

Prognostic Significance of Dual-Specificity Phosphatase 23 Expression in Acute Myeloid Leukemia

Xi Liu^{1,2}, Haihui Zhuang^{1,2}, Fenglin Li^{1,2}, Ying Lu^{1,2}, Renzhi Pei^{1,2}

¹Department of Hematology, The Affiliated People's Hospital of Ningbo University, Ningbo, People's Republic of China; ²Institute of Hematology, Ningbo University, Ningbo, People's Republic of China

Correspondence: Ying Lu; Renzhi Pei, Tel +86-574-87016871; Tel +86-574-87016870, Email rmluying@nbu.edu.cn; peirz@163.com

Background: Recently, an increasing number of studies have suggested dual-specificity phosphatase 23 (DUSP23) is a critical factor in the development of diffuse connective tissue disease and may be a valuable biomarker for primary human cancers. However, there is a lack of comprehensive studies on the prognostic significance of DUSP23 expression in acute myeloid leukemia (AML).

Methods: RNA sequencing data from The Cancer Genome Atlas (TCGA) (AML = 173), Genotype-Tissue Expression (GTEx) (healthy controls = 70) and GEO (AML = 461, healthy controls = 76) databases were used to compare DUSP23 expression between AML patients and healthy controls. The overall survival (OS) of DUSP23 in AML was evaluated using Kaplan-Meier Cox regression. Furthermore, univariate Cox regression and multivariate Cox regression analysis were used to determine whether DUSP23 was an independent prognostic factor for AML. We then verified the expression level and prognostic significance of DUSP23 in our cohort (AML = 128, healthy controls = 31). In addition, functional enrichment analysis of DUSP23-related DEGs was performed through gene set enrichment analysis (GSEA) and protein-protein interaction (PPI) network analysis.

Results: The expression level of DUSP23 is significantly higher in AML patients than in healthy controls in TCGA, GTEx, GEO databases and our cohort. By multivariate analysis, high expression of DUSP23 is a poor prognostic indicator of OS in the TCGA database. Next, we verified the role of DUSP23 as an adverse prognostic biomarker in our cohort. Enrichment analysis of related genes showed that DUSP23 may regulate important signal pathways in hematological tumors including the MAPK pathways. It is suggested by the PPI network that DUSP23, along with IMP3, MRPL4, MRPS12, POLR2L, and ATP5F1D may play a role in the process of AML.

Conclusion: The study demonstrated high expression of DUSP23 could serve as a poor independent prognostic biomarker in AML.

Keywords: DUSP23, expression, prognosis, AML, bioinformatics

Introduction

AML is highly heterogeneous and represents a cohort of clonal malignancies derived from myeloid precursors.¹ Existing drugs' poor therapeutic effects are attributed to leukemia's complex molecular mechanisms and chemoresistance.² The molecular basis of AML, aided by biomarkers, can play an essential role in AML diagnosis, prognostic stratification, residual leukemia monitoring, treatment response prediction, and targeted drug development. Therefore, it is necessary to discover novel biomarkers to better understand the molecular basis of AML.^{3,4}

Homoharringtonine (HHT) is widely used in clinical practice. Because of its anti-leukemic efficacy, cost-effectiveness, and minimal toxicity. HHT-resistant strains were constructed by our research team to explore the mechanism of HHT resistance. It was observed that the DUSP23 gene exhibited differential low expression in HHT-resistant cells than sensitive cells.⁵ This indicated that DUSP23 may serve as a therapeutic target and a potential prognostic factor in AML. DUSP23 is located on chromosome 1q23 and is characterized by a dual-specificity phosphatase catalytic domain.⁶ It belongs to the low-molecular-weight VHR-like subfamily of the dual-specificity phosphatase family. The expression of DUSP23 is tissue-specific, being expressed only in a few

normal tissues but more in fetal and tumor tissues, suggesting that its function may be related to embryonic development and tumor growth.⁷ So far, the expression of DUSP23 in AML and its prognostic value remain unclear.

The objective of this study was to assess the prognostic significance of DUSP23 in AML. Firstly, RNA sequencing data of AML patients and healthy controls were obtained from the TCGA, GTEx and GEO databases to examine the expression characteristics of DUSP23. Subsequently, the clinical significance of DUSP23 in AML was assessed by using Kaplan-Meier and Cox regression analyses and developing a nomogram prognostic model. Next, we verified the differences in DUSP23 expression between AML patients and healthy controls in our cohort and assessed its prognostic relevance. Additionally, functional enrichment analyses of DUSP23 were conducted using various methods, including GO, KEGG, GSEA, the PPI network, SSGSEA, and immune cell infiltration analysis, to provide a preliminary grasp of the underlying molecular mechanisms of DUSP23 in AML carcinogenesis and progression. Based on the above results, the prognostic value of DUSP23 for AML was confirmed.

Materials and Methods

Data Source

RNA-seq data and clinical information of AML patients for the TCGA and GTEx databases were obtained from the UCSC XENA browser (<https://portal.gdc.cancer.gov>;<https://xenabrowser.net/datapages/>),^{8–12} and the RNA-seq dataset GSE6891 was downloaded from the GEO databases <https://www.ncbi.nlm.nih.gov/geo/>.^{13–15} The TCGA databases include 173 AML patient samples, the GTEx databases include 70 healthy controls. The GSE6891 dataset, derived from the GPL570 platform, comprises 461 AML patient samples and 76 healthy controls. TPM values obtained from RNA-Seq were utilized, and a log2 transformation was performed for intrasample comparisons to ensure data consistency across different databases. Samples without survival data were excluded from the analysis.

Patients' Samples

A total of 128 AML patients and 31 healthy control bone marrow samples were collected at the Affiliated People's Hospital of Ningbo University. Written informed consent was obtained from all participants before their involvement in the study. This study was approved by the Research Ethics Committee of the Affiliated People's Hospital of Ningbo University. Patients were divided into two groups based on the cut-off value of DUSP23 mRNA expression. Non-M3 patients received standard anthracycline plus cytarabine therapy, while M3 patients received all-trans retinoic acid (ATRA) plus arsenic trioxide (ATO) therapy. Baseline clinical characteristics are shown in Table 1.

Table 1 Characteristics of AML Patients by High and Low DUSP23 Expression

Characteristics	Level	Low Expression of DUSP23	High Expression of DUSP23	p
n		71	37	
Age, n (%)	<= 60	30(42.3%)	22(59.5%)	0.089
	> 60	41(57.7%)	15(40.5%)	
WBC count($\times 10^9/L$), n (%)	<= 20	32(37.5)	25(67.6%)	0.026
	> 20	39(54.9%)	12(32.4%)	
BM blasts (%), n (%)	<= 20	5(7%)	3(8.1%)	0.841
	> 20	66(93%)	34(91.9%)	
Gender, n (%)	Male	42(59.2%)	20(54.1%)	0.611
	Female	29(40.8%)	17(45.9%)	
FLT3 mutation, n (%)	Negative	59(83.1%)	28(75.7%)	0.355
	Positive	12(16.9%)	9(24.3%)	
DNMT3A, n(%)	Negative	61(85.9%)	33(89.2%)	0.858
	Positive	10(14.1%)	4(10.8%)	

(Continued)

Table I (Continued).

Characteristics	Level	Low Expression of DUSP23	High Expression of DUSP23	p
KIT, n(%)	Negative	61(85.9%)	34(91.9%)	0.552
	Positive	10(14.1%)	3(8.1%)	
NPM1 mutation, n(%)	Negative	53(74.6%)	33(89.2%)	0.126
	Positive	18(25.4%)	4(10.8%)	
Cytogenetics, n (%)	t(8;21)	5 (55.6%)	5(71.4%)	0.672
	inv(16)	3(33.3%)	1(14.3%)	
	t(9;22)	1(11.1%)	1 (14.3%)	
Consolidation, n(%)	HSCT	24(33.8%)	9(24.3%)	0.31
	Chemotherapy	47(66.2%)	28(75.7%)	

Validation of Prognostic Gene by Quantitative Real-Time PCR (qRT-PCR) Analysis

RNA extraction from the tissue samples was conducted using Trizol reagent (Invitrogen, Shanghai, China) following the manufacturer's protocol. The obtained RNA (1000 ng) was then utilized for cDNA synthesis through a cDNA synthesis kit (Takara, Tokyo, Japan). qRT-PCR was performed using TB Green PCR Master Mix (Takara, Tokyo, Japan) on a 7500 Fast Real-Time PCR System (Applied Biosystems, Singapore), and the relative expression of DUSP23 was determined using the comparative cycle threshold (Ct) method.^{16,17} The $2^{-\Delta\Delta Ct}$ method was employed to assess gene expression relative to glyceraldehyde—3—phosphate dehydrogenase (GAPDH) for AML cells. The primers were as follows: DUSP23 forward: 5'-GCCATTGCTGAAATCCGACG-3'; reverse: 5'-CTGCTCATAGGTCTCGATGGA-3'; GAPDH forward: 5'-ATGGGGAAGGTGAAGGTCG-3'; reverse: 5'-GGGTCATTGATGGCAACAATATCCATA-3'.

