Zinc finger and BTB domain-containing protein 46 is essential for survival and proliferation of acute myeloid leukemia cell line but dispensable for normal hematopoiesis

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Abstract
Background: Zinc finger and BTB domain-containing protein 46 (Zbtb46) is a transcription factor identified in classical dendritic cells, and maintains dendritic cell quiescence in a steady state. Zbtb46 has been reported to be a negative indicator of acute myeloid leukemia (AML). We found that Zbtb46 was expressed at a relatively higher level in hematopoietic stem and progenitor cells (HSPCs) compared to mature cells, and higher in AML cells compared to normal bone marrow (BM) cells. However, the role of Zbtb46 in HSPCs and AML cells remains unclear. Therefore, we sought to elucidate the effect of Zbtb46 in normal hematopoiesis and AML cells.

Methods: We generated Zbtb46fl/fl and Zbtb46fl/flMx1-Cre mice. The deletion of Zbtb46 in Zbtb46fl/flMx1-Cre mice was induced by intraperitoneal injection of double-stranded poly (I).poly (C), and referred as Zbtb46 cKO mice. After confirming the deletion of Zbtb46, the frequency and numbers of HSPCs and mature blood cells were analyzed by flow cytometry. Serial intraperitoneal injection of 5-fluorouracil was administrated to determine the repopulation ability of HSCs from Zbtb46fl/fl and Zbtb46 cKO mice. The correlation between Zbtb46 expression and prognosis was analyzed using the data from the Cancer Genome Atlas. To investigate the role of Zbtb46 in AML cells, we knocked down the expression of Zbtb46 in THP-1 cells using lentiviral vectors expressing small hairpin RNAs targeting Zbtb46.

Results: The percentages and absolute numbers of HSPCs and mature blood cells were comparable in Zbtb46 cKO mice and its Zbtb46fl/fl littermates (Zbtb46fl/fl vs. Zbtb46 cKO, HPC: 801,310 ± 84,282 vs. 907,202 ± 97,403, t = 0.82, P = 0.46; LSK: 168,895 ± 7802 vs. 102,210 ± 5025, t = 1.65, P = 0.17; HSC: 19,753 ± 3116 vs. 17,608 ± 3508, t = 0.46, P = 0.67). The repopulation ability of HSCs from Zbtb46fl/flMx1-Cre mice was similar to those from Zbtb46fl/fl control (P = 0.26). Zbtb46 had elevated expression in AML cells compared to total BM cells from normal control. Knockdown of Zbtb46 in THP-1 cells led to a significant increase in cell apoptosis and reduced cell growth and proliferation.

Conclusion: Collectively, our data indicate that Zbtb46 is essential for survival and proliferation of AML cells, but dispensable for normal hematopoiesis.

Keywords: Zbtb46 transcription factor; AML; Hematopoietic stem cells
expressed in quiescent endothelial cells and inhibits cell proliferation through regulating cell cycle proteins as a shear-sensitive transcription factor.[5]

Emerging evidence supports the roles of the Zbtb46 gene in many kinds of cancers. Mutations in Zbtb46 are significantly associated with glioblastoma and astrocytoma risk.[6] In breast cancer, RP4-583P15.10 IncRNA, which is located downstream of the natural antisense strand of the Zbtb46 gene, is up-regulated and controls the progression of breast cancer by influencing the immune system.[7] The zbtb46-WFDC13 fusion gene is a novel candidate in high-grade serous ovarian carcinoma.[8] In prostate cancer, Zbtb46 is highly expressed and induces the expression of inflammatory response genes and contributes to neuroendocrine differentiation.[9,10] In leukemia stem cells (LSCs) in acute myeloid leukemia (AML), Zbtb46 is a member of 17-biomarkers to accurately predict the prognosis and clinical outcomes of current treatments.[11]

However, the function of Zbtb46 in normal hematopoiesis and hematological malignant cells is unclear. In the present study, we investigated the function of Zbtb46 in normal hematopoiesis using a Zbtb46<sup>fl/fl</sup>Mx1-Cre conditional knockout mouse model. Further, we evaluated the role of Zbtb46 in AML cells by knocking down the expression of Zbtb46 in THP-1 cells.

**Methods**

**Ethics statement**

All the animal experiments were approved by the Animal Committee of the Third Military Medical University (No. SYXX-2017-0002).

