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PLCB4 upregulation is associated with unfavorable prognosis in pediatric acute myeloid leukemia

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Abstract. Phospholipase C (*PLC*) is a membrane-associated enzyme that regulates several cellular behaviors including cell motility, growth, transformation and differentiation. *PLC* is involved in cancer migration, invasion and drug resistance. However, the expression status and prognostic role of *PLCB4* in acute myeloid leukemia (AML) remain unclear. In the present study, the complete clinical and mRNA expression data of 285 pediatric patients with *de novo* AML were obtained from the Therapeutically Available Research to Generate Effective Treatments database. The association between *PLCB4* expression and clinical and molecular features was explored. The expression of *PLCB4* was significantly higher in patients with AML who relapsed compared with those with long-term complete remission. Patients with *PLCB4* upregulation had significantly lower overall survival (OS) and event free survival (EFS) rate compared with those with low *PLCB4* expression. Multivariate Cox's regression analyses demonstrated that high *PLCB4* expression was an independent risk factor of adverse OS (P<0.01; HR, 2.081) and EFS (P<0.01; HR, 2.130). Following stratification analysis according to transplant status in cases of first complete remission, the patients with high expression of *PLCB4* had significantly lower OS and EFS rate in the chemotherapy group, but not the stem cell transplant group. Furthermore, *PLCB4*-associated genes were identified using Spearman's rank correlation analysis. KEGG pathway analysis revealed that *PLCB4* and its associated genes were mainly involved in three potential pathways, including the Rap1 signaling pathway. Overall, the findings of the present study suggest that increased *PLCB4* expression is associated with poor clinical outcome in pediatric patients with AML, and thus may represent a potential prognostic biomarker and therapeutic target for AML.

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Introduction

Acute myeloid leukemia (AML) is a highly heterogeneous disease, characterized by poor differentiation and abnormal proliferation of myeloid progenitors (1). AML is the most common type of acute leukemia in adults, with an estimated annual incidence of >20,000 new cases in the United States in 2019 (2). In 2013 it was reported that it was the leading cause of cancer deaths among children and individuals <35 years of age in China (3). Although ongoing advances in the treatment of AML have led to significant improvements in the clinical outcome, the overall survival (OS) rate remains low, mainly due to high recurrence rate and drug resistance (4). The specific mechanisms involved in these processes remain poorly defined.

Phospholipase C (*PLC*) plays an essential role in cell metabolism, as it hydrolyzes membrane-bound phospholipid phosphatidylinositol 4,5-biphosphate (PIP2) into two second messengers, inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) (5). A growing body of evidence indicates that increased expression of *PLC* promotes invasion and metastasis in several solid tumor types, including gastric carcinoma, breast cancer, hepatocellular carcinoma, pancreatic cancer, esophageal cancer and colorectal cancer (6,7).

Of the *PLC* isoforms, *PLCB1* promotes breast cancer cell migration via actin remodeling (8), and additionally regulates cell cycle progression in AML (9-12). *PLCB2* is significantly upregulated in human breast cancer and exhibits oncogenic functions and poor prognostic effects, by promoting cell division, motility and invasion (13,14).

PLCB4 encodes the β4 isoform of *PLC* isoenzymes, a superfamily that regulates the metabolism of inositol lipids (15). Increased *PLCB4* expression is associated with poor OS rate in patients with solid tumors, including mesothelioma, melanoma and gastrointestinal tumors (16-18). *PLCB4* contributes to solid tumor progression (16,17), however, its function in hematological tumors, particularly AML, has not been explored. Leukemia stem cells (LSC) are the source of drug resistance and relapse of AML, however, *PLCB4* expression in LSCs remain to be elucidated (19). In the present study, the effect of *PLCB4* expression and its clinical significance in AML were investigated. *PLCB4* expression may serve as a novel diagnostic or therapeutic target in pediatric patients with AML.

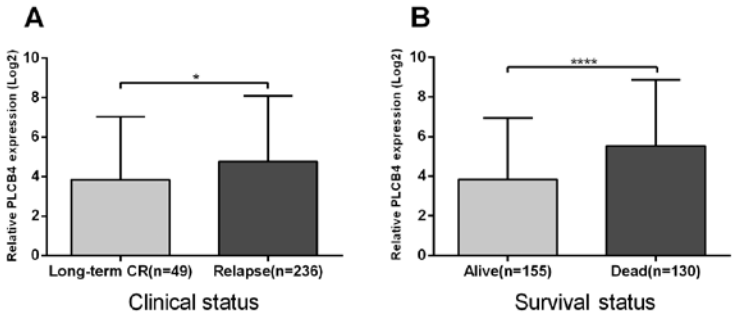

Figure 1. *PLCB4* expression in AML. (A) *PLCB4* expression in patients with AML of different clinical status (relapse and long-term CR). (B) *PLCB4* expression in AML patients of different survival status (alive and deceased) at 5-years follow-up. *P<0.05; ****P<0.0001. AML, acute myeloid leukemia; CR, complete remission; *PLCB4*, phospholipase C β4.