Prognostic Model Generation and Prediction

A nomogram was created using the RMS R package (version 6.3–0) to personalize the prediction of OS in AML patients, incorporating significant clinical characteristics and calibration plots. Calibration curves were used to assess the accuracy of the nomogram, which compared the observed rates with the nomogram-predicted probabilities. The optimal predictive values were represented by the 45° line. The discrimination ability of the nomogram was determined by the concordance index (C-index), which was computed using 8000 bootstraps resamples.¹⁸

DEG Analysis

The expression profile of high- and low-expression levels of DUSP23 (cut-off value of 50%) in AML samples (HTseq-Count) were compared using the DESeq2 R package to identify DEGs.^{19,20} A heatmap was used to visualize the top 20 DEGs (Figure 1B).

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichments Analysis of DEGs

A threshold of $|\log FC| > 1.5$ and $p_{adj} < 0.05$ was applied to select DEGs for functional enrichment analysis, and R (4.2.1) was used for this analysis. The clustered DEGs were visualized as a heatmap using ggplot2[3.3.6] in R.

GSEA

GSEA was performed using the R (4.2.1) to identify functional and pathway differences between the high- and low-expression groups of DUSP23. Significance was determined based on an adjusted P-value < 0.05 and FDR q-value < 0.25 .

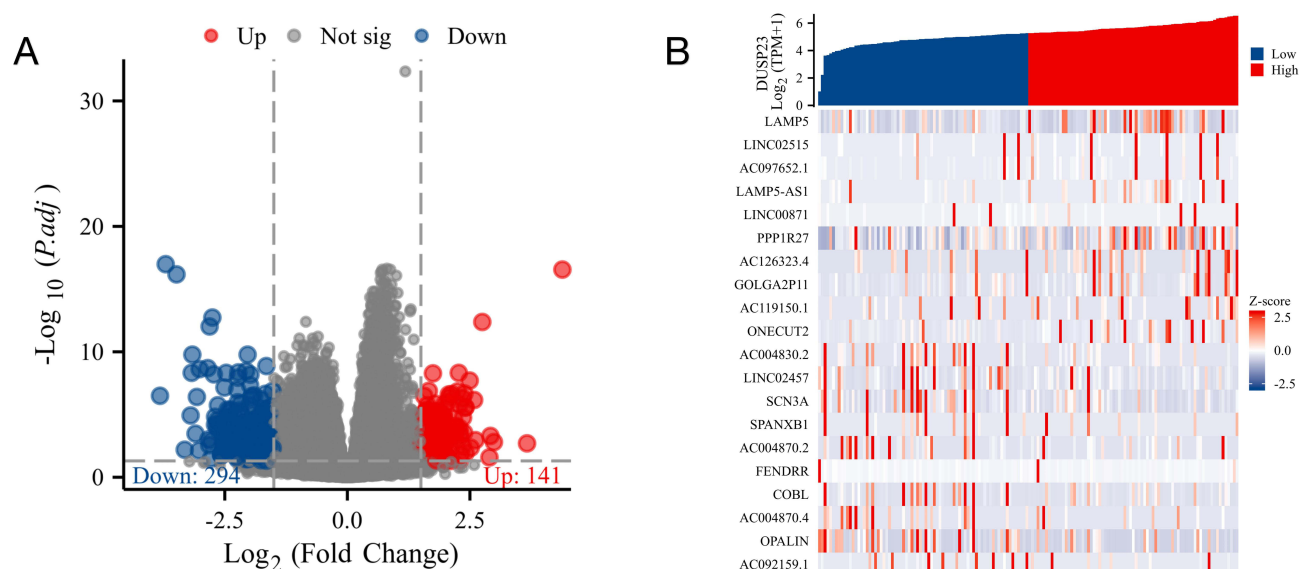


Figure 1 DEGs of the high- and low-DUSP23 expression groups in AML. **(A)** Volcano plot of DUSP23-related DEGs. **(B)** Heatmap of DUSP23-related DEGs.

PPI Network

The PPI network was derived from the STRING database (<https://string-db.org/>) to estimate the interactional correlations of the DEGs.²¹ A confidence score >0.45 was considered significant. Hub proteins and key nodes in the constructed PPI network were identified using the Cytoscape plugin CytoHubba.^{5,22}

Immune Infiltration Analysis

SSGSEA analysis was performed using the GSVA package in R (version 3.6.3) to analyze immune infiltration in DUSP23. Data on 24 types of infiltrating immune cells, as previously described, were obtained. The correlation between DUSP23 expression and the enrichment scores of the 24 types of immune cells was assessed using Spearman correlation.^{23,24} Moreover, the enrichment scores between high- and low-DUSP23 expression groups were compared using the Wilcoxon rank-sum test.

Statistical Analysis

The statistical data obtained from TCGA and GTEx were analyzed using R-4.2.1. Prognostic factors were evaluated using Cox regression analysis and the Kaplan-Meier method. The median expression of DUSP23 was taken as the cut-off value. A statistically significant result was considered as a P-value less than 0.05 in all tests. Furthermore, the effectiveness of the transcriptional expression of DUSP23 in differentiating AML from healthy samples was assessed using ROC analysis on the pROC package. The area under the curve (AUC) value, computed in the range of 0.5 to 1.0, indicated a discrimination ability of 50–100%. OS is defined as the length of time from a specific point in time, such as diagnosis or treatment, that patients with a disease are still alive.

Results

DUSP23 Expression in Pan-Cancer and AML

In pan-cancer analysis, the expression profiles of DUSP23 in normal and malignant samples were determined. Remarkably, DUSP23 was upregulated in multiple malignancies like Bladder cancer, ovarian cancer, etc. ([Supplementary Figure 1](#)). In addition, DUSP23 was also abnormally highly expressed in AML compared with healthy controls ([Figure 2A](#)). Subsequently, the result from GSE6891 also confirmed that DUSP23 expression is higher in AML patient samples ([Figure 2B](#)). To further investigate the significance of DUSP23 expression, the Wilcoxon Rank Sum test was used to compare its expression levels in patients with various clinicopathological features. The results indicated that DUSP23 was significantly upregulated in patients with > 70% peripheral blood (PB) blasts ($P < 0.05$) than those with $\leq$