**Generation of Zbtb46 conditional knockout mice**

Conditional Zbtb46<sup>fl/fl</sup>Mx1-Cre knockout mice were generated by crossing loxP-flanked Zbtb46 (Zbtb46<sup>lox/lox</sup>) mice with Mx1-Cre transgenic mice. In Zbtb46<sup>lox/lox</sup>Mx1-Cre mice, Mx1-Cre was induced by intraperitoneal (IP) injection of 10 μg/kg of body weight of the interferon-α inducer, double-stranded poly(I):poly(C) (poly(IC); GE Healthcare Life Sciences, Lithuania) every second day for a total of three injections. All data were obtained from mice at 4 to 8 weeks of age following poly(I:C) induction.

**Flow cytometry**

Single-cell suspensions were prepared from bone marrow (BM; femurs and tibiae), spleen, and thymus. Red cells were lysed with ammonium-chloride-potassium buffer. Cells were incubated for 30 min on ice with the antibodies. The following biotin-conjugated mouse antibodies were used (all from BioLegend, San Diego, CA, USA): anti-Gr-1 (Category number, #108404); anti-Ter119 (#116204), anti-B220 (#101318), anti-CD34 (#103208), and anti-CD135 (#103180), for lineage markers in mice. The fluorochrome-conjugated antibodies used were as follows (all from BioLegend except for the ones specifically mentioned): streptavidin-PerCP-Cy5.5 (#405214), PE-anti-Sca-1 (#108108), allophycocyanin (APC)-Cy7-anti-c-Kit (#105826), PE-Cy7-anti-CD48 (#103424), and APC-anti-CD150 (#115910) for analysis of hematopoietic progenitor cells (HPCs), LSK cells (Lin-Sca-1<sup>-c-Kit</sup>); and hematopoietic stem cells (HSCs); streptavidin-APC-Cy7 (#405208), PE-anti-Sca-1, APC-anti-c-Kit (#105812), and PE-Cy5-anti-Flt3/CD135 (eBioscience, San Diego, CA, USA, #46-1351-82, and BV421-anti-CD34 (#152028) for analysis of lymphoid-primed multipotent progenitors (LMPs), long term HSCs (LT-HSCs), short term HSCs (ST-HSCs), and multipotent progenitors (MPPs); streptavidin-APC-Cy7, PE-anti-Sca-1, APC-anti-c-Kit, PE-Cy7-anti-CD16/32 (#101318), and BV421-anti-CD34 for analysis of common myeloid progenitors (CMPs), granulocyte-monocyte progenitors (GMPs), megakaryocyte-erythroid progenitors (MEPs), and CD34<sup>+</sup> LSKit (CD34<sup>+</sup>Lin-Sca-1<sup>-c-Kit</sup>). The antibodies used for testing the mature cell populations were as follows (all from Biolegend): PE-anti-Gr-1 (#108408) and APC-anti-MAC (#101211) for analyzing myeloid cells, APC-anti-Ter119 (#116212) and PE-anti-CD71 (#113808) for red cells, PE-anti-B220 (#103208) and APC-anti-IgM (#406509) for B cells, PE-anti-CD4 (#100408) and APC-anti-CD8a (#100712) for T cells. While to analyze apoptosis in the cell lines, a simplified staining protocol based on APC-annexin V and 4′,6-diamidino-2-phenylindole (DAPI) were used. The cells were analyzed using a BD FACSCanto (BD Bioscience, San Jose, CA, USA). All fluorescence-activated cell sorting (FACS) data were analyzed by FlowJo software, version 10 (Tree Star, Inc., San Carlos, CA, USA).

**Cell isolation**

Lineage negative (Lin<sup>-</sup>; Gr-1<sup>-Ter119</sup> B220<sup>-</sup> CD19<sup>-IgM</sup> IL-7R CD3<sup>-</sup>), HPC (Lin Sca-1<sup>-c-Kit</sup>-), LSK (Lin Sca-1<sup>-c-Kit</sup>~), MPP (Lin Sca-1<sup>-c-Kit</sup>CD48~), HSC (Lin Sca-1<sup>-c-Kit</sup>CD48<sup>-</sup> CD150<sup>-</sup>), and myeloid (Gr-1<sup>-MAC</sup>~) cells were sorted from the BM of C57/B6 mice through FACS (BD FACS AriaII). B cells (B220<sup>+</sup>IgM~) and red cells (CD71<sup>+</sup>Ter119~) were sorted from the spleen of C57/B6 mice. CD8<sup>+</sup> T (CD4<sup>-</sup>CD8a<sup>+</sup>) and CD4<sup>+</sup> T (CD4<sup>+</sup>CD8a<sup>-</sup>) were sorted from the thymus of C57/B6 mice to detect T cells.