Materials and methods

Patients. All patients and corresponding clinical information were obtained from the Therapeutically Available Research to Generate Effective Treatments (TARGET) database (https://ocg.cancer.gov/programs/target/) on 11 November 2018. Samples were excluded if clinical information and RNA expression data were incomplete. A total of 285 pediatric patients with *de novo* AML were enrolled (median age, 10 years; range, 0-23 years), including 147 males and 138 females. Of these patients, 36 accepted stem cell transplants (SCTs) in first complete remission (CR1), and the remaining patients accepted chemotherapy. Median follow-up time of the patients was 1,355 days (range, 2-4,037 days).

Gene expression analyses. The raw counts of mRNA expression profiles (level 3) were downloaded from the TARGET database and $\log_2$ transformed. Patients were divided into high- and low-*PLCB4* groups, using the median of *PLCB4* expression level as the cut-off value. A comparison of clinical and molecular characteristics was performed between patients with high and low *PLCB4* expression.

Functional and pathway enrichment analysis of PLCB4-associated genes in AML. Spearman's rank correlation analysis was performed to identify *PLCB4*-associated genes using R software (version 3.3.3; www.r-project.org). Correlation coefficient values <-4 or >4 were considered to be significantly correlated with *PLCB4* expression. Gene Ontology (GO; http://geneontology.org) functional analysis (20) and Kyoto Encyclopedia of Genes and Genomes (KEGG; http://david.ncifcrf.gov) pathway enrichment analysis (21,22) of the *PLCB4*-associated genes were performed using the online Database for Annotation, Visualization and Integrated Discovery database (version 6.8; https://david.ncifcrf.gov/) (23). P<0.05 was considered as the cut-off criterion for significant differences.

PLCB4 expression in leukemia stem cells. The expression of *PLCB4* in primitive progenitor cells was assessed, based on the GSE30377 database (https://www.ncbi.nlm.nih.gov/geo/), in which gene expression data was obtained from 23 primary human AML samples and sorted into stem cells and progenitors, according to the expression of CD34 and CD38 markers (24).

Statistical analysis. Continuous variables were presented as the median with interquartile range, and categorical variables were presented as frequencies and percentage proportions. Pearson's χ^2 or Fisher's exact tests were performed for categorical variables. Mann-Whitney U tests were performed to analyze the difference of continuous variables between two groups, while Kruskal-Wallis test were used for the comparison of multiple groups, and a Bonferroni test for the post hoc test. OS was defined as the time from diagnosis until death for any reason, or until the last follow-up. Event-free survival (EFS) was defined as the time from diagnosis to death, relapse, induction failure or last follow-up. Survival analysis was based on the Kaplan-Meier method and the log-rank tests to compare the differences between survival curves. Univariate and multivariate Cox's regression analyses were used to assess the association between *PLCB4* expression and prognosis, as well as other clinical variables. The sensitivity and specificity of the *PLCB4* expression signature was evaluated according to the area under the curve (AUC) of receiver operating characteristic (ROC) curves of 5-year OS and EFS. ROC curves were generated using the survival ROC packages based on R software (version 3.3.3; www.r-project.org/). The SPSS software (version 23.0; IBM Corp.) was applied for statistical analysis. The figures were generated using GraphPad Prism (version 6.01; GraphPad Software, Inc.) or R software. P<0.05 was considered to indicate a statistically significant difference.

Results

PLCB4 expression in AML. Amongst the 285 patients, 49 remained in long-term complete remission (CR) until the last follow-up, and 236 experienced relapse. *PLCB4* expression was significantly upregulated in patients with relapse (median, 4.858) compared with patients with long-term CR (median, 4.087) (P<0.05; Fig. 1A). Significant differences were also observed in the classification of survival status (P<0.01; Fig. 1B). Median *PLCB4* mRNA expression was significantly lower in patients who survived (median, 3.807) compared with those that had died (median, 5.473) at 5-years follow-up. No significant differences in *PLCB4* expression were observed amongst karyotype and gender classifications (P=0.263 and 0.509, respectively) (data not shown).

Association between PLCB4 expression and clinical characteristics. The clinical and molecular features of patients

Table I. Comparison of clinical and molecular characteristics with *PLCB4* expression in patients with acute myeloid leukemia.

