Analysis of the expression of PHTF1 and related genes in acute lymphoblastic leukemia

Xin Huang¹,², Suxia Geng², Jianyu Weng², Zesheng Lu², Lingji Zeng³, Minming Li³, Chengxin Deng³, Xiuli Wu³, Yangqiu Li³,⁴ and Xin Du²*

Abstract

Background: Previous study showed that downregulated BCL11B expression in T cell acute lymphoblastic leukemia (T-ALL) cell line Molt-4 inhibited cell proliferation and induce apoptosis, which may be related to PHTF1 gene overexpression. The objective of this study was to investigate the expression of PHTF1 and related genes in ALL and further explore its function in T-ALL cell lines.

Methods: Real-time PCR was used to determine the gene expression level of PHTF1 in hematologic malignancies. The PHTF1, BCL11B, FEM1B and Apaf-1 gene expression levels and correlations were analyzed in patients with primary ALL (including T-ALL and B-ALL) and healthy individuals (HIs). Inhibition and overexpression of PHTF1 by lentiviral transduction were performed using the Molt-4 and Jurkat cell lines. Cell growth and apoptosis were measured by the Cell Counting Kit-8 assay and flow cytometry, respectively. Upon PHTF1 overexpression, the BCL11B, FEM1B and Apaf-1 gene expression levels were determined by real-time PCR.

Results: PHTF1 overexpression was found in both T-ALL (p = 0.004) and B-ALL (p < 0.001) groups compared with HIs group. A trend toward a negative correlation between the PHTF1 and BCL11B genes was detected for the T-ALL group, while positively correlated expression was found for the PHTF1 and BCL11B genes in HIs (p = 0.001). FEM1b and Apaf-1 overexpression was found in recently diagnosed ALL patients compared with HIs (p < 0.05). Positively correlated expression was found for the PHTF1, FEM1b and Apaf-1 genes in patients with ALL (p < 0.05) and HIs (p < 0.05). Direct up-regulation of PHTF1 expression inhibited the proliferation of Jurkat and Molt-4 cells and effectively induced apoptosis in Molt-4 cells. Direct inhibition of PHTF1 expression had no significant effect on the proliferation or apoptosis of Jurkat and Molt-4 cells. FEM1b and Apaf-1 overexpression, which did not obviously alter the BCL11B expression level, was detected in PHTF1-transduced T-ALL cell lines.

Conclusions: PHTF1 overexpression is responsible for regulating cell proliferation and apoptosis in T-ALL cell lines. PHTF1 may be a tumor-suppressor like gene and a therapeutic target for triggering the PHTF1-FEM1b-Apaf-1 apoptosis pathway.

Keywords: PHTF1, BCL11B, FEM1b, Apaf-1, ALL, Overexpression, Proliferation, Apoptosis

Background

T cell acute lymphoblastic leukemia (T-ALL) results from clonal malignant T cell proliferation, is an aggressive malignancy that does not respond well to chemotherapy and has a poorer prognosis [1, 2]. The cellular biology and pathogenesis of T-ALL are relatively complex, and these might be related to the different original malignant T cell clone [3–5]. Complex acquired genetic aberrations include chromosomal translocations, gene rearrangements and mutations, resulting in the abnormal expression of oncogenes such as Notch1, TAL1 (T-cell acute
lymphoblastic leukemia 1), and BCL11B (B-cell chronic lymphocytic leukemia/lymphoma 11B), which may be associated with advanced disease and resistance to treatment [6–10].

The B-cell leukemia/lymphoma 11B (BCL11B) gene is a member of the BCL family and plays a crucial role in the development, proliferation, differentiation, and subsequent survival of T cells [11]. BCL11B gene alterations are related to the malignant T cell transformation that occurs in hematological malignancies [6, 12–15]. Remarkably, the BCL11B gene is responsible for regulating apoptosis and cell proliferation [16–18]. Previous studies [16–18] have shown that inhibition of BCL11B expression by siRNA selectively inhibits proliferation and effectively induces apoptosis in T-ALL cell lines (Jurkat and Molt-4) but not in normal mature T and CD34+ cells [17, 19]. Additionally, global gene expression profiling has revealed that BCL11B siRNA-mediated apoptosis in Molt-4 cells might be related to the PHTF1 gene [10].