**Treatment with 5-fluorouracil (5-FU)**

The cell cycle-dependent myelotoxic reagent, 5-FU, kills proliferating cells by acting as a DNA synthesis inhibitor. Administration of 5-FU (Sigma-Aldrich, Saint Louis, MO, USA) was carried out via IP injection at a dose of 150 mg/kg. The 5-FU treatments were repeated once a week for a total of 3 weeks. The survival of individuals was monitored daily and analyzed with Graphpad Prism software 6.0 (GraphPad Software, San Diego, CA, USA).

**Cell culture**

Five human AML cell lines THP-1, mv4-11, Kasumi, U937, and HL-60 were obtained from the American Type Culture Collection (https://www.atcc.org/). Total BM cells were used as normal control. The AML cell lines were cultured in Roswell Park Memorial Institute-1640 medium (RPMI) containing 10% (v/v) fetal bovine serum (FBS) and 1% penicillin-streptomycin. The human embryonic kidney...
cell line, 293T, was cultured in high-glucose Dulbecco modified Eagle medium (DMEM) containing 10% FBS. All the cells were maintained in a 37°C incubator with 5% CO2.

**BrdU-incorporation assay**

Cultured THP-1 cells were treated with the final concentration of 10 μmol/L bromodeoxyuridine (BrdU) (BD Biosciences) for 30 min before analysis. Cells incubated with BrdU were harvested and stained with fluorescein isothiocyanate (FITC)-anti-BrdU antibody according to the manufacturer’s instructions provided in the FITC BrdU flow kit (BD Biosciences) and the treated cells were analyzed by flow cytometry (BD FACSCanto).

**Lentiviral constructs and packaging**

To generate lentiviral vectors expressing Zbtb46-specific small hairpin RNAs (shRNAs), we cloned shZbtb46-1, 2, and 3 [Table 1] into a pLKO.1 vector followed by puromycin screening. The correct cloning of the shRNAs was confirmed by Sanger DNA sequencing. The 293T cells were transfected with shZbtb46-1, 2, 3, and pLKO.1-scrambled control along with the helper plasmids, psPAX2 and pMD.2G, using polyetherimide (PEI) transfection reagent (Sigma-Aldrich) for lentivirus packaging. Following co-culturing for 12 h, the medium containing PEI and vectors was replaced with fresh complete DMEM. The viral supernatants were collected at 48 and 72 h following transfection and stored at –80°C until use.

**Lentiviral infection of cells**

THP-1 cells were seeded onto six-well plates and cultured with RPMI containing 10% FBS. Lentiviral stocks of shZbtb46-1, 2, 3, and pLKO.1-scrambled were added to the medium. After overnight incubation at 37°C with 5% CO2, the supernatants were removed by centrifugation, and the cell pellets were washed once with cold phosphate-buffered saline and then re-suspended with fresh complete RPMI. Positive clones were selected in 3 μg/mL final concentration of puromycin for 5 days. The proliferation of shZbtb46-3 and pLKO.1-scrambled infected THP-1 cells was analyzed daily for 7 days. The cellular density was determined by manual cell counting using Counting Slides with a TC20 Automated Cell Counter (Bio-Rad, Hercules, CA, USA).

**RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis**

Total RNA was extracted from cells using RNAiso Plus reagent (TaKaRa, Dalian, China) and quantified by NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA). The complementary DNA (cDNA) was reverse transcribed using a PrimeScript™ reverse transcription (RT) reagent Kit with genomic DNA (gDNA) Eraser (Perfect Real Time; TaKaRa). Zbtb46 expression was determined by qRT-PCR with TB Green Premix Ex Taq™ II (Tli RNaseH Plus; TaKaRa) using a CFX96 Touch Real-Time PCR system (Bio-Rad). All primers used for the qRT-PCR were listed in Table 1. All procedures in this study were performed according to standard molecular biology protocols or as the manufacturer’s instructions.