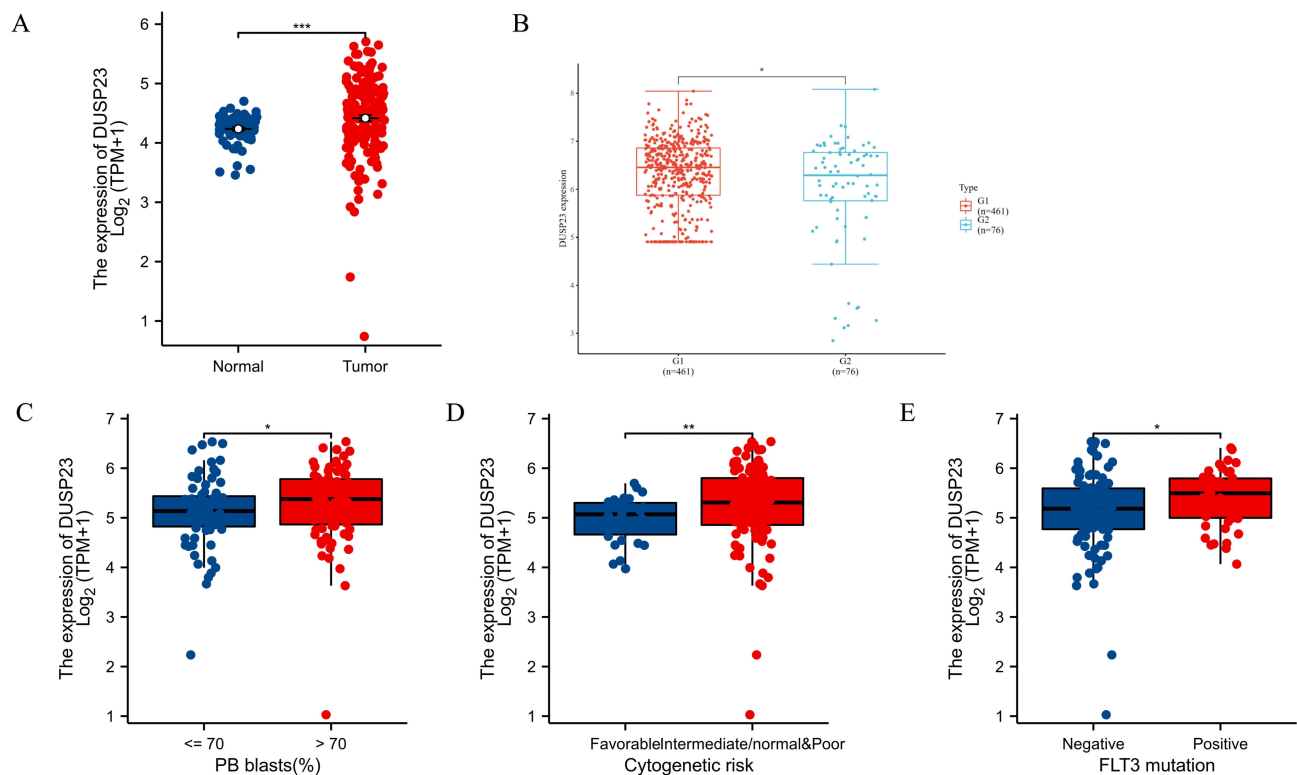


Figure 2 The association of DUSP23 expression with clinical characteristics. **(A)** Expression level of DUSP23 in paired normal and AML samples. Analysis between two groups: Wilcoxon Rank sum test; NS: $P \geq 0.05$ or higher; (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). **(B)** DUSP23 expression in AML from GEO databases (GSE6891). **(C)** PB blasts abundance, **(D)** Cytogenetic risk, **(E)** FLT3 mutation.

70% PB blasts, in patients with poor cytogenetic risk ($P < 0.01$) than intermediate/normal risk, and in FLT3 mutation-positive cases ($P < 0.05$) than FLT3 mutation-negative cases (Figure 2C–E). This may indicate that high DUSP23 expression is associated with an unfavorable prognosis.

The Relationship Between the Expression of DUSP23 and the Prognosis of AML Patients

The above results suggest that elevated expression of DUSP23 is associated with an unfavorable prognosis. Subsequently, the verification of whether DUSP23 is an independent predictive factor for unfavorable AML prognosis will be conducted. The clinical characteristics of TCGA patients were downloaded and divided into high and low expression groups according to the median of the expression of DUSP23 (Table 2). Notably, patients with higher DUSP23 expression had significantly higher WBC counts and PB blasts than those with lower DUSP23 expression ($P = 0.017$ and $= 0.014$, respectively; Table 2). There were also significant differences between the two groups in the incidence of each FAB classification and cytogenetic risk ($P = 0.006$ and $= 0.016$, respectively; Table 2). Higher DUSP23 expression was significantly correlated with FLT3 ($P = 0.01$; Table 2).

The study identified an association between poor OS and increased expression of DUSP23 in AML patients (Figure 3A). The robust prognostic value of DUSP23 for OS in AML patients was confirmed by ROC analysis, with a C-index of 0.647 (95% confidence interval: 0.580–0.714) (Figure 3B). In addition, cytogenetic risk (intermediate/normal and poor) and DUSP23 were also poor prognostic factors for AML patients (Figure 3C).

In addition, univariate Cox proportional hazards regression was used to assess factors influencing OS. The results revealed that high expression of DUSP23 ($P = 0.009$), poor & intermediate cytogenetic risk ($P < 0.001$), and age > 60 ($P < 0.001$) were predictive factors for worse OS (Table 3). Subsequently, multivariate Cox regression analysis including

Table 2 Association Between DUSP23 Expression and Clinicopathologic Features in AML Samples from the TCGA

Characteristics	Level	Low Expression of DUSP23	High Expression of DUSP23	p
n		75	75	
Age, n (%)	<= 60	45 (30%)	42 (28%)	0.620
	> 60	30 (20%)	33 (22%)	
WBC count ($\times 10^9/L$), n (%)	<= 20	45 (30.2%)	31 (20.8%)	0.017
	> 20	29 (19.5%)	44 (29.5%)	
BM blasts (%), n (%)	<= 20	32 (21.3%)	27 (18%)	0.403
	> 20	43 (28.7%)	48 (32%)	
PB blasts (%), n (%)	<= 70	43 (28.7%)	28 (18.7%)	0.014
	> 70	32 (21.3%)	47 (31.3%)	
Cytogenetic risk, n (%)	Favorable	22 (14.9%)	8 (5.4%)	0.016
	Intermediate/normal	35 (23.6%)	47 (31.8%)	
	Poor	18 (12.2%)	18 (12.2%)	
Gender, n (%)	Female	31 (20.7%)	36 (24%)	0.412
	Male	44 (29.3%)	39 (26%)	
Race, n (%)	Asian	1 (0.7%)	0 (0%)	0.670
	White	66 (44.3%)	69 (46.3%)	
	Black or African American	7 (4.7%)	6 (4%)	
FLT3 mutation, n (%)	Negative	57 (39%)	44 (30.1%)	0.010
	Positive	15 (10.3%)	30 (20.5%)	
IDH1 R132 mutation, n (%)	Negative	70 (47.3%)	65 (43.9%)	0.147
	Positive	4 (2.7%)	9 (6.1%)	
IDH1 R140 mutation, n (%)	Negative	67 (45.3%)	69 (46.6%)	0.547
	Positive	7 (4.7%)	5 (3.4%)	
IDH1 R172 mutation, n (%)	Negative	73 (49.3%)	73 (49.3%)	1.000
	Positive	1 (0.7%)	1 (0.7%)	
RAS mutation, n (%)	Negative	70 (47%)	71 (47.7%)	1.000
	Positive	4 (2.7%)	4 (2.7%)	
NPM1 mutation, n (%)	Negative	65 (43.6%)	51 (34.2%)	0.004
	Positive	9 (6%)	24 (16.1%)	
FAB classifications, n (%)	M0	11 (7.5%)	4 (2.7%)	0.006
	M1	11 (7.5%)	24 (16.4%)	
	M2	23 (15.8%)	15 (10.3%)	
	M3	9 (6.2%)	5 (3.4%)	
	M4	17 (11.6%)	12 (8.2%)	
	M5	3 (2.1%)	12 (8.2%)	
	M6	1 (0.7%)	1 (0.7%)	
	M7	0 (0%)	1 (0.7%)	
	+8	5 (4%)	3 (2.4%)	
	Complex	13 (10.4%)	11 (8.8%)	
Cytogenetics, n (%)	del(7)	4 (3.2%)	2 (1.6%)	0.551
	inv(16)	4 (3.2%)	4 (3.2%)	
	Normal	28 (22.4%)	41 (32.8%)	
	t(15;17)	6 (4.8%)	4 (3.2%)	

age, cytogenetic risk, and DUSP23 expression showed that age > 60 ($P < 0.001$), poor & intermediate cytogenetic risk ($P = 0.032$), and high expression of DUSP23 ($P = 0.008$) were independent prognostic factors for worse OS ($P < 0.05$).

