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 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.

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.

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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 log₂ 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 χ² 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 \textit{PLCB4} expression in patients with acute myeloid leukemia.

| Characteristic | Low \textit{PLCB4} (n=142) | High \textit{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) x10⁹/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 (%) | 0.240 |    | |
| Yes           | 11 (7.7) | 17 (11.9) | |
| No            | 110 (77.5) | 126 (88.1) | 0.017 |

\textit{PLCB4}, phospholipase C \(\beta4\); 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-\textit{PLCB4} groups, in order to determine the association of \textit{PLCB4} expression with AML (Table I). Patients with low \textit{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 and high 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 mutation frequencies of Fms-related tyrosine kinase 3 (FLT3) expression and the molecular characteristics of patients was statistically significant.

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. PCTY1B, PARD6B, RAB3IP, CALD1 and ALDH1A1 were the top 5 genes that positively correlated with the expression levels of PLCB4 according to the value

### 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.
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', 'G2/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

| Variables | OS             | P-value | EFS             | P-value |
|-----------|----------------|---------|-----------------|---------|
|           | HR (95% CI)    |         | HR (95% CI)     |         |
| 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

| Variables | OS             | P-value | EFS             | P-value |
|-----------|----------------|---------|-----------------|---------|
|           | HR (95% CI)    |         | HR (95% CI)     |         |
| 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 path | SLC16A2, HDAC2, PLCB4, THRBD, 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, COL1A1, 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.
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’, ‘G2/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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