tissues (\( P < 0.001 \)). PVT1 upregulation was correlated with TNM stage (\( P = 0.012 \)), histological grade (\( P = 0.031 \)), and lymph node metastasis (\( P = 0.028 \)). Patients with PVT1 higher expression have shown significantly poorer overall survival (\( P = 0.011 \)) than those with lower PVT1 expression. Using a multivariate Cox regression analysis, PVT1 expression (HR = 2.155, 95% CI = 1.618-6.994, \( P = 0.036 \)) was an independent predictor of OS in NSCLC.

Conclusions: lncRNA PVT1 expression is increased in NSCLC and associated with tumor progression. Therefore, lncRNA PVT1 expression is an independent prognostic factor of patients with NSCLC.

Keywords: Long noncoding RNA, PVT-1, prognosis, NSCLC

Introduction

Lung cancer is one of the leading causes of all cancer-related deaths worldwide, and with an incidence of over 200,000 new cases every year. Approximately, 85% of all lung cancer cases are categorized as non-small cell lung cancer (NSCLC) [1, 2]. Surgical resection, when possible, remains the only curative treatment for early stage NSCLC. However, nearly 50% of resected patients experience recurrence [3]. Therefore, strategies for elucidating the mechanism underlying progression and identifying novel biomarkers for NSCLC are urgently needed.

Long non-coding RNAs (lncRNAs) are evolutionarily conserved non-coding RNAs that are more than 200 nucleotides in length with no protein coding capacity [4]. Recent studies showed that lncRNAs play key roles in diverse biological processes, such as embryonic development, cell growth and tumorigenesis by regulating gene expression at the transcriptional and post-transcriptional levels [5, 6].

The lncRNA PVT1 is encoded by a gene that has been long known since it resides in the well-known cancer risk region 8q24 [7]. PVT1 exerts regulatory functions in various biological processes, such as proliferation, apoptosis, mobility and invasion [7]. Overexpression of PVT1 is a powerful predictor of tumor progression and patient survival in colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, and hepatocellular carcinoma [8-12]. However, its clinical significance and prognostic value in NSCLC have not been investigated until now.

Materials and methods

Patients and specimens

The present study was approved by the Human Ethics Committee of Shandong provincial Chest Hospital. The study methodologies conformed to the standards set by the Declaration of
Helsinki. Written informed consent was obtained from all patients. 145 paired NSCLC tissues and matched adjacent non-tumor tissues were obtained between May 2007 and February 2014. All patients recruited in this study were not subjected to preoperative radiotherapy and/or chemotherapy and were diagnosed with NSCLC based on histopathological evaluation. Complete clinicopathological data of the patients from which the specimens were collected were available. Each sample was snap frozen in liquid nitrogen and stored at -80°C prior to RNA isolation and qRT-PCR analysis.

RNA extraction and qRT-PCR analyses

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). qRT-PCR assays were performed to detect PVT1 expression using the Prime Script RT reagent Kit and SYBR Premix ExTaq (Takara) according to the manufacturer’s instructions. The primer sequences were as follows: Sense, 3’-CATCGGCGCTCAGCT-5’ and antisense, 3’-TCATGATGGCTGTATGTGCCA-5’ for PVT1; and sense, 3’-ATGGGGAAGGTGAAGGTCG-5’ and antisense, 3’-GGGGTCATTGATGGCAACAATA-5’ for GAPDH. All PCRs were performed in triplicate and GAPDH was used to normalize mRNA expression levels.

Statistical analysis

The continuous data were analyzed using an independent t-test between the two groups, whereas categorical data were analyzed by the χ² test or Fisher’s exact test, as appropriate. OS curves were plotted according to the Kaplan-Meier method, and the log-rank test was applied for comparison. The variables were used in multivariate analysis on the basis of the Cox proportional hazards model. P-values < 0.05 were considered statistically significant. All of the statistical analyses were performed using SPSS for Windows version 18.0 (SPSS, Chicago, IL, USA).

Results

LncRNA PVT1 is upregulated in NSCLC tissues

We examined the IncRNA PVT1 expression level in 145 paired NSCLC samples and adjacent non-tumor tissues by qRT-PCR, and normalized to GAPDH. IncRNA PVT1 level was significantly up-regulated in NSCLC tissues compared with corresponding adjacent non-tumor tissues (P < 0.001, shown in Figure 1), indicating that abnormal IncRNA PVT1 expression may be related to NSCLC pathogenesis. To assess the correlation between PVT1 expression and clinicopathological data, we divided the patients with NSCLC into a high PVT1 expression group (n = 74) and a low expression group (n = 71) according to the mean PVT1 expression level in the tumor tissues.

Correlations between the expression of PVT1 and the clinicopathological factors in NSCLC

To identify the clinical relevance of PVT1 expression in NSCLC, correlation between PVT1 expression and clinicopathological parameters such as age, gender, smoking status, tumor size, histological grade, lymph node invasion, and TNM stage was evaluated. As shown in Table 1, PVT1 upregulation was correlated with TNM stage (P = 0.012), histological grade (P = 0.031), and lymph node metastasis (P = 0.028). Taken together, these observations indicated that increased PVT1 expression is associated with the progression and development of NSCLC.