Then a Kaplan-Meier analysis was performed to examine the correlation between DUSP23 expression and AML patient prognosis. It was observed that poor prognosis was associated with high expression of DUSP23 in various subgroups, such as patients with age > 60 ($P < 0.001$; [Figure 4A](#)), BM blasts of > 20% ($P = 0.009$; [Figure 4B](#)), PB blasts <= 70% ($P = 0.02$; [Figure 4C](#)),

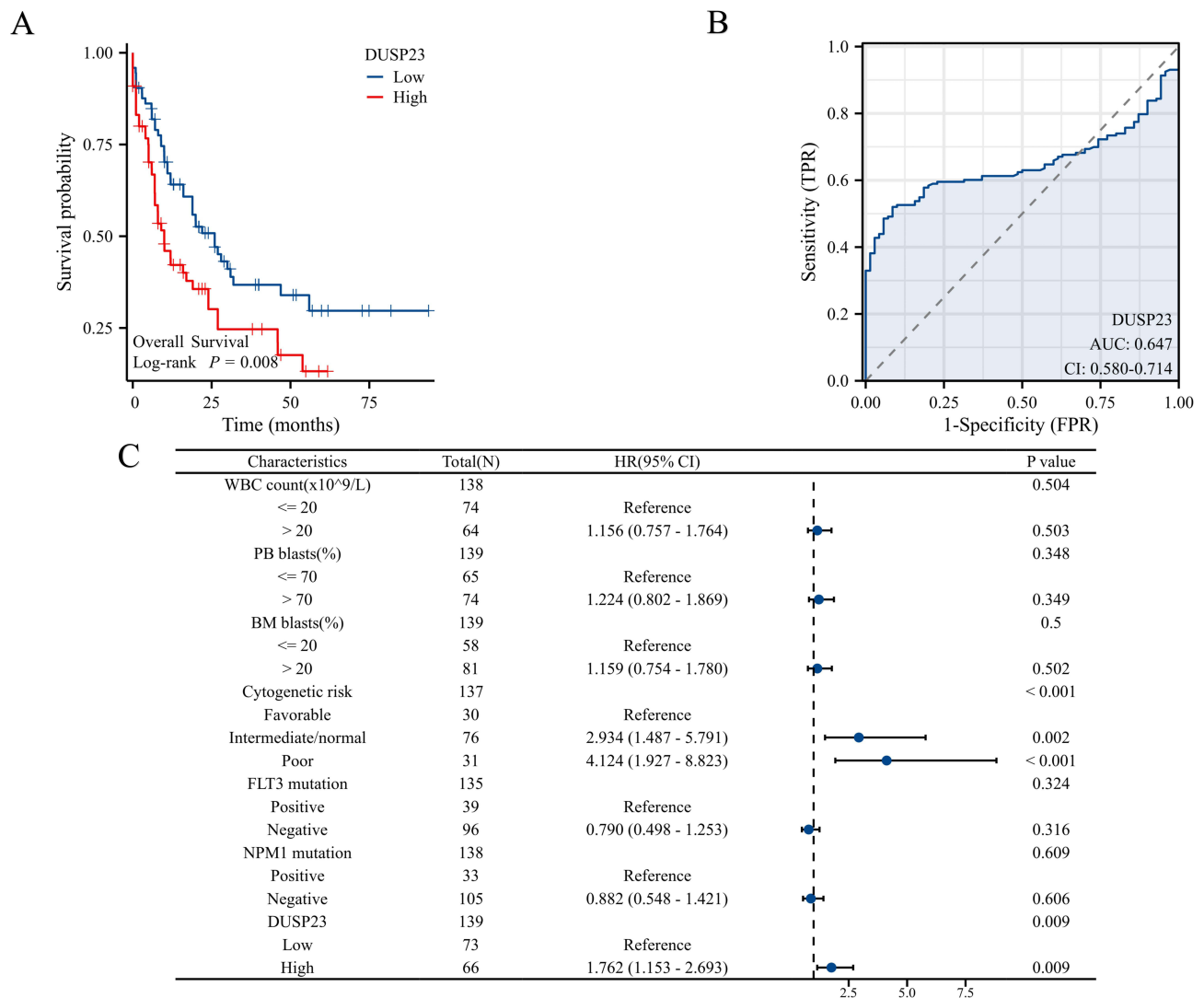


Figure 3 High expression of DUSP23 was associated with poor OS in AML patients. **(A)** Kaplan-Meier curves in all AML patients. **(B)** ROC analysis of DUSP23 in the prognosis of AML. **(C)** Forest plot showed that DUSP23 predicted poor prognosis in the subgroup of cytogenetic risk (Intermediate/normal and Poor) (HR=2.934, $P=0.002$ and HR=4.124, $P<0.001$), and DUSP23 high expression. (HR=1.762, $P=0.009$).

FLT3 mutation positivity ($P=0.05$), RAS mutation negativity ($P=0.023$), IDH1 R132 mutation negativity ($P=0.007$), R140 mutation negativity ($P=0.016$), R172 mutation negativity ($P=0.011$) (Figure 4D–H), and those with intermediate/normal and poor cytogenetic risk ($P=0.034$; Figure 4I).

Prognostic Model of DUSP23 in AML

Moreover, based on multivariable logistic regression analysis, we established a nomogram that included the prediction model. The established nomogram was well-calibrated and demonstrated good discriminative power, with a C-index of 0.727 (0.700–0.754) for OS prediction (Figure 5A). Additionally, calibration curves were used to assess the clinical net benefit of our model, and the results showed high consistency between the predicted survival probability and actual OS proportions at 1, 3, or 5 years (Figure 5B).

Validation of Prognostic Gene Expression by Clinical Samples

To verify the expression level of DUSP23 between AML patients and healthy controls, qPT-PCR was detected in our cohort. The results showed that DUSP23 was significantly higher in AML patients than in healthy controls, which was

Table 3 Univariate and Multivariate Cox's Regression Analysis of Factors Associated with OS in AML

Characteristics	HR (95% CI) Univariate Analysis	P-value Univariate Analysis	HR (95% CI) Multivariate Analysis	P-value Multivariate Analysis
WBC count ($\times 10^9/L$)		0.504		
> 20	Reference			
≤ 20	0.865 (0.567–1.321)	0.503		
PB blasts (%)		0.348		
> 70	Reference			
≤ 70	0.817 (0.535–1.247)	0.349		
BM blasts (%)		0.500		
> 20	Reference			
≤ 20	0.863 (0.562–1.326)	0.502		
Cytogenetic risk		< 0.001		
Favorable	Reference		Reference	
Poor&Intermediate/normal	3.184 (1.637–6.193)	< 0.001	2.130 (1.067–4.252)	0.032
Gender		0.912		
Male	Reference			
Female	0.976 (0.639–1.491)	0.912		
Age		< 0.001		
≤ 60	Reference		Reference	
>60	0.301 (0.195–0.464)	< 0.001	0.305 (0.193–0.482)	< 0.001
Race		0.685		
White	Reference			
Black or African American&Asian	0.833 (0.337–2.060)	0.693		
FLT3 mutation		0.324		
Positive	Reference			
Negative	0.790 (0.498–1.253)	0.316		
IDH1 R132 mutation		0.210		
Positive	Reference			
Negative	1.706 (0.691–4.215)	0.247		
IDH1 R140 mutation		0.737		
Positive	Reference			
Negative	0.886 (0.443–1.773)	0.733		
IDH1 R172 mutation		0.591		
Positive	Reference			
Negative	1.644 (0.229–11.829)	0.621		
RAS mutation		0.355		
Positive	Reference			
Negative	1.558 (0.570–4.264)	0.388		
NPM1 mutation		0.609		
Positive	Reference			
Negative	0.882 (0.548–1.421)	0.606		
DUSP23		0.009		
High	Reference		Reference	
Low	0.568 (0.371–0.868)	0.009	0.547 (0.350–0.855)	0.008

consistent with the TCGA and GEO databases. ($P = 0.0024$; [Figure 6A](#)). Patients were then divided into high and low expression groups based on the cut-off value of DUSP23 mRNA expression. As expected, individuals with high DUSP23 expression showed poor survival compared to those with low expression ($P = 0.0337$; [Figure 6B](#)), as shown by the K-M statistic.