**Statistical analysis**

Two-tailed Student’s t test was used to calculate the statistical significance of LMPP, Zbtb46 knockdown efficiency, and cell apoptosis. Differences between two or more independent groups were analyzed using multiple t tests for HSPC populations. P < 0.05 was considered to be significantly different.

**Results**

**Generation of Zbtb46 conditional knockout mice**

The function of Zbtb46 in HSCs and HPCs has not been reported previously. Therefore, to determine its role we first analyzed the expression of Zbtb46 in hematopoietic stem and progenitor cells (HSPCs) and mature hematopoietic populations. We found that Zbtb46 had relatively higher expression in HSPCs compared to the mature populations, including myeloid cells (Mac-1+Gr-1+), B cells (B220+IgM+), red cells (CD71-Ter119+), and mature T

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**Table 1: Primer list used in RNA interference, genotyping, and qRT-PCR.**

| Primers               | Sequences                                      |
|-----------------------|------------------------------------------------|
| shZbtb46-1            | CCGGGCTACTTCAAGAGCTCCTACTTCGAGATAGAGCTGAACAGGTTTTTGT |
| shZbtb46-2            | CCGGGCTTTTTCTTCAAGAGCTCCTACTTCGAGATAGAGCTGAACAGGTTTTTGT |
| shZbtb46-3            | CCGGGCTTTTTCTTCAAGAGCTCCTACTTCGAGATAGAGCTGAACAGGTTTTTGT |
| pLKO.1-scrambled      | CCGGTCTCTAGGTAAAGTCCCTGCTGCGGCTAGGACACTTAACCTGAGTACCGTT |}

qRT-PCR: Quantitative reverse transcription-polymerase chain reaction; Zbtb46: Zinc finger and BTB domain-containing protein 46; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.
cells (CD4+ T and CD8+ T) [Figure 1A]. This suggests that Zbtb46 has an important role in HSPCs. To evaluate the function of Zbtb46 in the normal hematopoietic system of mice, we generated the Zbtb46fl/fl mice [Figure 1B], then crossed these mice with Zbtb46fl/fl alleles with Mx1-Cre mice to generate Zbtb46fl/fl mice and Zbtb46fl/flMx1-Cre mice. The deletion of Zbtb46 was induced by three IP injections of double-stranded poly(I).poly(C) (poly(I:C); 10 mg/g of body weight) every second day for a total of three injections. The high efficiency of Zbtb46 deletion was confirmed by semiquantitative PCR analysis using genomic DNA from the BM cells of the Zbtb46fl/flMx1-Cre mice (hereafter referred to as Zbtb46 cKO mice) [Supplementary Figure 1A, http://links.lww.com/CM9/A240]. Accordingly, loss of Zbtb46 mRNA was confirmed by qRT-PCR of BM cells from Zbtb46 cKO mice [Figure 1C].

**Zbtb46 is dispensable for normal hematopoiesis in mice**

To evaluate the in vivo function of Zbtb46 in the hematopoietic system in mice, we analyzed the hematopoietic cells in the BM of Zbtb46fl/fl and Zbtb46fl/flMx1-Cre mice six weeks following the poly(I:C)-induction. The total number of BM cells are comparable in the Zbtb46 cKO mice and their Zbtb46fl/fl control littermates [Supplementary Figure 1B, http://links.lww.com/CM9/A240]. Flow cytometry analysis also revealed that the frequency of mature myeloid, B cells, red cells, and T cells was comparable in the BM cells, spleen and Thymus (except mature B cells in BM) from Zbtb46 cKO and the control mice [Supplementary Figure 1C–E, http://links.lww.com/CM9/A240]. This suggests that the loss of Zbtb46 did not affect the differentiation of mature blood cells.

