Long non-coding RNA PVT1 promotes tumor progression by regulating the Wnt pathway in human esophageal squamous cell carcinoma

Chengyun Li[1], Xiong Yue[2], Yitong Li[1], Chao Ma[1], Xinghua Wang[3], Junling Wang[1]

[1]Department of Toxicology, School of Public Health, Lanzhou University, Lanzhou, Gansu 730000, China;
[2]Department of Medical, Gansu Provincial Maternity and Child-Care Hospital, Lanzhou, Gansu 730000, China;
[3]Department of Gastrointestinal Surgery, Gansu Wuwei Tumor Hospital, Wuwei, Gansu 733000, China.

To the Editor: Esophageal squamous cell carcinoma (ESCC) is the seventh most common malignant cancer worldwide.[1] There is an urgent need to find sensitive and specific biomarkers to improve ESCC diagnosis and prognosis.[2] Tumor-specific long non-coding RNA (lncRNA) seems to be potential biomarkers for the diagnosis and treatment of cancer.[3] Our previous study has performed data mining analyses for ESCC and identification of the dysregulated lncRNAs from the Gene Expression Omnibus database (GEO, https://www.ncbi.nlm.nih.gov/sites/GDSbrowser).[4] The results demonstrated that lncRNA plasmacytoma variant translocation 1 (PVT1) gene expression is significantly up-regulated in ESCC tumor tissues. In the present study, we analyze the feasibility of using PVT1 as a tumor molecular marker and discuss its potential clinical applications.

We designed a experiment to investigate the PVT1 expression in ESCC tissues, microarray data, The Cancer Genome Atlas (TCGA) database, and ESCC-related human cell lines. In addition, the relationships between PVT1 expression and clinicopathological parameters and the prognosis of ESCC patients were assessed. The biological function of PVT1 *in vitro* was assessed using built vectors of silencing lentivirus. Furthermore, bioinformatics and proteins expression analyses were performed to investigate the regulatory mechanism of PVT1.

This study was approved by the Ethics Committee of Wuwei Tumor Hospital of Gansu (No. WWZLYY-LL-200603) and compliance with the Declaration of Helsinki. Written informed consent was provided by all patients before the start of the study. A total of 77 patients with ESCC (age 40–75 years) from Wuwei Tumor Hospital of Gansu (China) were recruited between 2019 and 2020 [Supplementary Table 1, http://links.lww.com/CM9/A992]. All patients were diagnosed using the Cancer Staging Manual and had no cancer other than ESCC. Non-cancerous tissues were located ≥5 cm from the tumor edge. Samples were stored in RNAlater (Ambion; Thermo Fisher Scientific, Inc.) at −80°C until subsequent experimentation. Patients' information was recorded, including sex, age, tumor-node-metastasis (TNM) stage, and pathological type. Statistical analysis was performed using SPSS Statistics 21.0 software (International Business Machines Corporation, Armonk, NY, USA). The paired *t* test, analysis of variance, Tukey's posthoc test, and x^2 test were used to compare differences between groups. Kaplan–Meier survival analysis was performed to assess the association between PVT1 expression and the survival of patients with ESCC. $P < 0.05$ was considered to indicate a statistically significant difference.

Integrated analysis of PVT1 expression was performed using 279 profiles of ESCC tumor tissues and normal esophagus tissues sequencing data from TCGA database [Supplementary Table 2, http://links.lww.com/CM9/A992]. In addition, PVT1 expression levels in 77 pairs of ESCC tumor tissues and their paired adjacent non-cancerous tissues were detected by real-time quantitative PCR (RT-qPCR). Results showed that the PVT1 expression was remarkably up-regulated (10.88 ± 2.41 *vs.* 13.46 ± 3.29; $P < 0.05$; Figure 1A). PVT1 expression levels in microarray detection and TCGA database were also remarkably raised in ESCC tumor tissues than non-cancerous esophagus tissues (fold change = 6.82 ± 1.79, 9.81 ± 1.57, and 6.94 ± 0.37; Figure 1B). As shown in Supplementary Table 3, http://links.lww.com/CM9/A992, the increased risk of 77 ESCC patients were linked with PVT1 increased expression (odds ratio [OR], 1.639; $P < 0.05$) and the TCGA database (OR, 1.327; $P < 0.05$). In this study, we found that the OR value of PVT1

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Chengyun Li and Xiong Yue contributed equally to this work.