Notably, patients with higher DUSP23 expression had markedly higher WBC counts than those with lower DUSP23 expression ($P = 0.026$; [Table 1](#)). Univariate Cox proportional hazards regression was used to assess factors influencing

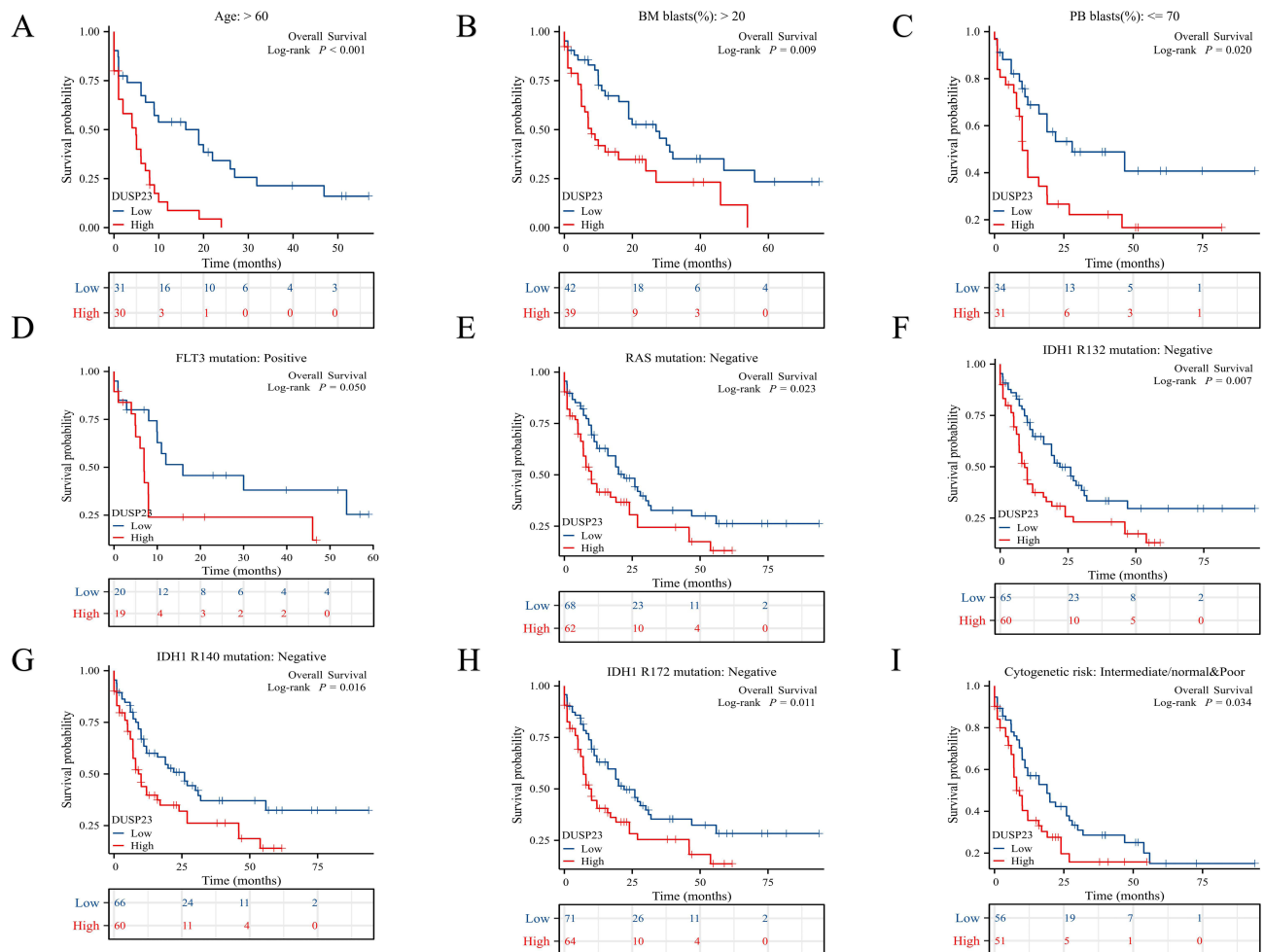


Figure 4 The impact of DUSP23 expression on survival of AML patients with specific subtypes. (A) Kaplan-Meier curves in AML patients with age ≥ 60 . (B) Kaplan-Meier curves in AML patients with BM blasts $> 20\%$. (C) Kaplan-Meier curves in AML patients with PB blasts $\leq 70\%$. (D–H) Kaplan-Meier curves in subgroups with FLT3 mutation-positive, RAS mutation-negative, IDH1 R132 mutation-negative, IDH1 R140 mutation-negative, R172 mutation-negative. (I) Kaplan-Meier curves in AML patients with cytogenetic risk intermediate/normal and poor.

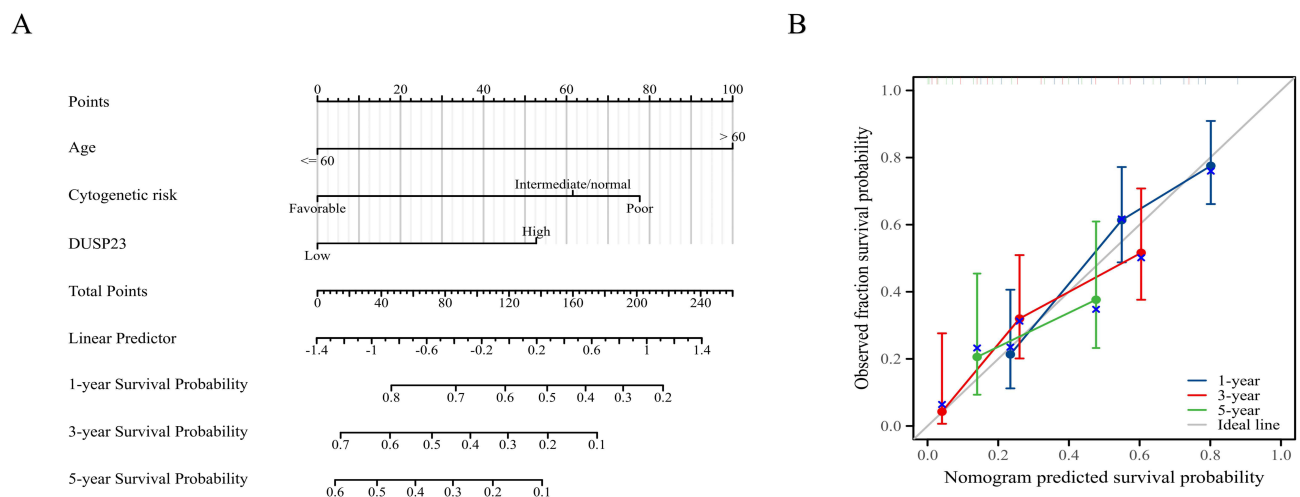


Figure 5 A prognostic predictive model of DUSP23 in AML. (A) Nomogram for predicting the probability of 1-, 3-, 5-year OS for AML. (B) Calibration plot of the nomogram for predicting the probability of OS at 1, 3, and 5 years.

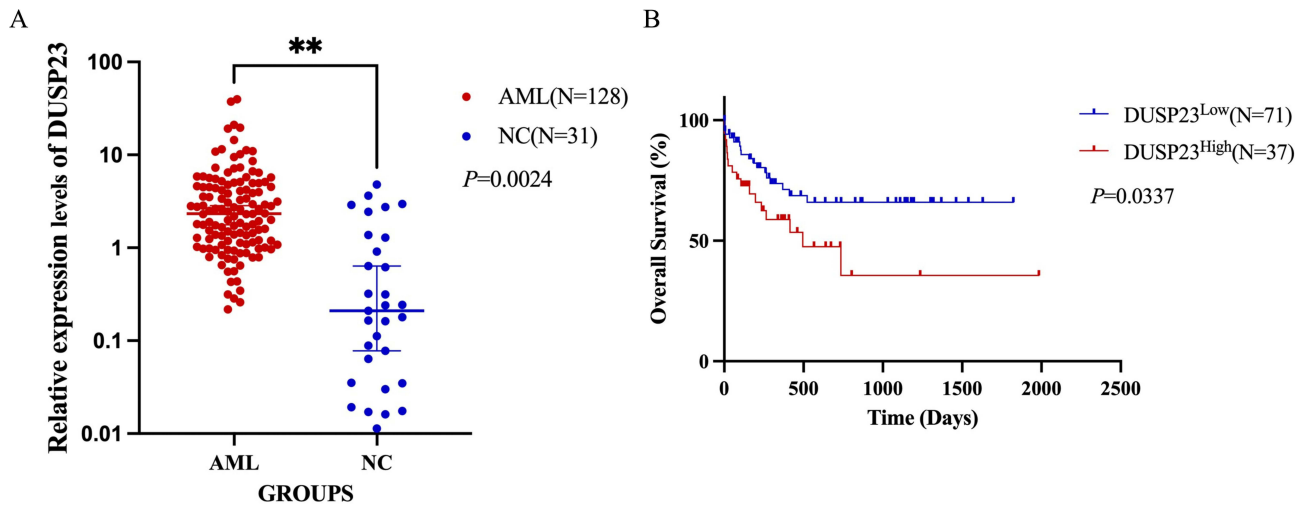


Figure 6 Quantitative real-time PCR results of DUSP23 expression. (A) qPT-PCR of DUSP23 (B) The overall survival analysis of DUSP23 related to AML patients (**P < 0.01).