Characteristic	Low *PLCB4* (n=142)	High *PLCB4* (n=143)	P-value
Age, median (range) years	11 (0-22)	10 (0-23)	0.493
Sex, n (%)			0.259
Male	78 (54.9)	69 (48.3)	
Female	64 (45.1)	74 (51.7)	
Race, n (%)			0.510
Caucasian	108 (76.1)	102 (71.3)	
African American	13 (9.2)	19 (13.3)	
Asian	6 (4.2)	3 (2.1)	
Other	7 (4.9)	6 (4.2)	
Unknown	8 (5.6)	13 (9.1)	
WBC, median (range) $x10^9$/l	59.8 (0.9-446)	28.6 (2-519)	<0.01
BM blast, median (range), %	73 (20-100)	73 (14-99)	0.473
PB blast, median (range), %	63 (0-97)	59 (0-97)	0.012
FAB subtypes, n (%)			<0.01
M0	2 (1.4)	5 (3.5)	
M1	16 (11.3)	21 (14.7)	
M2	29 (20.4)	41 (28.7)	
M4	52 (36.6)	13 (9.1)	
M5	22 (15.5)	32 (22.4)	
M6	2 (1.4)	2 (1.4)	
M7	1 (0.7)	8 (5.6)	
NOS	9 (6.3)	8 (5.6)	
Unknown	9 (6.3)	13 (9.1)	
Karyotype, n (%)			0.536
Normal	33 (23.2)	38 (26.6)	
Abnormal	100 (67.8)	97 (67.8)	
Unknown	9 (6.3)	8 (5.6)	
SCT in 1st CR, n (%)			0.400
Yes	16 (11.3)	20 (14.0)	
No	117 (82.4)	108 (75.5)	
CR status at end of course 1, n (%)			0.064
Yes	116 (81.7)	104 (72.7)	
No	24 (16.9)	37 (25.9)	
CR status at end of course 2, n (%)			0.298
Yes	122 (85.9)	120 (83.9)	
No	13 (9.2)	19 (13.3)	
MRD at end of course 1, n (%)			0.011
Yes	28 (19.7)	46 (32.2)	
No	76 (53.5)	59 (41.3)	
MRD at end of course 2, n (%)			0.210
Yes	17 (12.0)	23 (16.1)	
No	81 (57.0)	70 (49.0)	
Induction failure, n (%)	11 (7.7)	17 (11.9)	0.240
Relapse, n (%)	110 (77.5)	126 (88.1)	0.017

PLCB4, phospholipase C β4; WBC, white blood cells; BM, bone marrow; PB, peripheral blood; FAB, French-American-British classification; SCT, stem cell transplantation; CR, complete remission; MRD, minimal residual disease.

were compared between high- and low-*PLCB4* groups, in order to determine the association of *PLCB4* expression with AML (Table I). Patients with low *PLCB4* expression had higher white blood cell counts (median, 59.8) vs. patients with

high *PLCB4* expression (median, 28.6) (P<0.01). Significant differences were found in both FAB subtypes (P<0.01) and peripheral blood (PB) blast (P=0.012) between the two groups. There were no significant associations between *PLCB4* expression and race, gender, karyotype status, and PB and bone marrow blast percentages. Patients with high *PLCB4* expression had a significantly higher relapse rate than those with low *PLCB4* expression (88.1 vs. 77.5%; P=0.017). In addition, patients with high *PLCB4* expression had a higher incidence of minute residual disease (MRD) at the end of the first course of chemotherapy compared with those with low *PLCB4* expression (32.2 vs. 19.7%; P=0.011). No significant differences were observed at the end of the second course of chemotherapy (P=0.210) between patients with low and high *PLCB4* expression. Patients with high *PLCB4* expression had a tendency to have lower CR rates at the end of the first course (72.7 vs. 81.7%; P=0.064) and second course of therapy (83.9 vs. 85.9%; P=0.298) compared with those with low *PLCB4* expression; however, the differences were not statistically significant.

Association between PLCB4 expression and genetic mutations. Additionally, the association between *PLCB4* expression and the molecular characteristics of patients was investigated. No significant differences were detected in the mutation frequencies of Fms-related tyrosine kinase 3 (FLT3) internal tandem duplication (ITD) or point mutation, nucleophosmin 1 (NPM1), CCAAT enhancer binding protein α (CEBPA) and Wilms' tumor gene 1 (WT1) between the two *PCLB4* expression groups (Table II).

Table II. Comparison of genetic mutations and *PLCB4* expression in patients with acute myeloid leukemia.