Correspondence to: Dr. Chengyun Li, Department of Toxicology, School of Public Health, Lanzhou University, Lanzhou, Gansu 730000, China
E-Mail: 13814008655@163.com

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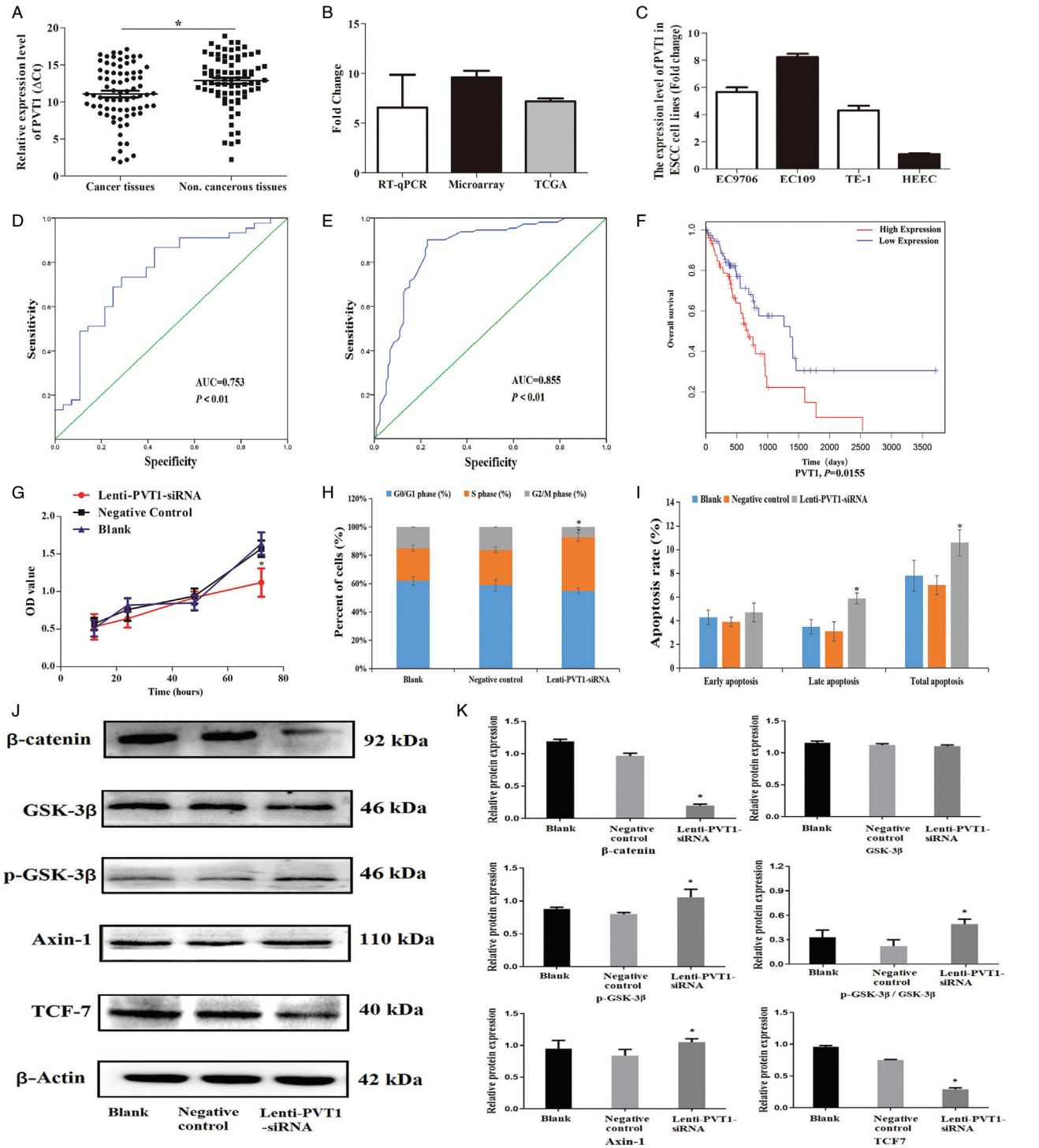

Figure 1: (A) PVT1 expression level was significantly up-reguated in ESCC tumor tissues ($n = 77$), $^{*}P < 0.05$. (B) The expression of PVT1 in ESCC tumor tissues, microarray data, and TCGA database. (C) RT-qPCR analysis of PVT1 expression in ESCC cell lines EC9706, EC109, and TE-1, and normal esophageal mucosa cell line HEEC. (D) ROC curve of the expression level of PVT1 in ESCC tissues for RT-qPCR ($n = 77$). (E) ROC curve of the expression level of PVT1 of ESCC tissues from TCGA database RNA sequencing ($n = 279$). (F) Kaplan–Meier survival analysis of the PVT1 expression and TCGA ESCC patients' overall survival time correlation. (G) Effect of lentivirus-PVT1-siRNA on EC109 cell lines, Cell proliferation was measured by MTT assay; (H) Effects of lentivirus-PVT1-siRNA on the cell cycle of EC109 cells, $^{*}P < 0.05$. (I) Effects of lentivirus-PVT1-siRNA on apoptosis of EC109 cells. (J) Typical western blotting bands. (K) Relatively optical density analyzed of proteins expression by Image pro plus software. ESCC: Esophageal squamous cell carcinoma; HEEC: Human esophageal epithelial cell; MTT: Thiazolyl blue tetrazolium bromide; PVT1: Plasmacytoma variant translocation 1 gene; ROC: Receiver operating characteristic; RT-qPCR: Real-time quantitative PCR; siRNA: Small interfering RNA; TCGA: The Cancer Genome Atlas.

expression in patients with ESCC and TCGA database were both greater than 1.0, which suggested that PVT1 may play an oncogene role during the ESCC development.