PHTF1 (putative homeodomain transcriptional factor) is a putative homeobox gene located at 1p11-p13 in the human genome [20]. This gene is evolutionarily conserved [21] and mainly expressed in the testis [20]. As a transcription factor, the PHTF1 gene is mainly involved in biological processes such as DNA-dependent transcription and the regulation of biological processes. However, studies on the PHTF1 gene in leukemia have not been reported. FEM1b (feminization-1 homolog b) has been identified as a binding partner for PHTF1 [22]. Previous in vitro experiments have suggested that human FEM1b is involved in apoptosis. FEM1b is a proapoptotic protein that interacts with the apoptosis-inducing proteins Fas, tumor necrosis factor receptor-1 (TNFR1), and apoptotic protease activating factor-1 (Apaf-1) [23]. Therefore, we hypothesized that the BCL11B gene and the PHTF1-FEM1b-Apaf-1 pathway may work together in tumor cell apoptosis. In this study, we analyzed the expression level of PHTF1 and its related genes for the first time in patients with ALL. To further explore its function in T-ALL cell lines, we performed experiments involving the down regulation or overexpression of PHTF1 in T-ALL cell lines using growth and apoptosis assays in vitro.

Results
Expression characteristics and correlation analysis of the PHTF1 and BCL11B genes in T-ALL and B-ALL patients and HIs
In order to characterize the expression of PHTF1 in primary T-ALL samples, we detected the expression level of PHTF1 in peripheral blood mononuclear cells (PBMCs) from 9 cases with T-ALL (median: 2.73 %, mean rank: 20.33, \( P = 0.004 \)), as well as 13 cases with B-ALL (median: 3.34 %, mean rank: 21.54, \( P < 0.001 \)). Overexpression of PHTF1 was found in both groups in comparison with HIs group, and there was no significant difference between the two ALL subtypes (Fig. 1a).

As previously reported [24], the BCL11B mRNA expression level in PBMCs from patients with T-ALL (median: 389.04 copies/10^5 β2M copies, mean rank: 26.56, \( P < 0.001 \)) was significantly higher than that in patients with B-ALL (median: 30.39 copies/10^5 β2M copies, mean rank: 7.69) (Fig. 1b).

Spearman’s rank correlation analysis of the expression levels of the PHTF1 and BCL11B genes was performed for patients with T-ALL and B-ALL. No significant correlation was found for the PHTF1 and BCL11B genes in the
B-ALL patients ($rs = 0.044, P = 0.887$) (Fig. 2a). However, a negative correlation trend was detected for the $PHTF1$ and $BCL11B$ genes in the T-ALL patient ($rs = -0.083, P = 0.831$) (Fig. 2b). A positively correlated expression level for the $PHTF1$ and $BCL11B$ genes was found in the HIs ($rs = 0.891, P = 0.001$) (Fig. 2c).

**Overexpression characteristics and positive correlation of the $PHTF1$, $FEM1b$ and Apaf-1 genes in ALL patients and HIs**

$FEM1b$ and Apaf-1 overexpression was found in recently diagnosed ALL ($FEM1b$ median: 11.19 %, mean rank: 15.90, $P = 0.014$) ($Apaf-1$ median: 7.47 %, mean rank: 16.87, $P = 0.001$) patients compared with HIs ($FEM1b$ median: 4.31 %, mean rank: 8.65) ($Apaf-1$ median: 2.52 %, mean rank: 7.20) (Fig. 3).

Positively correlated expression for the $PHTF1$, $FEM1b$ and Apaf-1 genes was found in ALL patients ($PHTF1$ vs. $FEM1b$: $rs = 0.864, P < 0.001$ and $FEM1b$ vs. Apaf-1: $rs = 0.682, P = 0.005$) (Fig. 4a, b) and HIs ($PHTF1$ vs. $FEM1b$: $rs = 0.939, P < 0.001$ and $FEM1b$ vs. Apaf-1: $rs = 0.830 P = 0.003$) (Fig. 4c, d).

**Inhibition or overexpression of $PHTF1$ by lentiviral transduction in T-ALL cell lines**

To investigate the potential role of $PHTF1$ in leukemogenesis, we first transfected Jurkat and Molt-4 cells with an active shRNA pair. We found that the $PHTF1$ expression level in Jurkat (Fig. 5a) and Molt-4 (Fig. 5b) cells was downregulated approximately 3-fold compared with a scrambled lentiviral transduction control group (CON 1).