Gene mutation	Low PLCB4 (n=142)	High PLCB4 (n=143)	P-value
FLT3-ITD, n (%)			0.156
Yes	26 (18.3)	21 (14.7)	
No	116 (81.7)	122 (85.3)	
FLT3-PM, n (%)			0.159
Yes	13 (9.2)	7 (4.9)	
No	128 (90.1)	135 (94.4)	
NPM1, n (%)			0.268
Yes	7 (4.9)	12 (8.4)	
No	128 (90.1)	128 (89.5)	
CEBPA, n (%)			0.596
Yes	9 (6.3)	7 (4.9)	
No	131 (92.3)	134 (93.7)	
WT1, n (%)			0.052
Yes	17 (12.0)	8 (5.6)	
No	120 (84.5)	132 (92.3)	

PLCB4, phospholipase C β4; *FLT3*-ITD, internal tandem duplication of the *FLT3* gene; *FLT3*-PM, *FLT3* point mutation at codon 835-836; *NPM1*, nucleophosmin 1; *CEBPA*, CCAAT-enhancer binding protein α; *WT1*, Wilms tumor gene 1.

Association of PLCB4 expression and prognosis. To determine the prognostic value of *PLCB4* expression in AML, Kaplan-Meier curves were generated to examine the association between *PLCB4* expression and patient survival. Patients with high *PLCB4* expression had shorter OS (median, 28.5 vs. 60.7 months) and EFS (median, 12.5 vs. 16.3 months) time (Fig. 2A and B). The prognostic value of *PLCB4* expression was further confirmed using Cox's regression analyses (univariate and multivariate). As presented in Table III, univariate analysis indicated that *PLCB4* overexpression (HR, 1.905; P<0.01), *FLT3*-ITD-positive (HR, 1.681; P=0.017), and *WT1*-mutated (HR, 1.827; P=0.029) were associated with shorter OS time, while the mutations in *NPM1* (HR, 0.451; P=0.081) and *CEBPA* (HR, 0.215; P=0.031) were favorable for OS time. Furthermore, patients with *PLCB4* overexpression (HR, 1.903; P<0.01), as well as *FLT3*-ITD-positive (HR, 1.634; P=0.023), and *WT1*-mutated (HR, 1.988; P=0.013) genotypes had shorter EFS time. *NPM1*-mutated (HR, 0.403; P=0.046) and *CEBPA*-mutated (HR, 0.185; P=0.018) genotypes were associated with longer EFS time. Multivariate analysis revealed high *PLCB4* expression was an independent prognostic factor for shorter OS time (P<0.01; HR, 2.081) and EFS (P<0.01; HR, 2.130) in AML. Notably, when patients were stratified according to transplant status in CR1, in the chemotherapy group, patients with high *PLCB4* expression had significantly shorter OS (P<0.01) and EFS (P<0.01) times compared with those with low *PLCB4* expression (Fig. 2C and D). The results were unaffected by multivariate adjustments for clinical and genetic mutation variables: OS (P<0.01; HR, 2.239) and EFS (P<0.01; HR, 2.311) times. No significant differences between high and low *PLCB4* expression groups of patients undergoing SCT were observed (OS, P=0.812; EFS, P=0.833) (Fig. 2E and F). Overall, these data suggest *PLCB4* overexpression may be an independent predictor of poor prognosis in patients receiving chemotherapy, but not undergoing SCT in CR1.

Discriminative capacity of PLCB4 expression. In order to evaluate the clinical utility of *PLCB4* expression as a prognostic biomarker of AML, the AUC of ROC curves were used to determine the discriminative capacity of *PLCB4* expression to predict 5-year survival rates. The AUC values were high for the 5-year ROC curves of OS and EFS (AUC, 0.654 and 0.657, respectively; Fig. 3A and B), and similar results were observed for OS and EFS times of patients treated with chemotherapy in CR1 (AUC, 0.649 and 0.65, respectively; Fig. 3C and D). Overall, these findings suggest that *PLCB4* expression may serve as a potential prognostic biomarker of AML.

Functional and pathway enrichment analysis of PLCB4-associated genes in AML. In order to obtain insights into the biological functions and potential mechanisms of *PLCB4* in AML, *PLCB4*-associated genes were identified using Spearman's rank correlation analysis. A total of 648 mRNAs were significantly correlated with *PLCB4* expression. Of these, 14 genes were negatively correlated and 634 genes were positively correlated. *PCYT1B*, *PARD6B*, *RAB3IP*, *CALD1* and *ALDH1A1* were the top 5 genes that positively correlated with the expression levels of *PLCB4* according to the value

