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LETTER TO THE EDITOR

Long noncoding RNA *PVT1* promotes tumor growth and predicts poor prognosis in patients with diffuse large B-cell lymphoma

Dear Editor,

Diffuse large B-cell lymphoma (DLBCL) is the most common and aggressive subtype of non-Hodgkin lymphoma (NHL), accounting for about 40% of all NHL cases [1]. Lacking symptoms at early time and efficient therapeutic methods made DLBCL one of the most life-threatening types of hematopoietic malignancy [2, 3]. Therefore, identifying novel therapeutic biomarker for early detection and prognosis prediction is urgently needed.

Thanks to large-scale gene expression profiling between DLBCL and normal B cells, vast groups of genes have been found deregulated. Moreover, various long non-coding RNAs (lncRNAs) have been reported aberrantly expressed in DLBCL [4, 5]. Previous studies showed that lncRNA plasmacytoma variant translocation 1 (*PVT1*) is an important epigenetic regulator with critical roles in human tumors [6]. Indeed, by searching the online database (starBase pan-cancer project database, version 3.0; http://starbase.sysu.edu.cn/), we found that *PVT1* was elevated in most kinds of tumors (Supplementary Figure S1A). However, the overall biological role and clinical significance of *PVT1* in DLBCL remains largely unknown.

In order to determine the possible involvement of *PVT1* in DLBCL, we retrieved 286 formalin-fixed, paraffin-embedded (FFPE) DLBCL tissues and 62 normal lymph node FFPE tissues from DLBCL patients treated at Renji hospital (Shanghai, China) between January 1st, 2012 and December 31st, 2017. Furthermore, we also collected 46 paired fresh DLBCL tissues and normal lymph node tissues. The clinical information of the enrolled DLBCL patients was recapitulated in Supplementary Table S1. The materials and methods used in this study were detailed in Supplementary information. We firstly assessed *PVT1* expression via real-time PCR (RT-PCR) in 46 paired fresh DLBCL tissues and normal lymph node tissues. The expression of *PVT1* was found to be significantly increased in DLBCL tissues as compared to their normal counterparts ($P < 0.001$; Supplementary Figure S1B). *PVT1* upregulation in tumor tissues was detected in 39 (84.8%) patients with DLBCL (Supplementary Figure S1C). Furthermore, in situ hybridization (ISH) staining on 286 DLBCL and 62 normal lymph node FFPE tissues confirmed that *PVT1* expression was remarkably increased in DLBCL FFPE tissues ($P < 0.001$; Supplementary Figure S1D). These results suggested that the *PVT1* was significantly upregulated in DLBCL tissues.

In order to explore the clinical significance of *PVT1* in DLBCL patients, we then analyzed the correlation between *PVT1* expression and clinicopathological features in DLBCL patients. We found that the expression of *PVT1* was positively correlated with the expression of myelocytomatosis oncogene (*MYC*) in 46 fresh DLBCL tissues ($P < 0.001$; Supplementary Figure S2A). Additionally, the ISH score of *PVT1* was positively correlated with tumor size and $Ki67^+$ cell rate in those 286 DLBCL (both $P < 0.001$; Supplementary Figure S2B and C). Both univariate (Chi-squared test) and multivariate (Logistic regression) analyses confirmed that high *PVT1* expression was associated with multiple extranodal site involvement (ESI) in DLBCL patients ($P = 0.007$; Supplementary Table S1). DLBCL patients were then divided into low and high *PVT1* expression groups according to the ISH score for survival analysis (Supplementary Figure S2D). Kaplan-Meier analysis was performed to assess the prognostic significance of *PVT1* expression in 286 DLBCL patients. The results showed that patients with low *PVT1* expression

Abbreviations: CI, confidence interval; DLBCL, diffuse large B-cell lymphoma; ESI, extranodal site involvement; FFPE, formalin-fixed, paraffin-embedded; HR, hazard ratio; IPI, international prognostic index; ISH, in situ hybridization; lncRNA, long non-coding RNA; *MYC*, myelocytomatosis oncogene; NCCN, national comprehensive cancer network; NHL, non-Hodgkin lymphoma; OS, overall survival; PFS, progression-free survival; *PVT1*, plasmacytoma variant translocation 1; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; RR, relative risk; RT-PCR, real-time PCR; shRNA, short hairpin RNA; siRNA, small interfering RNA