Similarly, we transduced Jurkat and molt-4 cells using a lentivirus expressing the $PHTF1$ gene. The $PHTF1$ expression level was approximately 2291-fold (Jurkat) (Fig. 5c) and approximately 1100-fold (Molt-4) (Fig. 5d) higher compared with the lentiviral transduction control group (CON 2).

**$PHTF1$ overexpression inhibits the proliferation of Jurkat and Molt-4 cells**

The effect of $PHTF1$ on Jurkat and Molt-4 cell growth was next evaluated. Compared with the negative control group, $PHTF1$ had markedly lower proliferation in both Jurkat ($n = 3, P = 0.004$) (Fig. 6a) and Molt-4 cells ($n = 3, P = 0.007$) (Fig. 6b). However, we found no obvious proliferation inhibition after $PHTF1$ knockdown in Jurkat (Fig. 6c) and Molt-4 cells (Fig. 6d).

**$PHTF1$ overexpression remarkably induces apoptosis in Molt-4 cells**

We then explored the effects of $PHTF1$ overexpression on T-ALL apoptosis in in vitro assays. The ratio of...
apoptosis in Molt-4 cells was 78.67 ± 29.26 %, and it was 15.50 ± 5.97 % for the negative control group (n = 3, P = 0.021) (Fig. 7a). Interestingly, the apoptosis ratio for Jurkat cells was 15.87 ± 5.65 %, while it was 9.57 ± 2.35 % for the negative control group. No significant difference between these two groups was found (n = 3, P = 0.150) (Fig. 7b). Notably, we could observe an increase in the trend toward apoptosis for Jurkat cells after PHTF1 upregulation; however, we found no significant apoptosis after PHTF1 knockdown in the Jurkat (Fig. 7c) and Molt-4 cells (Fig. 7d).

**PHTF1-related gene expression in Jurkat and Molt-4 overexpressing cells**

To further examine the mechanism by which PHTF1 regulates apoptosis, we investigated the expression of PHTF1-related genes in PHTF1 (PHTF1) or control (CON 2) infected Jurkat and Molt-4 cells. In infected Jurkat cells, the BCL11B expression level was unchanged, while FEM1b and Apaf-1 were upregulated approximately 2.4- and 4.4-fold, respectively (Fig. 8a). In transfected Molt-4 cells, the BCL11B expression level was also unchanged, while FEM1b and Apaf-1 were upregulated approximately 6.3- and 29.4-fold, respectively (Fig. 8b).

**Discussion**

Despite significant improvement in our understanding of T-ALL biology and pathogenesis [25], knowledge of the T cell activative signaling pathways involved in T-ALL remains limited. Thus, novel molecular insights and therapeutic approaches are urgently needed. Thus, based on previous finding that BCL11B siRNA-mediated apoptosis in the Molt-4 T-ALL cell line might be related to the PHTF1 gene [10], we focused our attention on the characteristics of BCL11B and PHTF1 gene expression in T-ALL patients. We found a positive correlation between the PHTF1 and BCL11B genes in healthy individuals. In contrast, a trend toward a negative correlation was found for the PHTF1 and BCL11B genes in the T-ALL patient although there was no statistical significance, which may be due to the limited number of samples. However, this result may indicate that there are different expression patterns for both of these genes in T-ALL, and for further confirmation of the relationship between PHTF1 and BCL11B is needed in a larger cohort of T-ALL samples. Low BCL11B expression is associated with poor prognosis, particularly in the standard risk group for thymic T-ALL [26]. PHTF1 and BCL11B genetic disorders may contribute to T-ALL pathogenesis.

To further explore its function in T-ALL cell lines, we downregulated and overexpressed PHTF1 in T-ALL cell lines and examined cell line growth and apoptosis using in vitro assays. PHTF1 up-regulation inhibits the proliferation of Jurkat and Molt-4 cells and effectively induces apoptosis in Molt-4 cells. The BCL11B expression level was unchanged, while FEM1b and Apaf-1 were upregulated. Interestingly, compared with the Jurkat cells, the remarkable apoptosis of Molt-4 cells may be related to the higher FEM1b and Apaf-1 expression level. Based on the reports regarding the expression characteristics of tumor suppressor genes in leukemia, such as wilms tumor 1 (WT1), which was consistently found to be highly expressed in peripheral blood (PB) or bone marrow (BM) in acute myeloid leukemia (AML) and is used for inhibiting tumor targeting [27], we hypothesized that PHTF1 is involved in negative regulation of tumor growth. Therefore, we considered that PHTF1 has tumor-suppressive activity and triggers the PHTF1-FEM1b-Apaf-1 apoptosis pathway using in vitro assays. However, direct inhibition of PHTF1 expression, and the BCL11B expression level was unchanged (data not shown), had no significant effect on the proliferation or apoptosis of Jurkat and Molt-4 cells. Therefore, we considered that PHTF1 might be the downstream gene of the BCL11B.