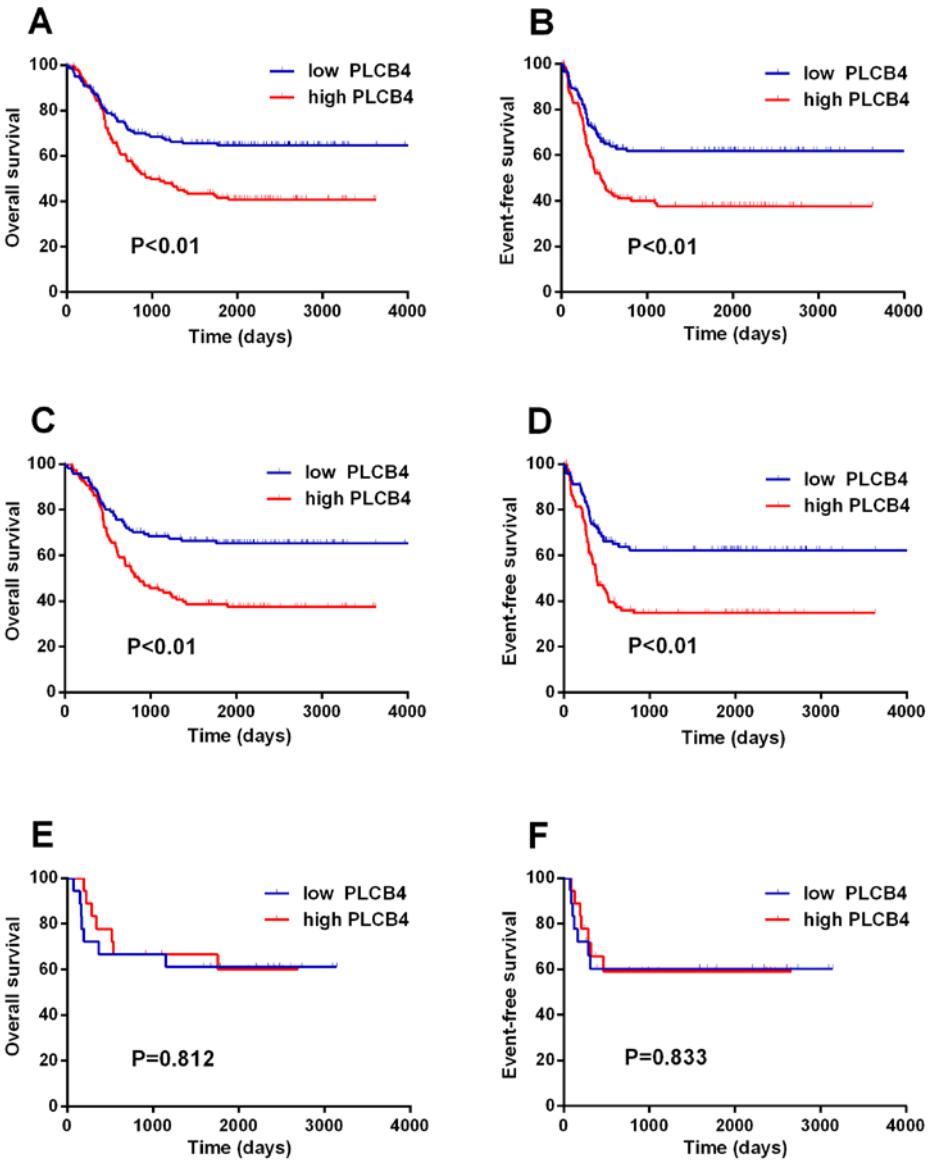

Figure 2. Survival analysis of patients with AML according to *PLCB4* gene expression. (A and B) Patients with high *PLCB4* expression had significantly shorter OS and EFS times compared with patients with low expression. (C and D) Patients that were treated with chemotherapy in CR1 and had high *PLCB4* expression had significantly shorter OS and EFS times compared with patients with low expression. (E and F) No significant differences were observed in OS and EFS times between high- and low-*PLCB4* expression groups in patients undergoing SCT in CR1. *PLCB4*, phospholipase C β4; SCT, stem cell transplants; OS, overall survival; EFS, event-free survival; CR1, first complete remission.

of the correlation coefficient. Subsequently, GO functional and KEGG pathway enrichment analysis were conducted, based on the genes that were correlated with *PLCB4* expression. The correlated genes were significantly enriched in the pathways associated with 'regulation of transcription', 'G_2/M transition of mitotic cell cycle', 'centriole replication', 'cilium assembly' and 'calcium-dependent cell-cell adhesion via plasma membrane cell adhesion molecules' (Fig. 4). KEGG pathway analysis predicted three potential pathways that were associated with *PLCB4* and its correlated genes and were regulated during AML, including the thyroid hormone signaling pathway, RAP1 signaling and platelet activation (Table IV).

mRNA expression of PLCB4 in leukemia stem cells. Amongst all cell populations that were assessed, the expression of *PLCB4* was highest in $CD34^+CD38^-$ cells compared with both $CD34^-/CD38^+$ and $CD34^+CD38^-$ cells (P=0.020 and 0.029, respectively; Fig. 5).