OS. The results showed that high DUSP23 expression (P = 0.038), consolidation (P < 0.001) and age > 60 years (P = 0.013) were predictive factors for worse OS (Table 4). Subsequent multivariate Cox regression analysis including age, consolidation and DUSP23 expression showed that consolidation (P = 0.015) and high DUSP23 expression (P = 0.042) were independent prognostic factors for worse OS (P < 0.05). These findings highlight the potential of DUSP23 as a valuable prognostic indicator for survival in AML.

Biological Function Enrichment of the DUSP23 Gene in AML

Next, the gene expression profiles of high- and low-DUSP23 expression groups were utilized to further explore the biological function of DUSP23 in AML. There were 435 DEGs between high- and low-DUSP23 expression groups,

Table 4 Univariate and Multivariate Analysis of the Prognostic Significance of DUSP23 in AML

Characteristics	HR (95% CI) Univariate Analysis	P-value Univariate Analysis	HR (95% CI) Multivariate Analysis	P-value Multivariate Analysis
WBC count(x10^9/L)				
> 20	Reference			
<= 20	0.625 (0.311–1.255)	0.186		
Age				
<= 60	Reference		Reference	
>60	2.463 (1.208–5.019)	0.013	1.885 (0.860–4.129)	0.113
Consolidation				
HSCT	Reference		Reference	
Chemo	0.197 (0.076–0.514)	<0.001	0.284 (0.101–0.797)	0.015
DNMT3A mutation				
Positive	Reference			
Negative	0.149 (0.020–1.086)	0.06		
KIT mutation				
Positive	Reference			
Negative	0.169 (0.023–1.239)	0.08		
DUSP23				
High	Reference		Reference	
Low	2.027 (1.041–3.943)	0.038	2.060 (1.025–4.136)	0.042

including 141 up-regulated and 294 down-regulated genes ($|\log \text{fold change (logFC)}| > 1.5$, $P < 0.05$) (Figure 1A). The top ten up- and down-regulated DEGs between the groups were presented in the heat map (Figure 1B).

To gain insights into the functional implications of the 435 DEGs concerning DUSP23 expression levels in AML, GO and KEGG functional enrichment analyses were performed. The target genes showed statistical significance in the GO terms related to biological processes such as embryonic organ development, regionalization, and morphogenesis, molecular functions including DNA-binding transcription activator activity, DNA-binding transcription activator activity/RNA polymerase II-specific, and carbohydrate binding, and cellular components such as collagen-containing extracellular matrix, endoplasmic reticulum lumen, and collagen trimer. The DEGs were found to be enriched in the KEGG pathways such as *Staphylococcus aureus* infection, complement, and coagulation cascades (Supplementary Figure 2).

Finally, GSEA enrichment analysis results show that DUSP23 may be related to multiple signaling pathways in hematological tumors, including BCR signaling, MAPK pathway, RAS regulation, PTEN regulation, HEDGEHOG signaling, WNT signaling, MYC pathway, and TP53 regulation (Figure 7A–H and Supplementary Table 1).

Identification of Hub Genes Associated with DUSP23 Expression

To perform a more in-depth analysis of the data, a PPI network related to DEGs was constructed and analyzed. The top 10 hub genes were identified using the maximal neighborhood component (MNC) and degree methods with the cytoHubba plug-in in Cytoscape (Figure 8A and B). It was discovered that nine hub genes were shared between the two gene lists, namely ATP5F1D, NDUFB7, POLR2L, MRPS12, MRPL2, UBA52, IMP3, UQCRCQ, and MRPL4. Significant associations were found between DUSP23 and ATP5F1D ($P < 0.001$, correlation coefficient: 0.702), NDUFB7 ($P < 0.001$, correlation coefficient: 0.731), POLR2L ($P < 0.001$, correlation coefficient: 0.702), MRPS12 ($P < 0.001$, correlation coefficient: 0.780), MRPL2 ($P < 0.001$, correlation coefficient: 0.498), UBA52 ($P < 0.001$, correlation coefficient: 0.637), IMP3 ($P < 0.001$, correlation coefficient: 0.512), UQCRCQ ($P < 0.001$, correlation coefficient: 0.591), and MRPL4 ($P < 0.001$, correlation coefficient: 0.708) (Figure 8C). Finally, the impact of hub gene levels on patient prognosis was examined, and it was discovered that IMP3, MRPL4, MRPS12, POLR2L, and ATP5F1D are closely linked to unfavorable clinical outcomes in individuals with AML (Figure 8D–H).

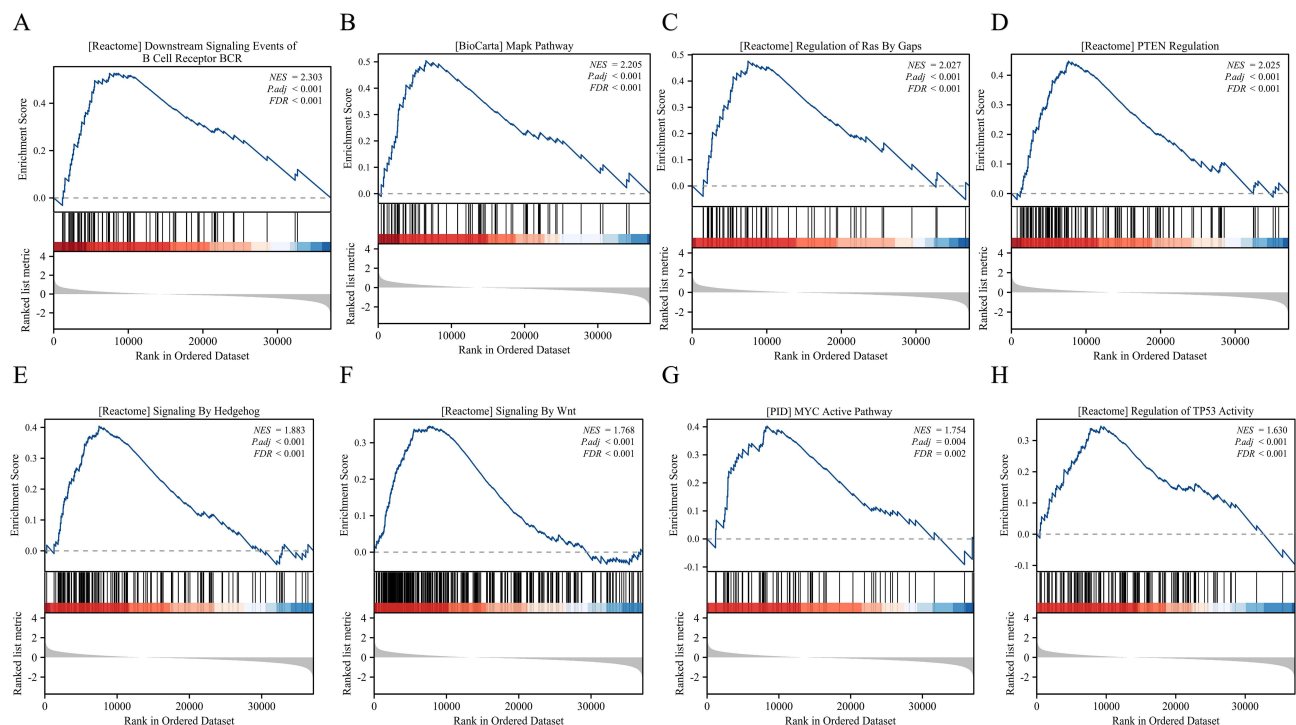


Figure 7 Enrichment plots from the gene set enrichment analysis (GSEA). (A–H) ES, enrichment score; NES, normalized ES; ADJ P- val, adjusted P-value.

