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LETTER TO THE EDITOR Open Access

Inhibition of BCL11B induces downregulation of PTK7 and results in growth retardation and apoptosis in T-cell acute lymphoblastic leukemia

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Abstract

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive subtype of leukemia with poor prognosis, and biomarkers and novel therapeutic targets are urgently needed for this disease. Our previous studies have found that inhibition of the B-cell leukemia/lymphoma 11B (*BCL11B*) gene could significantly promote the apoptosis and growth retardation of T-ALL cells, but the molecular mechanism underlying this effect remains unclear. This study intends to investigate genes downstream of *BCL11B* and further explore its function in T-ALL cells. We found that *PTK7* was a potential downstream target of *BCL11B* in T-ALL. Compared with the healthy individuals (HIs), *PTK7* was overexpressed in T-ALL cells, and *BCL11B* expression was positively correlated with *PTK7* expression. Importantly, *BCL11B* knockdown reduced *PTK7* expression in T-ALL cells. Similar to the effects of *BCL11B* silencing, downregulation of *PTK7* inhibited cell proliferation and induced apoptosis in Molt-4 cells via up-regulating the expression of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (*TRAIL*) and *p27*. Altogether, our studies suggest that *PTK7* is a potential downstream target of *BCL11B*, and downregulation of *PTK7* expression via inhibition of the *BCL11B* pathway induces growth retardation and apoptosis in T-ALL cells.

To the Editor:

The B-cell leukemia/lymphoma 11B (*BCL11B*) gene plays an important role in the development of T-cell acute lymphoblastic leukemia (T-ALL) [1, 2]. Our previous studies have shown that down-regulation of *BCL11B* effectively inhibits proliferation and induces apoptosis of T-ALL cells [3, 4]. PTK7 (protein tyrosine kinase 7), the target protein of sgc8 DNA aptamer, has been identified as a potential biomarker for T-ALL [5]. However, the detailed role and downstream molecular mechanisms of *BCL11B* and relationship between *BCL11B* and *PTK7* remain undefined. In this study, we determined the *BCL11B* target genes in T-ALL patients using the Gene Expression Omnibus (GEO) database and used specific siRNAs (small interfering ribonucleic acid) to down-regulate the expression of this gene in T-ALL cell lines to explore the mechanism.

A total of 220 de novo T-ALL patients from the GEO database and 36 peripheral blood mononuclear cells (PBMCs) of T-ALL from our center were used for analysis and validation. In GSE13159 and GSE28497 datasets, we found that the *BCL11B* was highly expressed in T-ALL ($P < 0.001$, Fig. 1a and S1a). These