Receiver operating characteristic (ROC) curve analysis showed that the PVT1 can distinguish ESCC patients' tumor tissues and non-tumor tissues with high diagnostic power (area under the curve [AUC], 0.753; $P < 0.05$, 95%

confidence interval: 0.717–0.839) and the TCGA database (AUC, 0.855, $P < 0.05$, 95% confidence interval: 0.804–0.906) [Figure 1D and 1E].

Supplementary Table 4, http://links.lww.com/CM9/A992 shows that PVT1 was up-regulated in 77 ESCC tumor tissues and associated with the tumor degree of differentiation and TNM stages of patients with ESCC ($P < 0.05$). Kaplan–Meier survival analysis demonstrated that patients with high PVT1 expression had a poor prognosis ($P < 0.05$; Figure 1F). In addition, the PVT1 was up expressed in ESCC cell lines (EC9706, EC109, and TE-1) which compared with normal human esophageal epithelial cell [Figure 1C].

To explore the potential biological function of PVT1 in ESCC progression, EC109 cells were stably transfected with lentivirus-PVT1-small interfering RNA (siRNA) or negative control lentivirus. As presented in Supplementary Figure 1A and 1B, http://links.lww.com/CM9/A992, the transfection efficiency was almost 100% and decreased by 25.07 times in EC109 cells by transfected with lentivirus-PVT1-siRNA ($P < 0.05$; Supplementary Figure 1C, http://links.lww.com/CM9/A992).

The thiazolyl blue tetrazolium bromide assay demonstrated that the proliferative ability of EC109 cells significantly decreased following transfection with lentivirus-PVT1-siRNA than blank and negative controls ($P < 0.05$; Figure 1G). Cell cycle assay detection results showed that the lentivirus-PVT1-siRNA transfection significantly decreased the number of EC109 cells in the G_2/M phase, while increasing the number of cells in the S phase, which compared with the blank and negative control group ($P < 0.05$; Figure 1H and Supplementary Figure 1D, http://links.lww.com/CM9/A992). Flow cytometry detection showed that the late cell apoptosis rate and the total cell apoptosis rate were significantly increased in the lentivirus-PVT1-siRNA stable transfection group, which compared with the blank and negative control group ($P < 0.05$; Figure 1I and Supplementary Figure 1E, http://links.lww.com/CM9/A992).

Based on the TCGA database and the competing endogenous RNA theory, we analyzed the PVT1 coexpressed ESCC differentially expressed mRNAs functionally enriched and potentially regulated signaling pathways. We have found that there were 83 Gene Ontology terms and 43 pathways enrichment score more than two times ($P < 0.05$). Results suggested that the most enriched function of up-regulated mRNAs was "Regulation of cellular response" and "Biological process," such as "Cell division," "Biological regulation," etc. [Supplementary Figure 1F, http://links.lww.com/CM9/A992]. In addition, we found that the predominantly enriched tumor-associated signaling pathways were in "Cancer," "Wnt signaling pathway," and "MAPK signaling pathway" [Supplementary Figure 1G, http://links.lww.com/CM9/A992]. Notably, the results of our previous study also revealed that the Wnt signaling pathway is involved in regulating the cancer cell cycle and affecting the process of ESCC. Thus, to further verify whether PVT1 regulates the development of ESCC through the Wnt signaling pathway, western blot was used to analyze the differences in expression levels of the key proteins. Results showed that the protein expression levels of b-catenin and TCF-7 were downregulated in the lentivirus-PVT1-siRNA group, p-GSK-3β/GSK-3β and Axin1 was up-regulated in the lentivirus-PVT1-siRNA group than blank and negative controls ($P < 0.05$; Figure 1J and 1K). The results demonstrated that the Wnt pathway was inhibited by transfection with lentivirus-PVT1-siRNA.

The present study demonstrated that up-regulated PVT1 expression in ESCC tissues was associated with a higher occurrence rate of ESCC, which was similar to other reports concerning PVT1.[5] ROC curve analysis demonstrated that PVT1 may be a valid diagnostic biomarker of ESCC. Cell functional experiments revealed that the lentivirus-PVT1-siRNA transfection inhibits the proliferation of EC109 cells by affecting the process of the cell cycle. In addition, our results also revealed that the PVT1 may affect the function of EC109 cells by regulating and activating the Wnt signaling pathway.

To conclude, up-regulated PVT1 can induce the ESCC tumorigenesis through regulating the cell cycle and Wnt signaling pathway. These results provide a novel insight that PVT1 may be prospective biomarkers and therapeutic target for patients with ESCC.

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Conflicts of interest

None.

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