Discussion

Despite improvements in the prognosis of AML, several clinical challenges remain for this disease. High relapse rates remain the major cause of treatment failure in patients with AML and CR1, who are treated with intensive chemotherapy alone (1). The present study demonstrated that increased *PLCB4* expression was associated with a high risk of relapse and death, whereas low expression of *PLCB4* was associated with favorable prognosis in patients with AML. ROC curve and Cox's regression analyses for OS and EFS time of patients with AML further confirmed that *PLCB4* expression was considered as an independent prognostic indicator. Furthermore,

Table III. Univariate and multivariate analyses of prognostic factors for OS and EFS in AML patients.

A, Univariate analysis

	OS		EFS	
Variables	HR (95% CI)	P-value	HR (95% CI)	P-value
PLCB4	1.905 (1.335-2.718)	<0.01	1.903 (1.334-2.714)	<0.01
Age	1.022 (0.992-1.054)	0.156	1.014 (0.983-1.045)	0.382
Sex	1.308 (0.927-1.845)	0.127	1.288 (0.913-1.817)	0.150
Race	1.005 (0.797-1.268)	0.963	0.958 (0.759-1.209)	0.718
FAB	1.071 (0.981-1.169)	0.127	1.091 (0.998-1.191)	0.055
WBC	1.000 (0.998-1.002)	0.894	1.000 (0.998-1.002)	0.841
BM blast	0.998 (0.990-1.007)	0.703	0.997 (0.989-1.006)	0.512
PB blast	0.996 (0.990-1.002)	0.206	0.996 (0.990-1.002)	0.175
Karyotype	1.103 (0.737-1.651)	0.634	1.110 (0.741-1.662)	0.612
SCT in 1st CR	0.842 (0.482-1.471)	0.545	0.804 (0.460-1.404)	0.442
FLT3-ITD	1.681 (1.099-2.571)	0.017	1.634 (1.069-2.497)	0.023
FLT3-PM	0.474 (0.194-1.158)	0.101	0.445 (0.182-1.088)	0.076
NPM1	0.451 (0.184-1.103)	0.081	0.403 (0.164-0.986)	0.046
CEBPA	0.215 (0.053-0.868)	0.031	0.185 (0.046-0.748)	0.018
WT1	1.827 (1.065-3.134)	0.029	1.988 (1.158-3.412)	0.013

B, Multivariate analyses

	OS		EFS	
Variables	HR (95% CI)	P-value	HR (95% CI)	P-value
PLCB4	2.081 (1.440-3.008)	<0.01	2.130 (1.447-3.137)	<0.01
FAB	-	-	1.099 (1.004-1.203)	0.040
FLT3-ITD	1.709 (1.066-2.742)	0.026	1.699 (0.994-2.902)	0.052
FLT3-PM	-	-	0.526 (0.192-1.440)	0.211
NPM1	0.317 (0.126-0.798)	0.015	0.340 (0.132-0.875)	0.025
CEBPA	0.213 (0.053-0.864)	0.030	0.194 (0.048-0.791)	0.022
WT1	1.536 (0.835-2.825)	0.167	1.807 (0.964-3.386)	0.065

Multivariate analysis included variables with P<0.1 in univariate analysis of OS and EFS. HR ≥1.0 indicated a higher risk for OS and EFS, whilst HR ≤1.0 indicated a lower risk. *PLCB4*, phospholipase C β4; CI, confidence interval; HR, hazard ratio; EFS, event-free survival; OS, overall survival; WBC, white blood cell; BM, bone marrow; PB, peripheral blood; FAB, French-American-British classification; SCT, stem cell transplantation; CR, complete remission; *FLT3*-ITD, internal tandem duplication of the *FLT3* gene; *FLT3*-PM, *FLT3* point mutation at codon 835-836; *NPM1*, nucleophosmin; *CEBPA*, CCAAT-enhancer binding protein α; *WT1*, Wilms tumor gene 1.

Table IV. Kyoto Encyclopedia of Genes and Genomes pathway analysis prediction of potential pathways in which *PLCB4* and *PLCB4*-associated genes were enriched in acute myeloid leukemia.

Pathway ID	Pathway name	Genes
hsa04919	Thyroid hormone signaling pathway	*SLC16A2*, *HDAC2*, *PLCB4*, *THRB*, *SLCO1C1*, *TBC1D4*, *ITGB3*, *PIK3R3*, *MED12L*
hsa04015	RAP1 signaling pathway	*PARD6B*, *IGF1R*, *MAGI3*, *PLCB4*, *TIAM1*, *TEK*, *RAPGEF6*, *RAPGEF5*, *ITGB3*, *RAPGEF2*, *EGF*, *PIK3R3*
hsa04611	Platelet activation	*PLCB4*, *PPP1R12A*, *COL2A1*, *PRKG2*, *ITGB3*, *PIK3R3*, *COL11A1*, *COL5A1*

PLCB4, phospholipase C β4; has, Homo sapiens.