In order to further characterize the role of PHTF1 in T-ALL, it is of interest to analyze the downstream genes regulated by PHTF1. Because overexpression of PHTF1 was found in both groups (T-ALL and B-ALL) in comparison with HIs group, we characterized the expression of FEM1b and Apaf-1, which are mainly involved in apoptosis in ALL patients and HIs. In a previous study [22], western blotting and immunofluorescence assays revealed the presence of PHTF1 and FEM1b in the same cells, and association between these proteins was demonstrated by co-immunoprecipitation. Previous in vitro
experiments have suggested that the human FEM1b gene is involved in apoptosis. Human FEM1b is 99% identical to the mouse protein, and it is reportedly capable of associating with the intracellular tail of the death membrane receptors Tnfrsf6 (tumor necrosis factor receptor superfamily, member 6; also known as Fas) and Tnfrsf1a (also known as TNFR1) [28]. Proteasome inhibitor treatment of SW620, HCT-116, and DLD-1 cells led to upregulation of the FEM1b protein and was associated with apoptosis induction. Blockade of FEM1b upregulation with morpholino antisense oligonucleotides suppressed proteasome inhibitor-induced apoptosis in these cells. The authors of this study have demonstrated that FEM1b can induce apoptosis when overexpressed in some cell lines. The proapoptotic protein FEM1b could represent a novel molecular target for overcoming apoptosis resistance in colon cancer therapy [29]. As a binding protein for FEM1b, Apaf-1 is a central component of the intrinsic apoptosis pathway. High Apaf-1 expression elevates erythroid apoptosis in iron overload myelodysplastic syndrome [30]. Zermati et al. have suggested that Apaf-1 deficiency contributes to tumor progression not only by decreasing activation of the apoptotic caspase but also by reducing DNA damage-induced cell cycle arrest, thus weakening the cytostatic effects of chemotherapy and radiotherapy [31]. FEM1b and Apaf-1 overexpression was found in samples from patients recently diagnosed with ALL, and a positively correlated expression level for the PHTF1, FEM1b and Apaf-1 genes was found in ALL patients and His.

**Fig. 4** Correlation analysis of the PHTF1, FEM1b and Apaf-1 expression levels. Positively correlated expression for the PHTF1, FEM1b and Apaf-1 genes was found in ALL patients (a) PHTF1 vs. FEM1b, (b) FEM1b vs. Apaf-1) and His (c) PHTF1 vs. FEM1b, (d) FEM1b vs. Apaf-1)
characterization of these genes at the gene expression level. To further explore its function in B-ALL, further studies will be performed to address this question.

**Conclusions**

In conclusion, PHTF1 overexpression is responsible for regulating the cell proliferation and apoptosis of T-ALL cell lines. Based on these preliminary findings, and reports regarding the expression characteristics of tumor suppressor genes in leukemia, such as Wilms tumor 1 (WT1), which was consistently found to be highly expressed in peripheral blood (PB) or bone marrow (BM) in acute myeloid leukemia (AML) and is used for inhibiting tumor targeting, we hypothesized that PHTF1 is involved in negative regulation of tumor growth. PHTF1 may be a tumor-suppressor like gene and a therapeutic target for triggering the PHTF1-FEM1b-Apaf-1 apoptosis pathway.

**Methods**

**Samples**

Nine newly diagnosed and untreated patients with T-ALL, and thirteen newly diagnosed and untreated patients with B-ALL were recruited. The diagnoses for all patients were based on cytomorphology, immunohistochemistry, cytoimmunological and cytogenetic analysis. Peripheral blood mononuclear cells (PBMCs) from ten healthy individuals (HIs) served as controls. The details of the samples are listed in Table 1. Peripheral blood was collected by heparin anticoagulation, and PBMCs were separated using the Ficoll-Hypaque gradient centrifugation method. All procedures were conducted in accordance with the guidelines of the Medical Ethics committees of the Health Bureau of Guangdong Province, China.
**Cell culture**

Cells from the well-characterized human T-cell acute lymphoblastic leukemia (T-ALL) cell lines Jurkat and Molt-4 were obtained from American Type Culture Collection (ATCC, Manassas, Virginia, USA) and maintained in RPMI-1640 media (Gibco, New York, USA) containing 10% fetal bovine serum (FBS, NATOCOR, Argentina) at 37 °C and 5% CO₂.