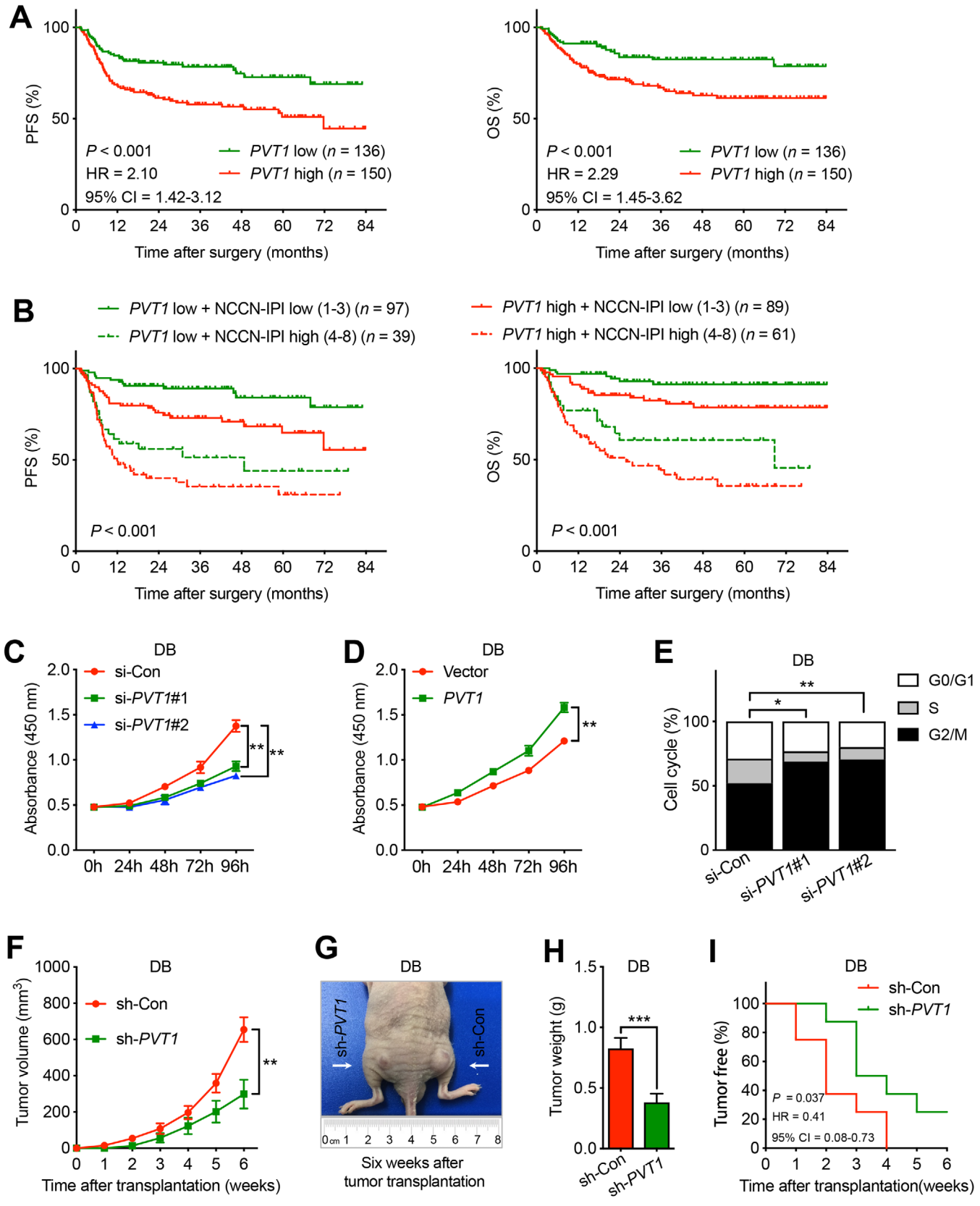

FIGURE 1 *PVT1* is a prognostic factor for DLBCL patients and plays an important oncogenic role in DLBCL *in vitro* and *in vivo*. A. Kaplan-Meier analysis of the correlation between *PVT1* expression and the PFS (left panel) or OS (right panel) in 286 DLBCL patients. B. The significance of combination of *PVT1* expression and NCCN IPI score in DLBCL survival (PFS and OS). C. Cell proliferation analysis of DB cells transiently transfected with *PVT1* siRNA (si-*PVT1*#1 *and* si-*PVT1*#2). DB cells transfected with control siRNA (si-Con) were served as

had increased progression-free survival (PFS; hazard ratio [HR] = 2.10; 95% confidence interval [CI] = 1.42-3.12; $P < 0.001$) and overall survival (OS) rate (HR = 2.29; 95% CI = 1.45-3.62; $P < 0.001$; Figure 1A). The 5-year PFS and OS rates of high and low *PVT1* expression groups were 51.0% *vs*. 72.8% and 61.4% *vs*. 82.5%, respectively. Multivariate Cox proportional hazards model showed that high *PVT1* expression was associated with reduced PFS (relative risk [RR] = 1.85; 95% CI = 1.19-2.89; $P = 0.006$) and OS rates (RR = 1.91, 95% CI = 1.13-3.23; $P = 0.015$; Supplementary Table S2). Moreover, we analyzed the PFS and OS by combine *PVT1* expression and national comprehensive cancer network international prognostic index (NCCN IPI) to assess the prognosis prediction ability. We found that this combination could further divide the DLBCL patients into four groups, with the group of both low *PVT1* expression and low NCCN IPI possess the best PFS and OS while both high *PVT1* expression and high NCCN IPI group possess the worst (Figure 1B). Taken together, our results provide evidence showing the potential of *PVT1* as an important prognostic factor for DLBCL patients.

