Plant homeodomain finger protein 2 as a novel IKAROS target in acute lymphoblastic leukemia

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Aim: Clinical significance of plant homeodomain finger 2 (*PHF2*) expressions is explored in acute lymphoblastic leukemia (ALL) patients. **Methods:** mRNA level was examined by qPCR. The retroviral gene expression, shRNA knockdown and chromatin-immunoprecipitation are used to observe IKAROS regulation on *PHF2* transcription. **Results:** *PHF2* expression is significantly reduced in subsets of ALL patients, and *PHF2*^{low} expression correlates with leukemia cell proliferation and an elevation of several poor prognostic markers in B-cell ALL. IKAROS directly promotes *PHF2* expression and patients with *IKAROS* deletion have significantly lower *PHF2* expression. Casein kinase II (CK2) inhibitor significantly promotes *PHF2* expression in an IKAROS-dependent manner, and casein kinase II inhibitor treatment also results in an increase of *PHF2* expression and enrichment of IKAROS and H3K4me3 at *PHF2* promoter in primary cells. **Conclusion:** Our results demonstrate that the IKAROS promotes *PHF2* expression, and suggest that *PHF2*^{low} expression works with the *IKAROS* gene deletion to drive oncogenesis of ALL.

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Histone demethylases and their role in the regulation of post-translational modifications of chromatin have greatly contributed to our understanding of epigenetics in tumorigenesis. Plant homeodomain finger 2 (*PHF2*) (Lysine demethylase 7C [KDM7C]) is a member of the Jumonji C (JmjC) family and contains a plant homeodomain finger and a JmjC domain. *PHF2* demethylates H3K9me2 (lysyl dimethylation), which, subsequently participates in epigenetic regulation of transcription [1].

PHF2 is a positive epigenetic regulator and is associated with tumor suppression in several types of cancer. *PHF2* gene deletion, or hypermethylation in its promoter region, is associated with breast cancer, whereas its downregulation is associated with head and neck cancers [2,3]. *PHF2* positively regulates the expression of genes such as *HNF4*, *CEBPα* and *NF-κB*, and subsequently aids with the expression of genes driven by these factors [4–6]. *PHF2* is also in association with p53 which is involved in tumor suppression in human cancer [7]. It is also reported that *PHF2* promotes the differentiation of tumor-initiating cells and induces the loss of tumor-initiating ability in the cells [8]. Thus, *PHF2* is well recognized as a tumor suppressor.

Many reports indicate that histone demethylases are aberrantly expressed in human cancers and may potentially provide additional therapeutic targets in the treatment of cancer [9–11], although the underlying mechanism of neoplastic transformation remains unknown. For example, *JMJD3* is highly expressed in T-cell ALL (T-ALL) [12]; *KDM5B* is highly expressed in acute lymphoblastic leukemia (ALL) [13]; *PHF8* promotes breast cancer oncogenesis and governs the response to retinoic acid in acute myeloid leukemia (AML) [14,15]; and *PHF2* is downregulated in colon cancer [4,7] and breast cancer [2]. It is also reported that low *PHF2* expression is associated with increased aggressiveness of the clear cell variant of renal cell carcinoma and is a poor prognostic factor [16]. Until now, however, there have been no reports about the *PHF2* expression in leukemia patients and its roles in oncogenesis in ALL.

Genetic alterations of *IKZF1* encoding, the lymphoid transcription factor IKAROS, are a hallmark of high-risk B-progenitor ALL such as BCR-ABL1 positive (Ph+) and Ph-like ALL, and are associated with poor outcome, even in the era of contemporary chemotherapy incorporating tyrosine kinase inhibitors in the treatment of Ph+ ALL [17]. Recently, we reported the IKAROS global binding profile in ALL cells and found that IKAROS regulates the expression of its targets through chromatin remodeling in ALL [18–21]. Our chromatin immunoprecipitation assays with sequencing (ChIP-seq) data showed IKAROS binding peaks in the promoter region of *PHF2*. We also found that casein kinase II (CK2) inhibitors increase the tumor suppressor activity of IKAROS and act as a functional activator of the *IKZF1* gene product [18–20]. However, it is still unclear whether and how IKAROS regulates *PHF2* expression.

Here, we examined *PHF2* expression in ALL patients, and analyzed the correlation between *PHF2* expression and several high-risk markers in ALL patients. We also identified that *PHF2* is a direct target of IKAROS, which binds to and promotes *PHF2* expression in ALL leukemic cells.