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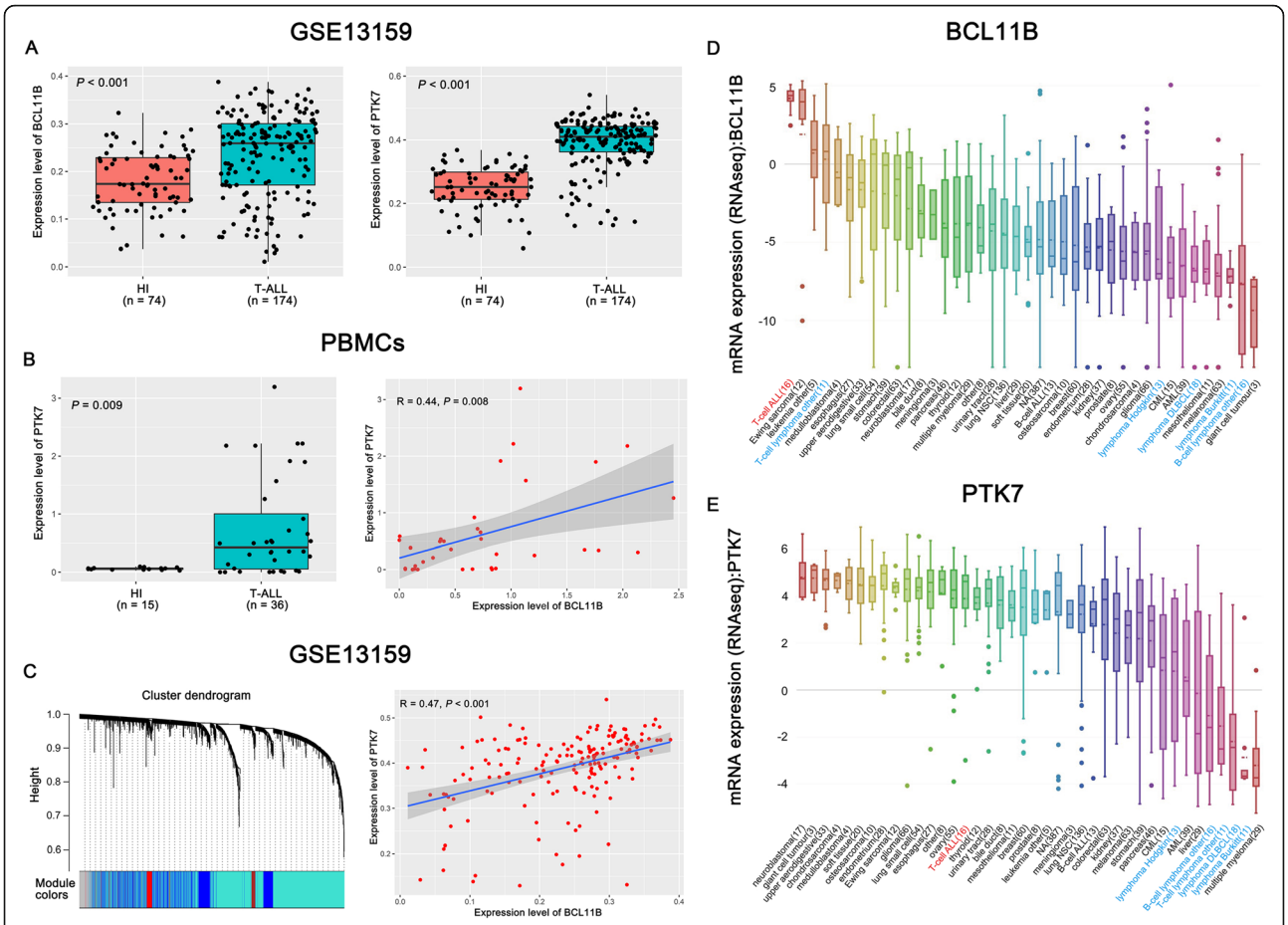

Fig. 1 Positive correlation between *BCL11B* and *PTK7* in T-cell acute lymphoblastic leukemia (T-ALL). (**a**) The expression levels of *BCL11B* (left panel) and *PTK7* (right panel) in T-ALL patients and healthy individuals (HIs) in the GSE13159 dataset. (**b**) Validation of the correlation between *BCL11B* and *PTK7*. Expression levels of the *PTK7* gene in PBMCs from T-ALL patients and His (left panel). Linear correlation analysis of the *BCL11B* and *PTK7* genes in T-ALL samples (right panel). (**c**) *BCL11B* and *PTK7* are positively correlated in the GSE13159 dataset. Left panel: co-expression modules were obtained by weighted gene co-expression network analysis (WGCNA). The colored row beneath the dendrogram shows the module assignment determined by the Dynamic Tree Cut. A cluster dendrogram demonstrated that 10,459 differentially expressed genes were enriched in 11 co-expression network modules, while *BCL11B* and *PTK7* were located in the same blue module. Right panel: a positive correlation was detected between *BCL11B* and *PTK7*. Both *BCL11B* (**d**) and *PTK7* (**e**) are highly expressed in T-ALL compared to lymphoma cell lines. Data were obtained through the Cancer Cell Line Encyclopedia (CCLE). Detailed methods are available in Materials and Methods in Additional file 1

results were consistent with our previous study [6]. Moreover, similar results of *PTK7* were found in GSE13159, GSE28497 and PBMCs ($P < 0.05$, Fig. 1a-b and S1b), which was also in line with previous report [7]. Next, to identify genes downstream of *BCL11B*, the *BCL11B* co-expression network was further characterized by weighted gene co-expression network analysis (WGCNA). Interestingly, Bioinformatics analysis [8] showed a significant positive correlation between the expression of *BCL11B* and *PTK7* in GSE13159, GSE28497 and PBMCs ($P < 0.05$, Fig. 1b-c and S1c). Furthermore, we studied the expression of *BCL11B* and *PTK7* in different cell lines from the Cancer Cell Line Encyclopedia (CCLE). Previous studies have shown that *BCL11B* is overexpressed in the acute type of adult T-cell leukemia/lymphoma (ATLL), and it is under-expressed in other lymphoma type. Consistently, *BCL11B* and *PTK7* was highly expressed in the T-ALL cells line but had low expression in the lymphoma cell lines (Fig. 1d-e). Based on these findings, we proposed that *PTK7* might be an important gene downstream of *BCL11B* in T-ALL.