**Lentivirus production and transduction**

Two recombinant lentivirus vectors based on the U6-shRNA-Ubi-EGFP and U6-NC-Ubi-EGFP vectors (purchased from Shanghai GeneChem Co., Ltd) were constructed to target the human PHTF1 gene (NM_006608) and as scrambled negative control, respectively. The target sequence of the PHTF1 shRNA was ACCCTAACTCTCAGGTAAA, and that for the negative control was TTCTCCGAACGTGTCACGT.

Two recombinant lentivirus vectors based on the Ubi-PHTF1-3FLAG-SV40-EGFP and Ubi-3FLAG-SV40-EGFP vectors (purchased from Shanghai GeneChem Co., Ltd) were constructed to express the human PHTF1 gene (NM_006608) and green fluorescence protein gene (negative control), respectively.

The transduced cells were divided into four groups: shRNA transduction (RNAi), scrambled control (CON 1), scrambled control (CON 2), and viral vector (PHTF1). The results demonstrated that the cells infected with PHTF1 shRNA have no significant difference compared with controls. The results represent the mean ± SEM (n = 3). NS no significance.

**Fig. 6** PHTF1 overexpression inhibits the proliferation of Jurkat and Molt-4 cells. 

- **a** Jurkat cells infected with PHTF1 (PHTF1) or control (CON 2) lentivirus were seeded in a 96-well plate and incubated for 72 h. The CCK8 assay demonstrated that cells infected with PHTF1 have lower viability compared with control. The results shown are the mean ± SEM (n = 3). P = 0.004 for bar 1 versus bar 2.

- **b** Molt-4 cells infected with PHTF1 (PHTF1) or control (CON 2) lentivirus were seeded in a 96-well plate and incubated for 72 h. The CCK8 assay demonstrated that the cells infected with PHTF1 have lower viability compared with controls. The results represent the mean ± SEM (n = 3). P = 0.007 for bar 1 versus bar 2.

- **c** Jurkat cells infected with PHTF1 shRNA (RNAi) or control (CON 1) lentivirus were seeded in a 96-well plate and incubated for 72 h. The CCK8 assay demonstrated that cells infected with PHTF1 shRNA have no significant difference compared with controls. The results represent the mean ± SEM (n = 3). NS no significance.

- **d** Molt4 cells infected with PHTF1 shRNA (RNAi) or control (CON 1) lentivirus were seeded in a 96-well plate and incubated for 72 h. The CCK8 assay demonstrated that cells infected with PHTF1-shRNA have no significant difference compared with controls. The results represent the mean ± SEM (n = 3). NS no significance.
PHTF1 gene transduction (PHTF1), and transduction control (CON 2). The lentiviral titers ranged from 1 to $2 \times 10^8$ TU/mL. Transduction was performed by ‘spin-infection’, and cells and lentiviruses (MOI = 5 for Jurkat, MOI = 10 for molt4) plus 10 μg/mL polybrene were mixed and spun at 900 × g for 40 min. The transfected cells were then cultured for 2–3 days prior to analysis by flow cytometry. For GFP cell analysis, GFP+ cells were cultured for 3 days and then FACS purified using the Aria II System (Becton–Dickinson Immunocytometry Systems, San Jose, CA, USA).

RNA extraction and cDNA synthesis
RNA was extracted using the TRizol kit (Invitrogen, Carlsbad, CA, USA), and it was then reverse-transcribed.
into first-strand cDNA using random hexamer primers and the reverse transcriptase Superscript II Kit (TaKaRa, Dalian, PR China) according to the manufacturer’s instructions.