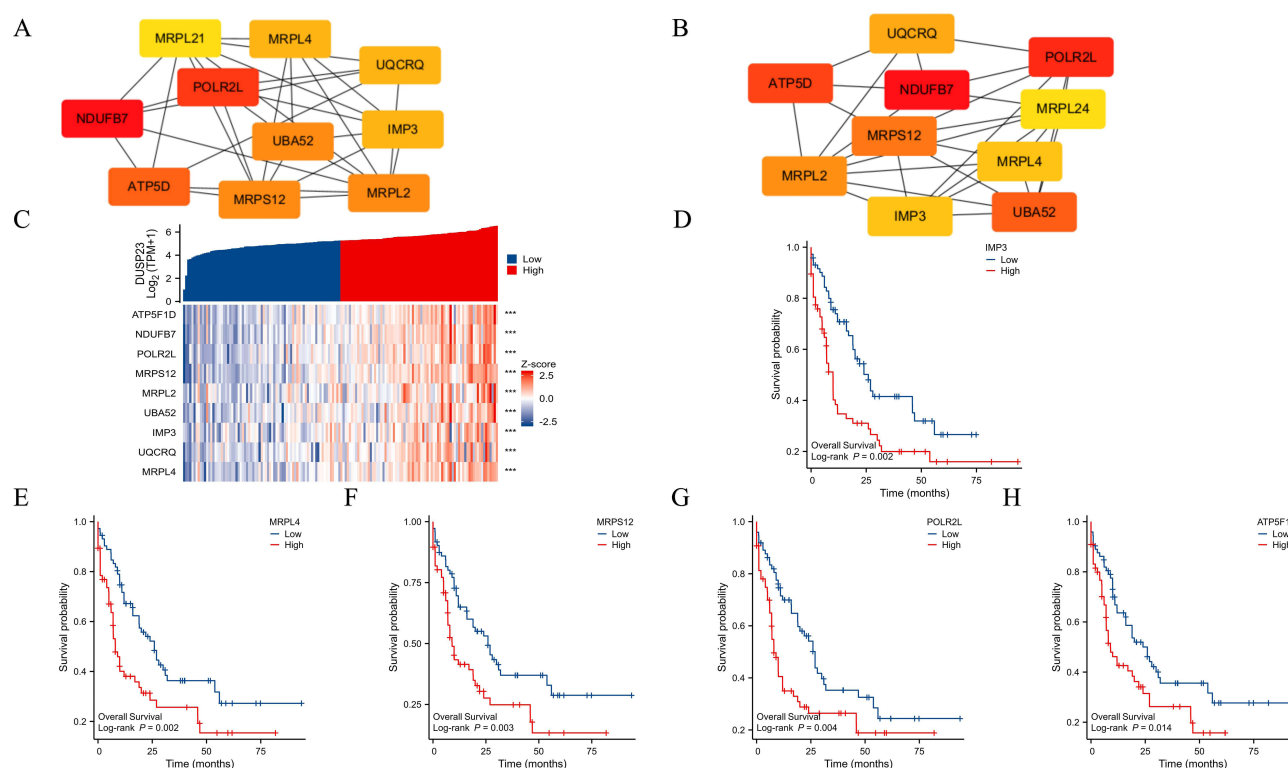


Figure 8 PPI network construction and clinical significance of hub genes. (A and B) The top 10 hub genes were selected based on (A) MNC and (B) degree. (C) The association of DUSP23 with nine hub genes (ATP5F1D, NDUFB7, POLR2L, MRPS12, MRPL2, UBA52, IMP3, UQCRCQ, and MRPL4) (*** $P < 0.001$). (D–H) The difference in OS between patients with high and low IMP3, MRPL4, MRPS12, POLR2L, and ATP5F1D expression levels is shown by Kaplan-Meier curves.

Immune Infiltration Analysis in AML

In the AML microenvironment, a positive correlation was found between the expression level of DUSP23 and the number of various immune cells, including NK CD56 bright cells, NK cells, TFH, TReg, Eosinophils, NK CD56dim cells, aDC, CD8 T cells, Mast cells, Cytotoxic cells, Neutrophils, iDC, Macrophages, Th17 cells, Th1 cells, and DC, as revealed by the correlation analysis of immune cell infiltration quantified by SSGSEA. Particularly, a significant association with DUSP23 was exhibited by NK CD56 (bright) cells, indicating that DUSP23 may serve as a significant immune marker in AML (Figure 9).

Discussion

The present study showed that the mRNA expression level of DUSP23 was significantly higher in AML patients than healthy controls, and DUSP23 high expression was an independent adverse prognostic factor for OS. In addition, The MAPK signaling pathways may be stimulated by increased DUSP23 expression levels, which may be important for the development of AML.

By exploring the association between DUSP23 gene expression levels and the main clinical features in TCGA-LAML cohorts, it was revealed that DUSP23 was significantly upregulated in AML patients. Notably, high levels of DUSP23 were associated with higher PB blast levels, intermediate/normal & poor cytogenetic risk, FLT3 mutation, and poor prognosis. FLT3 mutations are detected in approximately 30% of AML patients and are associated with unfavorable outcomes.²⁵ Therefore, we speculate that abnormally high expression of DUSP23 may have a negative impact on the survival of AML patients.

qPT-PCR was further performed to validate the differential expression of DUSP23 in AML and healthy controls. The results revealed a significant upregulation of DUSP23 in AML patients, and poor survival was observed in those with high DUSP23 expression. Using multivariate Cox regression analysis, we identified high DUSP23 expression as an independent prognostic factor along with consolidation. Therefore, DUSP23 can be considered as a novel adverse prognostic factor in AML patients.

Furthermore, GSEA showed enrichment of MAPK, RAS, PTEN, WNT, MYC, and TP53 pathways. The MAPKs are a family of serine-threonine kinases that play a crucial role in regulating the proliferation and differentiation of both normal and malignant