CX-4945 is an inhibitor of CK2. We reported that CK2-induced IKAROS phosphorylation result in the IKAROS inactivation. CK2 activity is higher in leukemic cells, and CK2 inhibitor reduces the IKAROS phosphorylation and restores IKAROS tumor suppressor activity [18–21]. Here, we found that CX-4945 treatment increases *PHF2* expression, whereas *IKZF1* knockdown blocks these CX-4945-induced changes. In addition, we found that deletion of the *IKZF1* gene is significantly associated with low *PHF2* expression in ALL. We also found that IKAROS promotes *PHF2* expression by chromatin remodeling through enrichment of H3K4me3 to the promoter. Our findings indicate that *PHF2* is significantly downregulated in ALL, and that an *IKZF1* deletion may be responsible for the low expression of *PHF2* in high-risk ALL. *PHF2* low expression may work in conjunction with *IKZF1* deletion to drive oncogenesis in ALL. The significance of the correlation may also be valuable in an integrated prognostic model for high-risk ALL although it requires large prospective trials to confirmation.

Materials & methods

Subjects & samples

Subjects with newly diagnosed ALL (age 12–77 years old) were recruited between June 2008 and June 2016. There was a total of 164 patients (107 B-cell and 57 T-cell ALL) who were recruited at the First Affiliated Hospital of Nanjing Medical University and Zhongda Hospital Southeast University. Diagnoses were based on the WHO Diagnosis and Classification of ALL (2008). The study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University and Zhongda Hospital Southeast University, Nanjing, China.

Therapy

The therapy protocol has been previously published (CALLG2008) [22]. Induction was with vincristine, daunorubicin, cyclophosphamide, L-asparaginase, and prednisone-based regimens. Early consolidation used cyclophosphamide, cytarabine, thioguanine, high-dose methotrexate/L-asparaginase and mitoxantrone. Late consolidation used vincristine, daunorubicin, L-asparaginase, prednisone; cyclophosphamide, vincristine, cytarabine, epipodophyllotoxin and dexamethasone; or high-dose methotrexate/L-asparaginase, epipodophyllotoxin and cytarabine. Maintenance therapy used 6-mercaptopurine and methotrexate. Subjects with *BCR/ABL1*-positive ALL received Imatinib starting day 15 of introduction therapy.

Cytogenetic & molecular analyses

Cytogenetics and detection of the most common *IKZF1* deletion, IKAROS 6 (Ik6), were analyzed as previously described [20]. qPCR was performed on StepOne Plus Real-time PCR system (Applied Biosystem-ThermoFisher, CA, USA). Gene expression values of genes of interest (GOI) were achieved in each sample by a formula derived from a scatter graph of cycle threshold (Ct) values from serial dilutions of a template standard as described [20,23].

Expression levels of GOIs were normalized to housekeeping genes expressed as gene expression value of GOI/18s rRNA. Subjects were allocated into high or low *PHF2* expression cohorts (4th quartile vs 1st–3rd quartiles) with a cut-off value determined by SPSS 20.0 (IBM, NY, USA) [20].

The qPCR for *PHF2* expression was analyzed as above in Nalm6, CEM cells and primary ALL cells. Results were normalized to those obtained with *18s rRNA* and presented as fold induction over vector controls. Primers: *18s rRNA*, sense: 5'-GTAACCCGTTGAACCCCAT-3', antisense: 5'-CCATCCAATCGGTAGTAGCG-3'; *PHF2* sense: 5'-CAAGCGGGTCCTCAACGT-3', antisense: 5'-CAAGCGGGTCCTCAACGT-3'.

Cell culture, plasmids & retroviral gene transfer

The Nalm6 cell line has been previously described [20]. The CCRF-CEM (CEM) cell lines were obtained from the American Type Culture Collection (VA, USA). Cells were cultured in RPMI-1640 medium (Cellgro, MA, USA) supplemented with 10% fetal bovine serum (Hyclone, UT, USA). HEK 293T cells were cultured in DMEM (Cellgro) supplemented with 10% fetal calf serum and 1% L-glutamine (Cellgro). Cells were incubated at 37°C in a humidified atmosphere with 5% CO₂. Primary human B- and T-cell ALL cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (GE-Hyclone, UT, USA). CX-4945 was purchased from Sigma (MO, USA). Cells were cultured with or without CX-4945 and collected for total RNA isolation. Human *IKZF1* retroviral construct and retroviral production have previously been described [18,20,24–26].