**Real-time polymerase chain reaction**

Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) quantitative detection of the β2 microglobulin (β2M), BCL11B, PHTF1, FEM1b, and Apaf-1 genes in cDNA from PBMCs was performed using TaqMan real-time PCR. To precisely determine the BCL11B copy number, a duplex vector, including a fragment of the BCL11B and β2M genes was constructed and used as a reference (the duplex vector was a gift from Prof. C. A. Schmidt, Ernst-Moritz-Arndt University Greifswald, Germany). Another vector including the PHTF1, FEM1b, and Apaf-1 genes was constructed based on DNA concentration, and it was measured by spectrophotometry and confirmed by quantitative gel electrophoresis. Standard dilutions of the vector ranging from 10^7 to 10^0 copies were prepared.

Briefly, PCR was performed in a 25 μL total volume containing 2 μL cDNA, 25 pmol of each primer, 10 nmol of each dNTP, 1.5 U AmpliTaq Gold (Applied Biosystems, Branchburg, NJ, USA), 5 pmol 6FAM-TAMRA probe, and PCR buffer containing 4.5 mM MgCl2. After an initial denaturation at 95 °C for 5 min, 50 cycles of 95 °C for 15 s and 64 °C for 1 min were performed.

Primers and probes for β2M, BCL11B, PHTF1, FEM1b, and Apaf-1 gene amplification were synthesized by Invitrogen (Carlsbad, CA, USA) (Table 2). The absolute amounts of BCL11B and β2M were measured in two independent assays, and the BCL11B content per 100,000 β2M copies was calculated using the following formula: n = 100,000 × BCL11B/β2M. The amounts of the PHTF1, FEM1b, and Apaf-1 copies were calculated using the following formulas: n = PHTF1/β2M × 100 %, n = FEM1b/β2M × 100 %, and n = Apaf-1/β2M × 100 %.

The absolute amounts of BCL11B and β2M were measured in two independent assays, and the BCL11B content per 100,000 β2M copies was calculated using the following formula: n = 100,000 × BCL11B/β2M [12]. In this study, we used this method to measure BCL11B expression. The standard for other genes (PHTF1, FEM1b and Apaf-1) was constructed using another triple plasmid (synthesized by Invitrogen), and we determined the β2M, PHTF1, FEM1b and Apaf-1 copy number, which were compared relative to the gene expression level of the β2M reference gene between different clinical samples.

**Cell proliferation assay**

Cell proliferation was detected using the Cell Counting Kit-8 (CCK-8) assay according to the manufacturer’s instructions. Briefly, Jurkat and Molt-4 cells transfected with or without lentiviruses at a density of 5 × 10^5 cells/mL were seeded into a 96-well plate (100 μL/well). CCK-8 reagent (10 μL) was then added to each well, and the cells were cultured for 3 h at 37 °C and 5 % CO2. After incubation, the absorption value (450 nm) of each well was measured with a 680-type microplate reader (BioRad, Berkeley, CA, USA).
Table 1 The details of samples used in study

| Diagnosis | Subtype | Numbers | Age (year) |
|-----------|---------|---------|------------|
|           |         | Total   | Male       | Female     | Range | Median |
| ALL       |         | 22      | 17         | 5          | 15–55 | 22.5   |
|           | T-ALL   | 9       | 7          | 2          | 17–27 | 20     |
|           | B-ALL   | 13      | 10         | 3          | 15–55 | 24     |
| His       |         | 10      | 6          | 4          | 22–35 | 25.5   |

Table 2 Sequences of primers and probes for real-time PCR (TaqMan method)

| Primer/probe | Sequence |
|--------------|----------|
| BCL11B-F     | 5′-CACCCCGACGAAGATGACCAC-3′ |
| BCL11B-R     | 5′-CGGCCCGGTTCACAGTGAATG-3′ |
| BCL11B-P     | 5′-FAM-TCAACCGAGAAAGGCATCTGTCACAAGGA-TAMRA-3′ |
| β2M-F        | 5′-CTCGGCTACTCTCTCTCT-3′ |
| β2M-R        | 5′-TACATGTCCTGATCCACTAATCT-3′ |
| β2M-P        | 5′-FAM-CTCAGTGCACTCCAGAAGTGGCA-TAMRA-3′ |
| PHTF1-F      | 5′-GGAAAAGTGATGACTGCAAGAAAC-3′ |
| PHTF1-R      | 5′-AAACCACTCATGCTTGGG-3′ |
| PHTF1-P      | 5′-FAM-TCTGACTACCATGATGGCT-TAMRA-3′ |
| FEM1B-F      | 5′-CCTGCTTGTTCACATGACAGGAG-3′ |
| FEM1B-R      | 5′-TGACTCTTGCTACAGGGATCTTATCT-3′ |
| FEM1B-P      | 5′-FAM-CGGAGCTACACGATCGACGAA-TAMRA-3′ |
| APAF1-F      | 5′-TGACGCTGCTTGGCTTGG-3′ |
| APAF1-R      | 5′-CATGGGAGCTACAGGAGGATCTTATCT-3′ |
| APAF1-P      | 5′-FAM-TGGAGCTTCTTCTATGCTGCGT-TAMRA-3′ |