In normal hematopoiesis, HSCs not only have self-renewal capacity to give rise to more identical HSCs but also possess multi-potency to differentiate into all mature blood cells.[12] Given that Zbtb46 had relatively higher expression in HSPCs compared with mature populations, we examined these stem and progenitor cell compartments by flow cytometry. We observed that Zbtb46 cKO mice showed similar frequencies and absolute numbers of LT-HSCs (Lin-Sca-1+c-Kit+CD48-CD150+ or Lin-Sca-1+c-Kit+CD34-Flt3-), LSKs (Lin-Sca-1+c-Kit+CD34+), MPPs (Lin-Sca-1+c-Kit+CD34+Flt3+), and HPC (Lin-Sca-1+c-Kit+) but a slight increase in ST-HSCs (Lin-Sca-1+c-Kit+CD34+Flt3-) in comparison with their wild-type littermates [Figure 2A and 2B]. The frequencies...
and absolute numbers of the myeloid lineages including CMP, GMP, and MEP, were unaffected [Figure 2A and 2C]. LMPPs are the main source for the generation of B cells, T cells, and monocytes.\[13\] Our result indicated that the number of LMPP (Lin-Sca-1+c-Kit+Flt3+) declined slightly in the Zbtb46\(cKO\) mice although the difference was not significant [Figure 2A and 2D]. In summary, Zbtb46 conditional knockout in primary mice had no obvious phenotype in either progenitor or lineage cells.

In the hematopoietic system, 5-FU injection kills proliferating HSPCs and stimulates quiescent HSC entering the cell cycle for hematopoietic reconstitution.\[14\] 5-FU injection assay was performed to further explore whether the loss of Zbtb46\(cKO\) affected hematopoietic reconstitution in primary Zbtb46\(cKO\) and Zbtb46\(fl/fl\) mice. The survival of the treated animals was monitored daily for 3 weeks. The survival curve following 5-FU injection of Zbtb46\(fl/fl\) (\(n=11\)) and Zbtb46\(cKO\) mice (\(n=12\)) [Figure 2E]. Together these data suggest that Zbtb46 is dispensable for normal hematopoiesis in mice.

High Zbtb46 expression is associated with a poor prognosis in AML

It has been reported that Zbtb46 is a negative indicator of LSC.\[11\] However, the role of Zbtb46 in AML remains unclear. To further evaluate the role of Zbtb46 in human AML, we evaluated the expression of Zbtb46 in AML by analyzing public databases.\[15,16\] We found that the Zbtb46 gene was highly expressed in peripheral blood or BM from AML patients (542 individuals) compared to peripheral blood mononuclear cells from normal samples [74 individuals; Figure 3A]. Simultaneously, by analyzing the correlation between Zbtb46 expression and prognosis in the AML cohort in the Cancer Genome Atlas (TCGA),\[17\] we found that patients with low Zbtb46 expression had significantly better overall survival outcomes than those with high Zbtb46 expression [\(P = 0.028\); Figure 3B]. Further, we analyzed Zbtb46 expression in human total BM cells of normal control and AML cell lines including THP-1, U937, HL-60, mv4-11, and Kasumi-1. The results showed that Zbtb46 was highly expressed in most AML cell lines compared to the normal BM cells [Figure 3C]. Collectively, the results indicate that Zbtb46 may play important roles in AML cells.

Zbtb46 is essential for survival and proliferation of AML cells

To investigate the role of Zbtb46 in the AML cells, we knock down the expression of Zbtb46 in THP-1 cells using lentiviral vectors expressing shRNAs targeting Zbtb46. Three Zbtb46-specific shRNA (shZbtb46-1, 2, and 3) were cloned into the vector pLKO.1, and shZbtb46-3 was...
is essential for metastasis. However, in AML, Zbtb46 has been reported as a novel tumor promoter and knockdown efficiency [around 70%; Figure 4A]. First, we evaluated the proliferation rate of THP-1 cells with shZbtb46-3-mediated silencing of Zbtb46 and found that the growth rate of Zbtb46-silenced THP-1 was significantly reduced following the silencing of Zbtb46 compared to pLKO.1-scrambled control [Figure 4B]. We further assessed the incorporation of the thymidine analog, BrdU, to investigate the cell cycle kinetics of Zbtb46-silenced THP-1 cells and corresponding control cells (THP-1 cells infected with pLKO.1-scrambled). While 32% of THP-1 cells incorporated BrdU when infected with pLKO.1-scrambled, 21% of BrdU+ cells were detected in the Zbtb46-silenced THP-1 cells [Figure 4C and 4D], which indicated that the silencing of Zbtb46 reduced the proliferation of THP-1 cells. We next analyzed apoptosis in Zbtb46-silenced THP-1 cells and its control by staining with Annexin V and DAPI. The percentage of early (Annexin V+/DAPI-) and late (Annexin V+/DAPI+) apoptotic cells were remarkably increased in the shZbtb46-3 knockdown group [Figure 4E and 4F]. These results suggest that Zbtb46 is essential for the survival and proliferation of AML cells.