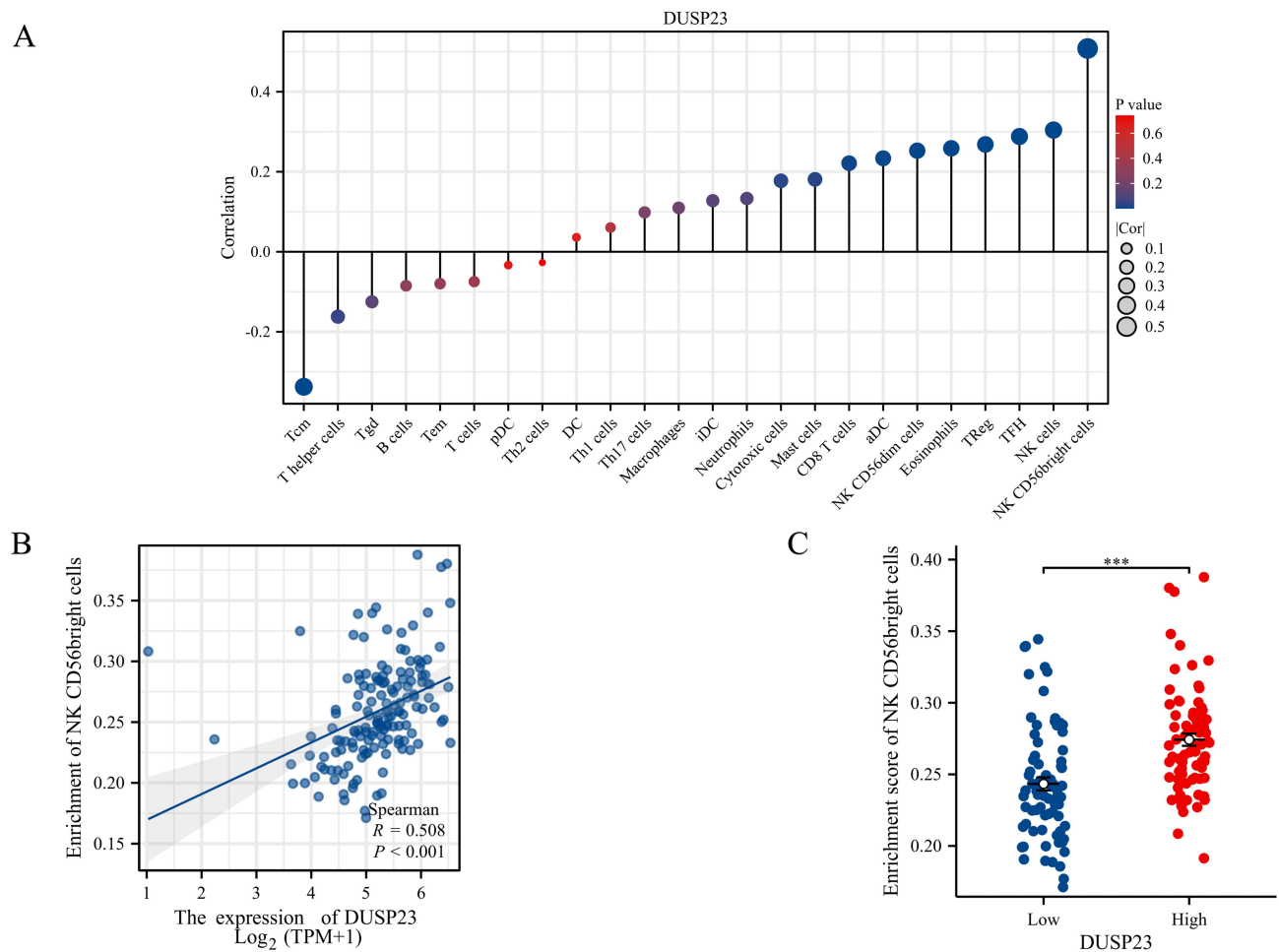


Figure 9 Correlation analysis between the level of DUSP23 expression and immune cell infiltration. **(A)** The relative contents of 24 kinds of immune cells in AML. **(B)** Correlation between the relative enrichment score of NK CD56(bright) cells and the expression level (TPM) of DUSP23. **(C)** Infiltration of NK CD56(bright) cells between low- and high-DUSP23 expressed (** $P < 0.001$).

hematopoietic cells.^{26,27} MAPK contributes to the development of various cancers, such as colorectal, ovarian, and breast cancers, as well as AML.^{28–30} The molecular mechanisms responsible for maintaining a balance between activated RAS-GTP and inactive RAS-GDP are complex and involve multiple factors, including protein kinases, scaffolding proteins, phosphatases, GAPs, and GEFs.³¹ Mutations in genes encoding these components of the intricate network have been identified in inherited genetic syndromes called RASopathies, as well as in sporadic cancers, including AML. Notably, these mutations occur with a high frequency in juvenile myelomonocytic leukemia (JMML).³² PTEN is an important tumor suppressor gene frequently inactivated in human cancer. Its inactivation has been shown to have crucial roles in the generation of leukemia stem cells.³³ In AML, mutations in hematopoietic stem/progenitor cells result in upregulated Wnt signaling through various mechanisms. Wnt signaling is crucial for maintaining leukemic stem cells.³⁴ Although the impact of MYC protein expression in AML is not well understood, dysregulation of MYC has been implicated in AML. Most AML cases exhibit high levels of MYC protein, which is correlated with a worse prognosis.³⁵ TP53, a frequently mutated tumor suppressor gene in human cancer, remains intact in most AML cases, suggesting potential for harnessing its physiological tumor-suppressive roles. Therefore, pharmacological activators of the TP53 pathway may offer clinical benefits in AML. Conversely, despite the lower frequency of TP53 mutations in AML compared to other cancers, TP53 mutations are associated with chemoresistance and a high risk of relapse.³⁶ Thus, we speculated that the pathological mechanism of DUSP23 may be related to these signaling pathways.

In addition, through a series of rigorous screens, five hub genes that could accurately predict the prognosis of AML were found. IMP-3 binds to and promotes the translation of insulin-like growth factor II (IGF-II) mRNA among its many mRNA targets.³⁷ Reports suggest that reducing IMP-3 levels through siRNA-mediated knockdown decreases the proliferation of human

K562 chronic myeloid leukemia cells by reducing IGF-II biosynthesis.³⁸ Similarly, inhibiting IMP-3 in AML may potentially reduce cell proliferation. MRPL4 has been identified as a high-risk factor for prostate cancer (PC) and a potential diagnostic biomarker.³⁹ Additionally, MRPS12 has been implicated in ovarian cancer.⁴⁰ Several reports indicate that POLR2L plays a significant role in PC, glioblastoma (GBM), and HBV-related hepatocellular carcinoma (HCC).^{41–43} Targeted specific demethylation of ATP5D m1A using the dm1ACRISPR system significantly enhances the expression of ATP5D and glycolysis in cancer cells. In vivo, data confirm the involvement of m1A/ATP5D in tumor growth and cancer progression.⁴⁴ However, further experiments are required to verify the mechanism of action among these genes.

In the analysis of immune cell infiltration, higher levels of CD56 (bright) NK cells were found to be associated with high expression of DUSP23. CD56 (bright) NK cells, which are considered to be efficient cytokine producers with immunoregulatory properties, can also become cytotoxic upon appropriate activation and have been shown to play a role in various disease states, including cancer, autoimmunity, neuroinflammation, and infection.^{45,46} In this study, a positive correlation was observed between the infiltration of CD56 (bright) NK cells and the expression of DUSP23. Kaplan-Meier survival analysis indicated that poor prognosis in AML patients was associated with high expression of DUSP23. However, the relationship between CD56 (bright) NK cells and AML has not been fully clarified. Therefore, further exploration is required to elucidate the relationship between DUSP23 and CD56 (bright) cells and the potential involvement of DUSP23 and CD56 (bright) NK cells in immune escape in AML.

Our study revealed DUSP23 expression is higher in AML patients than healthy controls and high DUSP23 expression indicate a poor prognosis. Furthermore, we found that the potential biological function of DUSP23 in AML may be mediated by activation of MAPK signalling pathways. However, this was a retrospective follow-up study. To define the role of DUSP23 in AML more precisely, further research with a larger sample size of AML is needed. In addition, the mechanism of DUSP23 in AML needs to be confirmed in the future.

Conclusion

Our findings have revealed that an unfavorable impact on the OS of AML patients was observed in cases where the gene DUSP23 was highly expressed. In addition, high levels of DUSP23 were identified as an independent predictor of poor prognosis in AML patients.

Abbreviations

DUSP23, Dual-specificity Phosphatase 23; AML, acute myeloid leukemia; TCGA, the Cancer Genome Atlas; GTEx, Genotype-Tissue Expression; DEGs, differentially expressed genes; OS, overall survival; GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; GSEA, gene set enrichment analysis; PPI, protein-protein interaction; STRING, Search Tool for the Retrieval of Interacting Genes; ATRA, all-trans retinoic acid; ATO, arsenic trioxide; C-index, concordance index; AUC, area under the curve; WBC, white blood cell; MNC, the maximal neighborhood component; qRT-PCR, Quantitative real-time PCR; IGF-II, insulin-like growth factor II; JMML, juvenile myelomonocytic leukemia; PC, prostate cancer; GBM, Glioblastoma; HCC, HBV-related hepatocellular carcinoma.

Data Sharing Statement

The databases generated and/or analysed during the current study are available in the GEO, TCGA, and GETx repository, <https://portal.gdc.cancer.gov/>; <https://xenabrowser.net/datapages/>; <https://www.ncbi.nlm.nih.gov/geo/>.

Ethics Approval and Informed Consent

This study was conducted in accordance with the Declaration of Helsinki. This study was conducted under the approval of the Ethics Committee of the Affiliated People's Hospital of Ningbo University. All methods were carried out in accordance with relevant guidelines and regulations. (Approval NO.: the Affiliated People's Hospital of Ningbo University Ethics review 2023-research-107).

Author Contributions

Each author has carefully reviewed and endorsed the final manuscript. They have collectively contributed significantly to various aspects of the research, including conception, study design, execution, data acquisition, analysis, and interpretation. All authors actively participated in drafting, revising, and critically reviewing the article. They have provided final approval for the version submitted for publication, unanimously agreed on the selected journal, and committed to being accountable for all facets of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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