Apoptosis assays

Annexin-V binding assays were conducted 72 h after lentiviral transduction using the Apoptosis Detection Kit (eBioscience, San Diego, CA, USA) according to the manufacturer’s instructions. Briefly, cells were washed twice with PBS, resuspended in 100 μL binding buffer containing APC-conjugated Annexin V, and incubated in the dark for 15 min. Cells were then washed and suspended in 200 μL binding buffer containing PI. Annexin V positive cells were analyzed with an Accuri C6 flow cytometer (Becton–Dickinson Immunocytometry Systems, San Jose, CA, USA).

Statistical analyses

Differences in mRNA expression between two clinical groups were analyzed by the Mann–Whitney U test. Data are presented as medians. Spearman’s rank correlation analysis was used to analyze the PHTF1, BCL11B, FEM1b, and Apaf-1 mRNA level in different clinical samples. An independent-sample t test was used for all comparisons, and data are represented as mean ± SD. P values <0.05 were considered statistically significant.

Abbreviations

ALL: acute lymphoblastic leukemia; Apaf-1: apoptotic protease activating factor-1; ATCC: American Type Culture Collection; B-ALL: B-cell acute lymphoblastic leukemia; β2M: β2 microglobulin; BCL11B: B-cell leukemia/lymphoma 11B; CCK8: Cell Counting Kit-8; FEM1b: feminization-1 homolog b; HIs: healthy individuals; PBMCs: peripheral blood mononuclear cells; PHTF1: putative homeodomain transcriptional factor; qRT-PCR: real-time quantitative reverse transcription-polymerase chain reaction; siRNA: small interference RNA; shRNA: small hairpin RNA; T-ALL: T-cell acute lymphoblastic leukemia; TNFR1: tumor necrosis factor receptor-1.

Authors’ contributions

XD, YQL and XH conceived of the study, participated in its design and coordination and helped draft the manuscript. XH, SXG, LJZ, and XLW performed experiments and analyses. JYW, ZSL, MML and CXD were responsible for patient treatment and acquiring of clinical data. All authors read and approved the final manuscript.

Author details

1 Southern Medical University, 510515 Guangzhou, People’s Republic of China. 2 Department of Haematology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, 510080 Guangzhou, People’s Republic of China. 3 Institute of Hematology, Medical College, Jinan University, 510632 Guangzhou, People’s Republic of China. 4 Key Laboratory for Regenerative Medicine of Ministry of Education, Jinan University, 510632 Guangzhou, People’s Republic of China.

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Compliance with ethical guidelines

Competing interests

The authors declare that they have no competing interests.

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Table 1

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| β2M-R        | 5′-TACATGTCCTGATCCACTAATCT-3′ |
| β2M-P        | 5′-FAM-CTCAGTGCACTCCAGAAGTGGCA-TAMRA-3′ |
| PHTF1-F      | 5′-GGAAAAGTGATGACTGCAAGAAAC-3′ |
| PHTF1-R      | 5′-AAACCACTCATGCTTGGG-3′ |
| PHTF1-P      | 5′-FAM-TCTGACTACCATGATGGCT-TAMRA-3′ |
| FEM1B-F      | 5′-CCTGCTTGTTCACATGACAGGAG-3′ |
| FEM1B-R      | 5′-TGACTCTTGCTACAGGGATCTTATCT-3′ |
| FEM1B-P      | 5′-FAM-CGGAGCTACACGATCGACGAA-TAMRA-3′ |
| APAF1-F      | 5′-TGACGCTGCTTGGCTTGG-3′ |
| APAF1-R      | 5′-CATGGGAGCTACAGGAGGATCTTATCT-3′ |
| APAF1-P      | 5′-FAM-TGGAGCTTCTTCTATGCTGCGT-TAMRA-3′ |
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