We sought to further verify the association between *BCL11B* and *PTK7* in both T-ALL and non-T-ALL cell lines at the mRNA and protein levels. As shown in Fig. 2a, *BCL11B* and *PTK7* mRNA were highly expressed in T-ALL cell lines (Jurkat and Molt-4), but almost absent in *BCL11B*-negative Burkitt lymphoma cell line (Raji). Next, an aptamer was used to determine the cell surface protein expression of *PTK7* in Jurkat and Raji cells. Sgc-8, the PTK7-specific aptamer, was labeled with a Cy5 fluorescent reporter and incubated with Jurkat and Raji cells under different concentrations, which revealed

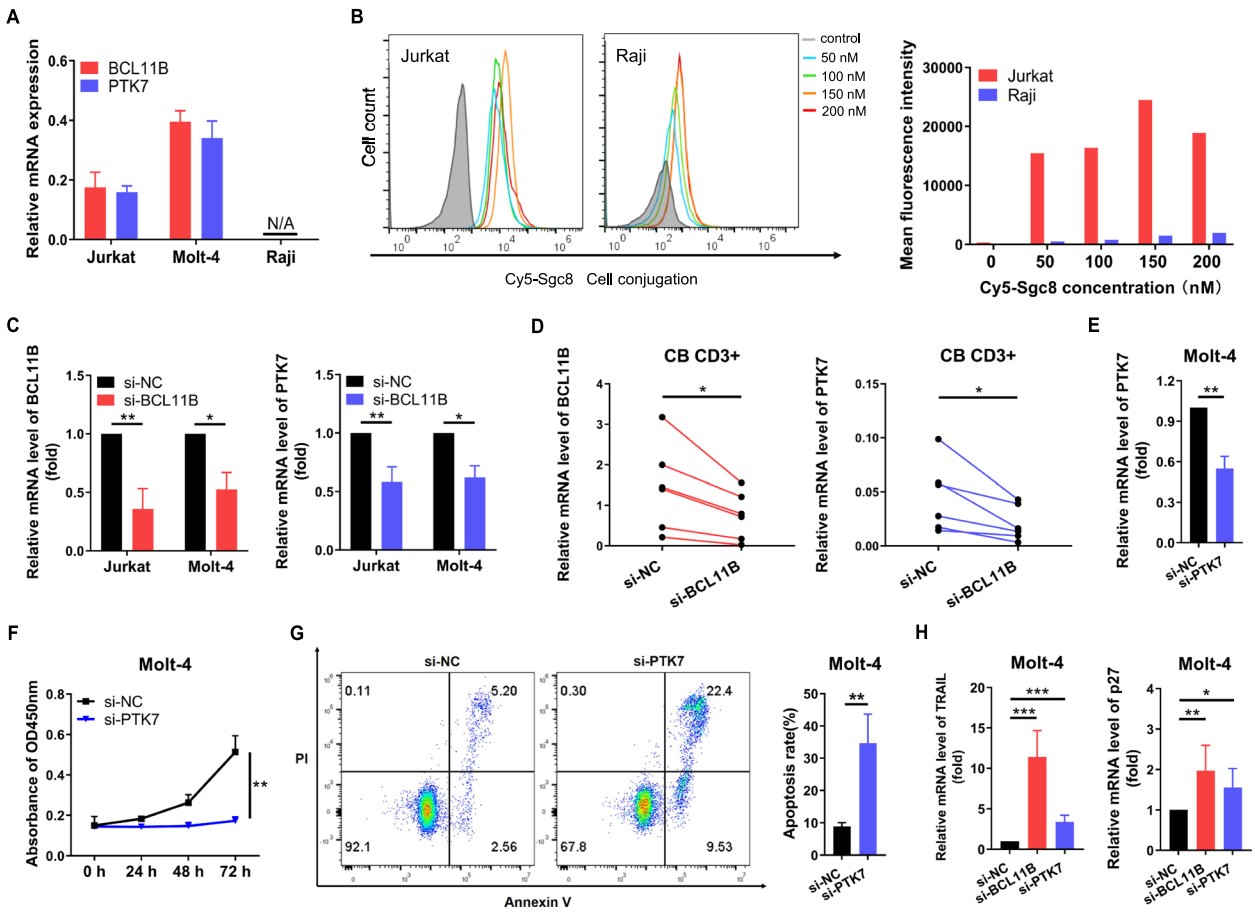

Fig. 2 Downregulation of *PTK7* induces cell growth retardation and apoptosis. (**a**) The *BCL11B* and *PTK7* mRNA expression levels in T-ALL (Jurkat and Molt-4) and non-T-ALL (Raji) cell lines. *GAPDH* was used as an endogenous reference. (**b**) Cultured Jurkat and Raji cells were treated with Cy5-Sgc8 for 1 h at different concentrations. Flow cytometry analysis of the specific cell binding of Cy5-Sgc8 to Jurkat cells. (**c**) Expression of *PTK7* in T-ALL cell lines 48 h after *BCL11B* siRNA transfection. (**d**) Effect of *BCL11B* siRNA on the *BCL11B* mRNA expression level by real-time quantitative polymerase chain reaction (qRT-PCR) 48 h after transfection in CB CD3+ T cells. Negative control siRNA-treated cells were used for comparison. (**e**) Knockdown efficiency in Molt-4 cells were analyzed after knocking down the *PTK7* gene. (**f**) Molt-4 cells were transfected with si-*PTK7* and then cell proliferation was assessed by Cell counting kit-8 (CCK-8) assay. (**g**) Apoptosis in Molt-4 cells transfected with si-PTK7 was measured by flow cytometry after 48 h. (**h**) Expression of the *TRAIL* and *p27* genes in Molt-4 cells 48 h after transfected with *BCL11B* siRNA and *PTK7* siRNA. Non-specific siRNA treated cells were used as negative control. Asterisks signify statistically significant differences (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$)

that Cy5-Sgc8 specifically bound to Jurkat cells but did not react with Raji cells (Fig. 2b). Excitingly, there was a significant decrease in *PTK7* mRNA expression after silencing *BCL11B* expression in the Jurkat and Molt-4 cells and cord blood (CB) CD3+ T cells (Fig. 2c-d). These results confirmed that *PTK7* might be regulated by the *BCL11B* signaling pathway in both T-ALL cell lines and human CD3+ T cells. Based on the above results, we attempted to further understand the role of *PTK7* in T-ALL cells. Compared to the negative control, the proliferation