Association between PVT1 expression and prognosis of NSCLC patients

Overall survival curves in high PVT1 group and low PVT1 group were shown in Figure 2. As was expected, patients with PVT1 higher expression have shown significantly poorer overall survival (P = 0.011) than those with lower PVT1 expression. Using a multivariate Cox regression analy-
IncRNA PVT1 and NSCLC prognosis

Table 1. Association of PVT-1 expression with clinicopathological variables in 145 NSCLC patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>High PVT1 expression</th>
<th>Low PVT1 expression</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>&lt; 55</td>
<td>66</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>≥ 55</td>
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<td>41</td>
<td>41</td>
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<tr>
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<tr>
<td>Female</td>
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<td>History of smoking</td>
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<tr>
<td>Ever</td>
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<td>41</td>
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</tr>
<tr>
<td>Never</td>
<td>53</td>
<td>33</td>
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</tr>
<tr>
<td>Tumor size</td>
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<tr>
<td>≤ 3 cm</td>
<td>55</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>&gt; 3 cm</td>
<td>90</td>
<td>51</td>
<td>51</td>
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<td>TNM stage</td>
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</tr>
<tr>
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<td>32</td>
</tr>
<tr>
<td>III/IV</td>
<td>67</td>
<td>42</td>
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<tr>
<td>Histological grade</td>
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<tr>
<td>Well and moderately</td>
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<tr>
<td>Poorly</td>
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<td>5</td>
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<tr>
<td>Lymph node metastasis</td>
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<tr>
<td>Positive</td>
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</tr>
</tbody>
</table>

Figure 2. Kaplan-Meier survival analysis of association between IncRNA PVT1 expression level and overall survival of 145 NSCLC patients.

Discussion

Despite advances in early detection and standard treatment, NSCLC is often diagnosed at an advanced stage and carries a poor prognosis. The overall 5-year survival rates including all stages for patients with ADC, LCC and SCC are only 17%, 15% and 11%, respectively [2]. Greater knowledge of the molecular origins and progression of NSCLC may lead to improvements in the prevention, diagnosis and treatment of the disease.

Traditionally, cancer was regarded as a genetic disease, but current research revealed that cancer development and progression involves epigenetic abnormalities [13]. Genetic continuity has been shown to involve epigenetic regulation such as DNA methylation, histone deacetylation and non-coding RNA regulation [14]. IncRNAs are more than 200 nucleotides in length with limited or no protein-coding capacity and serve as the primary regulatory ncRNA [15]. Increasing evidences showed that IncRNAs could play an important role in cellular development, differentiation, and many other biological processes [16]. Specific IncRNAs have also been shown to play a critical role in tumor progression and development. Examples include HOX transcript antisense RNA (HOTAIR) in colorectal cancer [17], metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) in lung cancer [18], and hepatocellular carcinoma upregulated long noncoding RNA (HULC) in hepatocellular carcinoma and pancreatic cancer [19, 20].
tated microRNAs: miR-1204, miR-1205, miR-1206, miR-1207-5p, miR-1207-3p and miR-1208 [21]. Several published reports have revealed that PVT-1 is involved in cancer pathophysiology. Kong et al found that the higher expression of PVT1 was significantly correlated with deeper invasion depth and advanced TNM stage of gastric cancer. Multivariate analyses revealed that PVT1 expression served as an independent predictor for overall survival (P = 0.031), suggesting that lncRNA PVT1 might serve as a candidate prognostic biomarker and target for new therapies in human gastric cancer [8]. The study findings by Huang et al suggested that the increased expression of lncRNA PVT1 in pancreatic ductal adenocarcinoma (PDAC) was correlated with tumor progression, and PVT1 might be a potential molecular biomarker for predicting the prognosis of patients with PDAC [9]. In the study by Takahashi et al, colorectal cancer cells transfected with PVT-1 siRNA exhibited significant loss of their proliferation and invasion capabilities. In addition, univariate and multivariate analysis revealed that PVT1 expression level was an independent risk factor for overall survival of colorectal cancer patients [10]. Ding et al found that the relative expression levels of PVT1 were significantly higher in HCC tissues compared with the corresponding non-cancerous tissues. Furthermore, Kaplan-Meier analysis indicated that the patients with high PVT1 expression exhibited poor recurrence-free survival (P = 0.021), and multivariate analysis demonstrated that high levels of PVT1 expression are an independent predictor for HCC recurrence (P = 0.042; hazard ratio, 1.653). Thus, the high expression levels of PVT1 in HCC may serve as a novel biomarker for predicting tumor recurrence in HCC patients, and as a potential therapeutic target [22]. However, until now, the clinical significance and prognostic value of PVT1 in NSCLC have not been investigated.

In the present study, we found that IncRNA PVT1 level was significantly up-regulated in NSCLC tissues compared with corresponding adjacent non-tumor tissues, indicating that abnormal IncRNA PVT1 expression may be related to NSCLC pathogenesis. PVT1 upregulation was correlated with TNM stage, histological grade, and lymph node metastasis. Taken together, these observations indicated that increased PVT1 expression is associated with the progression and development of NSCLC. As was expected, patients with PVT1 higher expression have shown significantly poorer overall survival than those with lower PVT1 expression. Using a multivariate Cox regression analysis, we found that PVT1 expression was an independent predictor of OS in NSCLC. In conclusion, we have proved that IncRNA PVT1 expression was increased in NSCLC and associated with tumor progression. The present study also demonstrated that IncRNA PVT1 expression was an independent prognostic factor of patients with NSCLC.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Wu-Zhang Wang, Department of Interventional Treatment, Shandong Provincial Chest Hospital, 46 Lishan Road, Lixia District, Jinan 250013, Shandong, China. Tel: 086-13793187175; Fax: 086-531-86568178; E-mail: drwangwuzhang@126.com

References

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