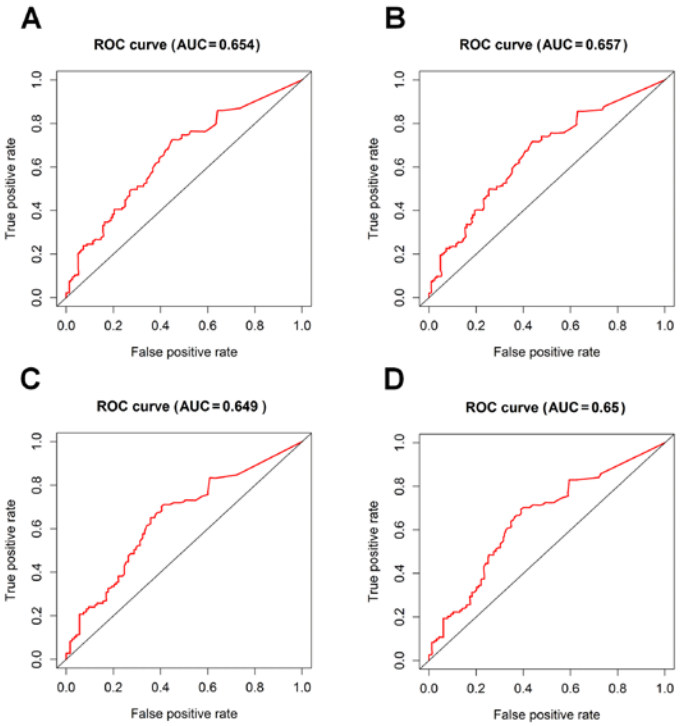

Figure 3. ROC analysis of phospholipase C β4 expression to predict 5-year OS and EFS. ROC predicts 5-year (A) OS and (B) EFS of all patients in the cohort, and (C) OS and (D) EFS of patients treated with chemotherapy. ROC, receiver operating characteristics; OS, overall survival; EFS, event-free survival; AUC, area under the ROC curve.

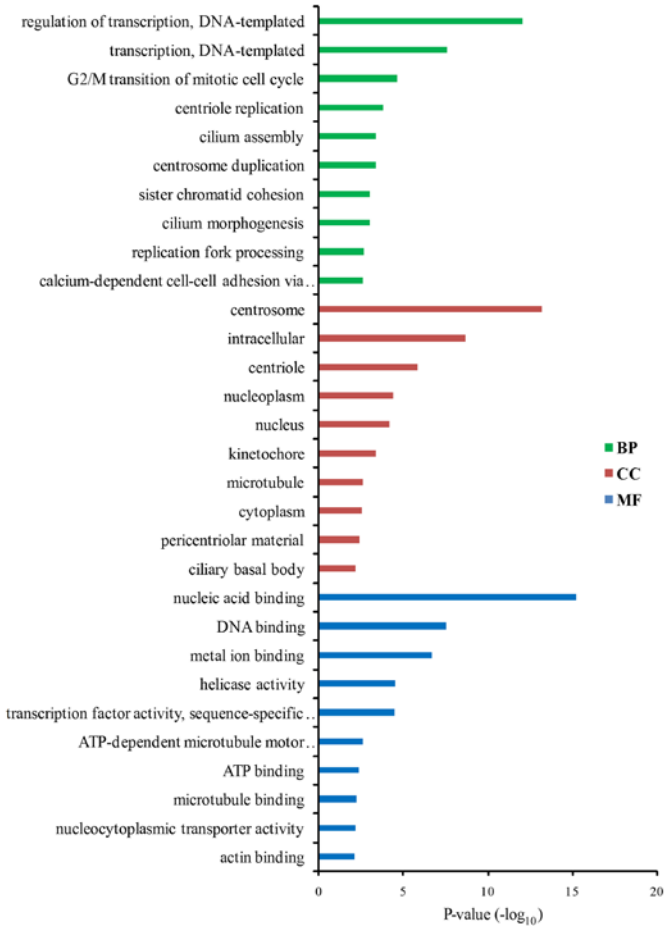

Figure 4. Functional enrichment analysis of *PLCB4* and associated genes in AML. GO analysis of *PLCB4* and associated genes using the Database for Annotation, Visualization and Integrated Discovery database in AML showed 143 GO terms enriched with these genes. The top 10 enriched GO terms were classified into CC, MF and BP. *PLCB4*; phospholipase C β4; GO, Gene Ontology; BP, biological processes; CC, cell components; MF, molecular functions; AML, acute myeloid leukemia.