of Molt-4 cells transfected with *PTK7*-siRNA was significantly decreased ($P < 0.01$, Fig. 2e-f). Meanwhile, the Annexin V/PI-positive cells demonstrated a significant increase for *PTK7*-siRNA transfected Molt-4 cells, reaching 34.66 ± 5.21% ($P = 0.008$, Fig. 2g). In addition, recent reports have shown that tumor necrosis factor (TNF)-related apoptosis-inducing ligand (*TRAIL*) and *p27* are found to be involved in the *BCL11B* pathway to regulate the cell cycle and apoptosis [3, 9, 10]. Interestingly, significant increases in the expression levels of TRAIL and p27 were detected in the *PTK7*-siRNA group, which was consistent with the trend exhibited in the *BCL11B*-siRNA group (Fig. 2h). These data suggested that *PTK7* was a downstream *BCL11B* target gene in T-ALL cell growth and apoptosis.

In summary, this is the first report demonstrating that *PTK7* is an important functional downstream target gene of *BCL11B* in T-ALL. Our study provides rationale for targeting *BCL11B/PTK7* in the development of therapeutics for T-cell malignancies.

Abbreviations

BCL11B: B-cell leukemia/lymphoma 11B; CCLE: Cancer cell line encyclopedia; GEO: Gene expression omnibus database; HI: Healthy individual; PTK7: Protein tyrosine kinase receptor 7; PBMCs: Peripheral blood mononuclear cells; siRNA: Small interfering ribonucleic acid; T-ALL: T-cell acute lymphoblastic leukemia; TRAIL: Tumor necrosis factor (TNF)-related apoptosis-inducing ligand; WGCNA: Weighted gene co-expression network analysis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40364-021-00270-3.

Additional file 1. Materials and Methods.

Additional file 2: Figure S1. Expression patterns of *BCL11B* and *PTK7* in the GSE28497 dataset. High expression of *BCL11B* (A) and *PTK7* (B) in T-ALL. (C) *BCL11B* and *PTK7* had a positive correlation.

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Not applicable.

Authors' contributions

Kehan Li, Cunte Chen and Rili Gao performed the experiments, wrote the paper, and analyzed the data. Xibao Yu, Youxue Huang, Zheng Chen, Zhuandi Liu, Shaohua Chen and Xin Huang helped analyze the data. Gengxin Luo provided primary cells and patient information. Grzegorz K. Przybylski, Yangqiu Li, and Chengwu Zeng designed the study and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data available on request.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of the affiliated hospitals of Jinan University. Written informed consent was obtained from all patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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