Quantitative chromatin immunoprecipitation

Quantitative chromatin immunoprecipitation (ChIP) assays were performed by incubating chromatin with antibodies against IKAROS [18–20], H3K4me3 and HDAC1 (Abcam, MA, USA), Mi2 (CA, USA) or normal rabbit IgG (Abcam) as a control [18–20,26]. Enrichment of the ChIP sample over input was evaluated by qPCR with more than or equal to three replicates using specific primers in the promoter region of *PHF2* (forward: 5'-TGCTAGGAGACTGAGCCAGGTT-3', reverse: 5'-ACCCCTGCTCGGAATTCC-3') or no IKAROS-binding region (IKAROS-negative-binding control primers, forward: 5'-GCTCGAAACCACCCTGATCA-3', reverse: 5'-TGCCGGCTAATTTTGTATTTTGTAGT-3'). Relative concentration of the qPCR product is presented as the fold change of the level of DNA-IKAROS and DNA-H3K4me3 samples compared with controls.

Luciferase assay

The *PHF2* promoter was cloned into pGL4.15 vector (Promega, WI, USA). The transient luciferase reporter assay was performed in HEK293T cells using the Promega luciferase assay reagents and measured with luminometer following the manufacture's instruction [18,20,26]. The firefly luciferase activities were calculated as fold change relative to values obtained from pGL4.15 vector only control cells, and expressed as a percentage of pcDNA3.1-*IKZF1* transfection-induced luciferase activity versus that of pcDNA3.1 vector. All transfection and reporter assays were performed independently, in triplicate, at least three-times.

For CX-4945 treatment, the cells are incubated with 20 µM CX-4945 for 24 h after the transfection.

IKZF1 shRNA knockdown

Nalm6 and CEM cells were transiently transfected with human *IKZF1* shRNA constructs in the green fluorescent protein vector (pGFP-v-RS) (OriGene, MD, USA) using the Neon Transfection System (Invitrogen, CA, USA). We used scrambled 29-mer shRNA cassette in the pGFP-v-RS vector as a control. Knockdown of IKAROS was confirmed by *IKZF1* mRNA level [18,20,26]. Primers used for qPCR are 5'-GGCGCGGTGCTCCTCCT-3' (*IKZF1*-F) and 5'-TCCGACACGCCCTACGACA-3' (*IKZF1*-R).

Statistical analyses

Median differences between the cohorts were evaluated using a Mann–Whitney U-test. Frequency differences were analyzed using univariate and multivariate Cox models. Relapse-free survival (RFS) and overall survival (OS) were estimated by the Kaplan–Meier method and compared by log-rank test. The starting point for the observation time for OS was date of diagnosis. RFS was estimated for subjects achieving complete remission starting at the time remission was declared. Living subjects were censored for survival at last follow-up. Statistical analyses used SPSS version 20.0. Data were represented as mean values with bars representing the standard error of the mean. Data shown are representative of three independent experiments. Determinations of statistical significance were

Figure 1. *PHF2* expression in acute lymphoblastic leukemia.
Comparison of *PHF2* expression in B-cell acute lymphoblastic leukemia and T-cell acute lymphoblastic leukemia with normal bone marrow controls.

Table 1. Significant correlation of <i>PHF2</i> low expression with high-risk markers in B-cell acute lymphoblastic leukemia.					
Characteristics	<i>PHF2</i> ^{high}	<i>PHF2</i> ^{low}	Univariate analyses (Chi-square tests)	Multivariate analyses (multivariate Cox model)	
			<i>p</i> -value	<i>p</i> -value	HR (95% CI)
<i>IKZF1</i> deletion (Ik6) (%)	46.3	8.0	0.001	0.005	0.021 (0.001–0.321)
Bone marrow blasts (%)median (range)	90.4 (38.0–100.0)	82.4 (28.0–96.0)	0.001	0.021	0.001 (0.000–0.335)
Spleen extramedullary infiltration (%)	46.8	23.1	0.034	0.412	2.018 (0.377–10.799)
PLT, × 10 ⁹ /l median (range)	32.0 (2.0–292.0)	62.0 (4.0–292.0)	0.048	0.143	1.008 (0.997–1.019)
Stem cell marker CD34 ⁺ (%)	82.3	60.0	0.021	0.728	1.325 (0.271–6.484)
Myeloid marker CD33 (%)	48.5	25.0	0.046	0.479	0.576 (0.125–2.651)
Time to reach CR after treatment >4 weeks (%)	48.7	19.0	0.015	0.006	0.119 (0.026–0.549)

CR: Complete remission; HR: Hazard ratio; PLT: Platelet.

performed using a Student’s *t*-test for comparison of two groups or analysis of variance for comparing more than two groups.