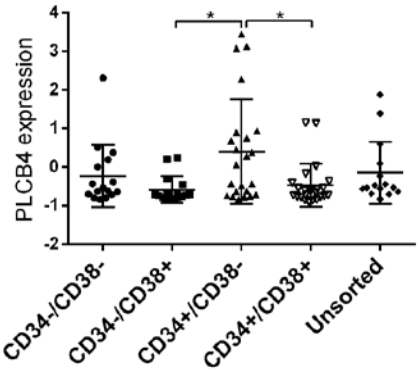

Figure 5. *PLCB4* mRNA expression in leukemia stem cell populations. Comparison of *PLCB4* mRNA expression differences among the following sorted cell populations: $CD34^+CD38^+$, $CD34^+CD38^-$, $CD34^-CD38^+$, $CD34^-CD38^-$ and unsorted cell populations according to the CD34 and CD38 markers of leukemia stem cells. $^*P<0.05$. *PLCB4*; phospholipase C β4.

high *PLCB4* expression was associated with an unfavorable outcome in patients with AML who received chemotherapy. Thus, *PLCB4* represents a predictive molecular marker for the effectiveness of chemotherapy. However, further verification is required in larger cohorts.

High *PLCB4* expression was reported in numerous cancer types and was associated with worse clinical outcomes for gastrointestinal tumors and mesothelioma, as well as melanomas (17,18). The present study suggests that *PLCB4* expression plays a vital role in tumor development and recurrence in patients with AML, however the underlying mechanisms of *PLCB4* in AML remain poorly understood. A previous study reported that *PLCB4* was upregulated in multidrug-resistant HL-60 cell lines compared with wild-type HL-60 cells (25), indicating its association with drug-resistance in leukemia.

The presence of MRD following induction and/or consolidation chemotherapy has been demonstrated to be a significant risk factor and predictive marker of relapse in patients with AML (5,26-29). A growing body of evidence suggests that MRD prior to hematopoietic cell transplantation is associated with adverse clinical prognosis in AML in CR1 (30,31). Notably, this study's findings indicated that a positive effect of *PLCB4* overexpression on the incidence of MRD was observed in patients with AML and CR1, demonstrating that *PLCB4* expression plays a role in the relapse of AML.

$CD34^+CD38^-$ leukemia stem cells are resistant to chemotherapy, immune-evasive, and are associated with a lower CR rate following induction and an unfavorable prognosis in AML (32,33). In the present study, *PLCB4* was found to be highly expressed in $CD34^+CD38^-$ populations and was significantly associated with ALDH1A1, an important marker of cancer stem cells (34). However, the specific mechanism of *PLCB4* in leukemia stem cells remain undefined.

To further clarify the impact of *PLCB4* expression on the response to treatment and clinical outcomes in patients with AML, the genes that were significantly correlated with *PLCB4* expression were identified in the current study. GO and KEGG analysis were performed to examine the potential functional pathways of *PLCB4*-associated genes involved in AML. RAP1 signaling regulates several biological processes, including cell polarity, proliferation, differentiation, adhesion and movement (35). Moreover, RAP signaling plays an essential role in the invasion and migration of leukemia cells through interaction with downstream target molecules (36). RAP guanine nucleotide exchange factors (RAPGEFs) act as a molecular switch by promoting the exchange of RAP1 from a GDP-bound state to the active GTP-bound state (37,38). Notably, RAPGEF6, RAPGEF5 and RAPGEF2 were significantly correlated with *PLCB4* expression. GO and KEGG analysis revealed *PLCB4* and *PLCB4*-associated genes were involved in 'regulation of transcription, DNA-templated', 'transcription, DNA-templated', 'G_2/M transition of mitotic cell cycle', 'centriole replication' and 'RAP1 signaling pathway'. Thus, RAP1 signaling may be involved in AML cell migration and invasion, via activation of *PLCB4*. Further studies to confirm this hypothesis experimentally are required.

To the best of our knowledge, the present study is the first to evaluate the prognostic value of *PLCB4* expression in AML. However, some potential limitations remain. The present study was based on information obtained from the TARGET database, which restricted the data available. Experiments on cell and animal models are required to understand and validate the role of *PLCB4* expression in AML. Despite these limitations, the present study identified a direct association between *PLCB4* expression and prognosis based on a large and representative population. Further studies are required to elucidate the potential molecular mechanisms of *PLCB4* in AML.

In conclusion, upregulation of *PLCB4* was associated with a poor clinical outcome in patients with AML. *PLCB4* may therefore be a potential prognostic biomarker and therapeutic target of AML.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LZ, SW and WZ contributed to the study design. DS contributed to downloading and processing the data. SW and JL analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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