**Discussion**

In this study, using a mouse model with conditional deletion of Zbtb46, we identified that Zbtb46 is dispensable for the maintenance of HSPCs in a steady state. Further, we also identified that Zbtb46 acts as an important factor in maintaining the survival and proliferation of AML cells.

Emerging evidence supports the important role of the Zbtb46 gene in cancers. In malignant prostate cancer, Zbtb46 has been reported as a novel tumor promoter and is essential for metastasis. However, in AML, Zbtb46 has been identified as a negative indicator of LSC, and the expression of Zbtb46 in AML LSCs negatively correlated with the clinical prognosis. In this study, we demonstrated that Zbtb46 was highly expressed in most AML cell lines. By analyzing the correlation between Zbtb46 expression and prognosis in the AML cohort from TCGA, we found that patients with low Zbtb46 expression had significantly better survival outcomes than those with high Zbtb46 expression. Human AML is organized as a hierarchy initiated by LSC that give rise to progenitors, and eventually to terminally differentiated blasts. Hence, Zbtb46 may function in a context-dependent manner. In LSC, high expression of Zbtb46 may impair their function, while in the more differentiated blasts, like in the AML cell lines, Zbtb46 is essential for survival and proliferation of the cells. Further studies using in vivo mouse models of AML would enable us to develop a clearer understanding of the underlying mechanisms.

HSCs give rise to all lineages of blood cells, and the balance between their proliferation and quiescence is carefully regulated by a complex network under homeostatic or stress conditions. Many genes may have limited effects on hematopoiesis under homeostatic conditions; however, they could play important functions under stress conditions. In this study, we have only identified the role of Zbtb46 under the homeostatic condition and found that Zbtb46 is dispensable for normal hematopoiesis in mice. Further studies are needed to clarify the role of Zbtb46 under stress conditions.

The limitations of the study are as follows: First, we evaluated the role of Zbtb46 only under homeostatic conditions. Further studies regarding the function of Zbtb46 under stress conditions would make this study more complete. Secondly, when we studied the function of Zbtb46 in THP-1 cells, we found that the silencing of Zbtb46 impaired the survival and proliferation of THP-1 cells. However, how Zbtb46 overexpression might affect
Figure 4: Knockdown of Zbtb46 significantly affected the survival and proliferation of AML cells. (A) Knockdown efficiency of the Zbtb46 gene mediated by three lentiviral vectors (shZbtb46-1, 2, 3) in THP-1 was determined by qRT-PCR and compared to pLKO.1-scrambled control. \( P < 0.05 \) compared to pLKO.1-scrambled. (B) The proliferation rate of the THP-1 cell line was significantly inhibited after the silencing of Zbtb46. \( ^{\dagger}P < 0.01 \) compared with pLKO.1-scrambled control. (C) Representative flow cytometry of pLKO.1-scrambled and shZbtb46-3 lentiviral silenced THP-1 stained with BrdU and DAPI. (D) The histograms revealed apoptosis frequency (sub-G0/G1, G0/G1, S, and G2-M phase in pLKO.1-scrambled and shZbtb46-3 interfering THP-1. \( ^{\dagger}P < 0.05 \) compared with pLKO.1-scrambled control. (E and F) The percentage of apoptotic cells in the shZbtb46-3 knockdown group and control cells (THP-1 cells infected with pLKO.1-scrambled) was analyzed by flow cytometry. \( ^{\dagger\dagger}P < 0.01 \) compared with pLKO.1-scrambled control. AML: Acute myeloid leukemia; APC: Allophycocyanin; BrdU: bromodeoxyuridine; DAPI: 4',6-Diamidino-2-phenylindole; FITC: Fluorescein isothiocyanate; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; qRT-PCR: Quantitative reverse transcription polymerase chain reaction; Zbtb46: Zinc finger and BTB domain containing protein 46.
AML proliferation and apoptosis remains unclear. Further studies that explore the effect of Zbtb46 overexpression in THP-1 cells will make our results more significant and relevant.

In this study, we demonstrated that Zbtb46 is dispensable for normal hematopoiesis but indispensable for survival and proliferation of AML cells. This may provide us a potential target in the clinical treatment of AML.

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**Conflicts of interest**

None.

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