Results

Clinical & laboratory variables in patients with high & low *PHF2* expression

PHF2 mRNA levels in bone marrow samples from adults with ALL, especially those with B-cell ALL (B-ALL), were significantly lower than those in healthy controls (Figure 1) and also in B-ALL (GSE7440 and GSE11877) and T-ALL (GSE10609, GSE26713) cohort studies (Supplementary Figure 1). We compared clinical and laboratory variables in subjects with B-ALL divided into cohorts with high or low *PHF2* mRNA levels (Tables 1 and Supplementary Table 1). The B-ALL cohort with low *PHF2* mRNA levels was associated with a high-median bone marrow blast percentage (90.4 vs 82.4%; *p* = 0.001), higher percentage of stem cell marker CD34⁺ (82.3 vs 60.0%; *p* = 0.021) and a higher myeloid marker CD33 (48.5 vs 25.0%; *p* = 0.046) in comparison to the high *PHF2* expression cohort (Table 1 and Supplementary Table 1). The associated median bone marrow blast percentage was confirmed in multivariate analyses (Table 1). The low *PHF2* expression cohort also had a higher frequency of Ik6 (+), the most common *IKZF1* deletion (46.3 vs 8.0%; *p* = 0.001); a lower median platelet (PLT) count and a higher percentage of splenomegaly (46.8 vs 23.1%; *p* = 0.034) (Table 1) compared with the high *PHF2* expression cohort. The associated Ik6(+) percentages were also confirmed in multivariate analyses (Table 1). There were no significant associations between clinical and laboratory variables in the low versus high *PHF2* expression cohorts in subject with T-ALL (data not shown).

Time to complete remission >4 weeks after chemotherapy induction is considered a poor prognostic marker in patients with ALL. We found that our cohort of B-ALL patients with low *PHF2* expression had a significantly

Figure 2. IKAROS binds to the promoter of PHF2 in Nalm6 B-cell acute lymphoblastic leukemia cells identified by ChIP-seq. (A) IKAROS-binding peaks on PHF2 promoter. The ChIP-seq data (GSE44218) was our previously published [18]; (B & C) quantitative ChIP data show the IKAROS bindings to the promoter of PHF2 in ALL cell lines (B) and primary patients' samples (C).

ChIP: Chromatin immunoprecipitation; Neg: IKAROS-negative-binding control primers.

higher percentage of delayed remission >4 weeks (48.7 vs 19.0%; $p = 0.015$) compared with the cohort with high PHF2 expression (Table 1).

We also analyzed the correlation of PHF2 expression with survival and found no significant difference in the median OS between the patients with PHF2 low and high expression (Supplementary Figure 2). While not statistically significant, we did note a trend toward a shorter median RFS in B-ALL patients with PHF2 low expression compared with the cohort with high PHF2 expression (9 months [5.581,12.419 months] vs 12 months [8.545,15.455 months]; $p = 0.139$) (Supplementary Figure 2).

IKAROS binds to the PHF2 promoter & regulates its expression in ALL

To address the potential mechanism underlying low PHF2 expression we analyzed transcription factor motifs in the PHF2 promoter region. ChIP-seq data identified IKAROS-binding peaks in the PHF2 promoter region in Nalm6 B-ALL (Figure 2A) and primary B-ALL cells (Supplementary Figure 3) [18,19]. IKAROS binding was confirmed by quantitative ChIP assay in ALL cell lines (Figure 2B) and primary ALL patient samples (Figure 2C). These data indicate a direct effect of IKAROS on PHF2 transcription. Consistent with these data, expression of IKAROS promotes PHF2 promoter activity by a luciferase reporter assay (Figure 3A) and increases PHF2 mRNA levels in Nalm6 and CEM cells (Figure 3B). Conversely, efficient IKAROS knockdown decreases PHF2 expression in Nalm6 and CEM cells (Figure 3C).

Correlation of IKZF1 deletion with PHF2 low expression in B-ALL patients

We next analyzed a possible correlation between IKZF1 levels with PHF2 in primary B-ALL cells [27–29]. We found IKZF1 mRNA levels were positively correlated with PHF2 expression in B-ALL (GSE11877) and T-ALL (GSE26713) cohorts (Supplementary Figure 4). We also found PHF2 expression was significantly lower in B-ALL cells with *Ik6* (0.03201 ± 0.000527 vs 0.11331 ± 0.05773 ; $p = 0.033$; Figure 3D). This is consistent with our

Figure 3. IKAROS promotes *PHF2* expression in acute lymphoblastic leukemia.