For further exploring the biological role of *PVT1* in DLBCL, we carried out both *in vitro* and *in vivo* analysis. Reducing *PVT1* expression by two small interfering RNAs (siRNAs) in four DLBCL cell lines (DB, OCI-Ly10, BJAB, and OCI-Ly1) all significantly reduced their proliferation rates (Figure 1C and Supplementary Figure S3A). Contrarily, elevating *PVT1* expression by transfecting *PVT1* overexpression plasmid promoted DLBCL cells proliferation (Figure 1D and Supplementary Figure S3B). Further cell cycle analysis showed that reduced *PVT1* expression in DB and OCI-Ly1 induced G0/G1 arrest (Figure 1E and Supplementary Figure S3C). Finally, reducing *PVT1* expression by short hairpin RNA (shRNA) in both DB and OCI-Ly1 impeded tumor growth on mouse xenograft models, as shown by reduced tumor volume, tumor weight, and tumor formation rate (Figure 1F–I and Supplementary Figure S4). Taken together, these results indicate that *PVT1* influenced DLBCL cells' proliferation capacity, possibly through regulating cell cycle progression.

Although the current standard treatment with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) has improved the outcome of DLBCL patients remarkably, there still existed over 30% of patients encounter relapse or refractory [2]. Recent years, the high throughput sequencing technologies have helped define the molecular and biological events with DLBCL development comprehensively [4]. Cumulative evidences suggested the importance of lncRNA in cancer development and progression [5–8]. A recent study into analyzing the lncRNA expression profile of DLBCL patients has suggested a six-lncRNA signature could efficiently predict the outcome of DLBCL patients [4]. Therefore, it is meaningful to find novel lncRNAs for pre-assessment of DLBCL patients' prognosis.

In the present study, we provided evidences that elevated *PVT1* expression was found in DLBCL tissues compared with normal lymph node tissues, and was positively associated with adverse clinicopathological outcomes. Moreover, the *PVT1* expression was also a potent independent poor prognostic factor in DLBCL. Consistently, genome-wide association study confirmed the association between *PVT1* variants with DLBCL development [9]. *PVT1* has been studied in a variety of pathological processes about tumorigenesis and tumor progression. However, the role of *PVT1* played in DLBCL remained to be clarified. Similarly, our results showed that silencing *PVT1* expression significantly inhibit DLBCL cell proliferation both *in vitro* and *in vivo*. Opposite effect was observed with *PVT1* overexpression. Uncontrolled cell proliferation is the hallmark of cancer cells, most of which are fulfilled through directly regulating cell cycle progression [10]. Our flow cytometric results indicated that reducing *PVT1* in DB and OCI-Ly1 cells induced G1 phase arrest and suppressed cell proliferation. However, similar effects were not observed among OCI-Ly10 and BJAB cells, suggesting other mechanisms might be used by *PVT1* in regulating DLBCL tumorigenesis.

In conclusion, our investigations first elucidated the dysregulation of *PVT1* expression in DLBCL, and determined the prognostic value of *PVT1* for DLBCL patients. Furthermore, we confirmed the oncogenic role of *PVT1* in DLBCL development both *in vitro* and *in vivo*. These would possibly provide rationale for future targeting *PVT1* in DLBCL.

control. D. Cell proliferation analysis of DB cells transiently transfected with *PVT1*overexpression plasmids (*PVT1*). DB cells transfected with empty vector (Vector) served as control. E. Cell cycle distributions of DB cells transfected with *PVT1* siRNA (si-*PVT1*#1 *and* si-*PVT1*#2). The cell cycle distributions were assessed by measuring DNA content of PI-stained cells using flow cytometry. DB cells transfected with control siRNA (si-Con) were served as control. F. Tumor growth curves of DB cells transfected with *PVT1* shRNA *in vivo*. DB cells transfected with control shRNA (sh-Con) were served as control. G. Representative images of mice at the 6th week after separately subcutaneous transplantation of DB cells transfected with *PVT1* shRNA. The mice transplanted with DB cells treated with control shRNA (sh-Con) were served as control. H. The mean tumor weight of mice received DB cells with or without *PVT1* knockdown. I. The tumor-free percentages of mice received DB cells with or without *PVT1* knockdown. Bar, SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Abbreviations: *PVT1*, plasmacytoma variant translocation 1; DLBCL, diffuse large B-cell lymphoma; SEM, standard error of mean; PFS, progression-free survival; OS, overall survival; shRNA, short hairpin RNA; siRNA, small interfering RNA; PI, propidium iodide

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The protocol of this study was approved by the Shanghai Ninth People's Hospital and Renji Hospital Affiliated to School of Medicine, Shanghai Jiao Tong University. The need for informed consent was waived.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The datasets are available from the corresponding authors on reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

MZ and LY designed and conducted the study. RY, TS, ML, and YS collected clinical samples and data. RY, ML, YS and QL performed the experiments. MZ and LY wrote the manuscript and prepared the figures and tables. All authors reviewed and approved the final manuscript.

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