(A) The activity of *PHF2* promoters measured by luciferase reporter assay following transfection with *IKAROS* or control vector in HEK293 cells or that with CX-4945 treatment. *** $p < 0.001$ compared with pcDNA3.1 vector only; * $p < 0.05$ compared with no CX-4945 treatment. (B) Expression of IKAROS promotes *PHF2* expression in ALL cells. Compared with Mig vector: *** $p < 0.01$ compared with Mig vector only; (C) IKAROS knockdown by shRNA suppresses *PHF2* expression in ALL cells. *** $p < 0.01$ compared with siCTL group. Data shown in A–C are representative of two to three independent experiments; (D) Comparison of *PHF2* expression in the patients with Ik6(+) versus that of Ik6(-) in B-ALL.

ALL: Acute lymphoblastic leukemia; B-ALL: B-cell acute lymphoblastic leukemia; siCTL: Scramble shRNA control.

Figure 4. CX-4945 promotes *PHF2* expression in an IKAROS-dependent manner.

(A) CX-4945 treatment induced increase of *PHF2* expression in Nalm6 and CEM cells; **(B)** effect of IKAROS knockdown by shRNA on CX-4945-induced suppression of *PHF2* expression. *** $p < 0.01$ compared with siCTL group; **(C)** CX-4945 promotes *PHF2* expression in primary cells from ALL patients. Data shown in A–C are representative of two to three independent experiments.

ALL: Acute lymphoblastic leukemia; siCTL: Scramble shRNA control.

finding that B-ALL patients with *PHF2* low expression have a significantly higher percentage of *Ik6* (Table 1). These data suggest that a *IKZF1* deletion may contribute to low *PHF2* expression in B-ALL patients.

CK2 inhibitor CX-4945 promotes *PHF2* expression by enhancing IKAROS activity

CX-4945 treatment facilitates the effect of IKAROS-mediated increase of *PHF2* promoter activity (Figure 3A). Through qPCR, we also showed that treatment of Nalm6 and CEM cells with CX-4945 promoted expression of *PHF2* mRNA in a dose-dependent manner (Figure 4A), and that *IKZF1* knockdown significantly blocked this effect (Figure 4B). The effect of CX-4945 on *PHF2* mRNA levels was also observed in primary B- and T-ALL cells (Figure 4C). Our results indicate that IKAROS not only binds to the *PHF2* promoter but that treatment with CX-4945 enhances IKAROS tumor suppressor activity, promoting *PHF2* expression in leukemia cells.

Enhanced IKAROS activity by CK2 inhibition increases H3K4me3 occupancy at the *PHF2* promoter

Epigenetic regulation is critically important in mediating precise gene expression. We reported IKAROS regulates gene expression through epigenetic regulation [18,19]. Here, we also explored the epigenetic mechanism by which IKAROS regulates *PHF2* expression. We performed ChIP assay for IKAROS, and HDAC1 and Mi2, the two key components of NuRD complex, and amplified the resulting *PHF2* promoter sequences. No significant HDAC1 and Mi2 bindings are observed in the *PHF2* promoter in both Nalm6 and CEM cells (Supplementary Figure 5A & B). CX-4945 treatment significantly increases the binding of IKAROS to the *PHF2* promoter region compared with

Figure 5. Chromatin switches upon CX-4945 treatment. (A–C) CX-4945 increases the enrichment of IKAROS (A, B) and H3K4me3 (C & D) in promoter of *PHF2* in ALL cell lines (A & C) and primary cells (B & D). Data shown are representative of three independent experiments.

untreated Nalm6 B-ALL and CEM T-ALL cells (Figure 5A), as well as primary B-ALL and T-ALL cells (Figure 5B). However, CX-4945 treatment shows no effect of HDAC1 and Mi2 binding on the promoter in Nalm6 and CEM cells (Supplementary Figure 5A & B). Instead, CX-4945 treatment increased H3K4me3 occupancy at the *PHF2* promoter region in Nalm6 and CEM cells (Figure 5C) and in primary B- and T-ALL cells (Figure 5D), indicating the IKAROS activate the *PHF2* expression by forming an open chromatin with H3k4me3.

Discussion

The discovery of histone demethylases has provided us with new insights into understanding the epigenetic landscape of the chromatin environment of cancer cells. The aberrant expression of histone demethylases in human cancers can potentially provide additional therapeutic targets in the treatment of cancer [30]. Prior studies have demonstrated that *PHF2* is H3K9 demethylase, which subsequently participates in the epigenetic regulation of transcription [1].

H3K9 modification provides a universal mechanism of epigenetic gene regulation, thus *PHF2* is suspected to be involved in a diverse set of biological functions including as a tumor suppressor. Here, our data indicate that *PHF2* is downregulated in ALL and that *PHF2* low expression is associated with leukemia cell proliferation and several poor prognostic markers. These data also reveal the oncogenic effect of *PHF2* low expression in ALL. Our data are consistent with previous reports which indicate that *PHF2* low expression is associated with poor outcomes in human cancer [1–2,4–5,7,16].

Unlike most other histone demethylases, which are overexpressed in human cancers [13,31–33], *PHF2* is deleted or hypermethylated in its promoter region in breast cancer and downregulated in head and neck cancers [2,3]. In addition, other reports confirm *PHF2* is downregulated in colon cancer and involved in the epigenetic regulation of p53 [7]. These reports indicate that *PHF2* low expression is involved in oncogenesis. Our data demonstrated that *PHF2* is downregulated in adult ALL patients compared with normal controls; however, the downstream targets of *PHF2* in the oncogenesis of ALL still need further clarification.

There have been few reports concerning the regulation of histone demethylase expression in human cancers. Histone demethylase 5B (KDM5B) is reported to be transcriptionally suppressed by IKAROS, HDAC1 and CK2 in ALL [13]. Here, we found that IKAROS directly promotes *PHF2* transcription in ALL, and that an *IKZF1*

gene deletion is associated with low *PHF2* expression in high-risk ALL. Interestingly, we found restoring IKAROS function by CK2 inhibition can dramatically increase the H3K4me3 enrichment in the promoter of *PHF2* in ALL cells. Thus, our data suggest that IKAROS promotes *PHF2* expression by forming active chromatin. These data not only suggested the oncogenic roles of IKAROS dysfunction-induced *PHF2* low expression, but also indicated that restoring IKAROS function is important to restoring the altered epigenomic state of IKAROS targets.

Conclusion

This is the first time to demonstrate that low *PHF2* expression is significantly associated with acute lymphoblastic leukemia. Indeed, we found that *PHF2* low expression was significantly associated with leukemia cell proliferation and several poor prognostic indicators in adult ALL patients. Moreover, we discovered that IKAROS directly regulates *PHF2* transcription, and that CK2 inhibition, by restoring IKAROS function, promotes *PHF2* transcription through histone modification. Our data, therefore, demonstrate the oncogenic effects of IKAROS dysfunction induce decreases in *PHF2* expression. This also suggests that this dysfunction may represent a possible underlying mechanism in high-risk ALL. We suggest that *PHF2* expression levels may be integrated into future adult ALL risk-stratification models.

Summary points

- *PHF2* expression is significantly reduced in subsets of acute lymphoblastic leukemia (ALL) patients.
- *PHF2*^{low} expression correlates with leukemia cell proliferation and poor prognostic markers in B-cell ALL.
- Patients with *IKAROS* deletion have significantly lower *PHF2* expression.
- *IKAROS*s directly promote *PHF2* expression.
- Casein kinase II inhibitor significantly promotes *PHF2* expression in an *IKAROS*-dependent manner through histone modification.
- *PHF2*^{low} expression works with the *IKAROS* gene deletion drive oncogenesis of ALL.
- Promotion of *PHF2* expression may be one of the mechanisms underlying therapeutic effect of Ck2 inhibitor on high-risk ALL.

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Availability of data & materials

The datasets generated from the patients during the current study are not publicly available in accordance with local health research ethics protocols but may be available from the corresponding author.

Authors' contribution

Z Ge, Y Gu, Q Han, Q Ge, G Gao, J Ma, H Song, J Hu, B Chen, S Dovat, C Song performed experiments and analyzed data; C Song, Z Ge, S Dovat designed and supervised data analysis; C Song, Z Ge, J Sloane, G Gao wrote the manuscript.

Financial & competing interests disclosure

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Ethical conduct of research

All the patients provided their written informed consent in accordance with the Declaration of Helsinki before enrollment in the study. The study was approved by the Institutional Review Board of the Nanjing Medical University and Zhongda Hospital Southeast University, Nanjing, China.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/epi-2017-0092

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