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ORIGINAL ARTICLE

Expression of CDK9 in Newly Diagnosed Patients with Acute Myeloid Leukemia and its Clinical Significance

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SUMMARY

Background: **This study investigated the role of cyclin-dependent kinase 9 (CDK9) expression levels and prognosis in acute myeloid leukemia (AML) by examining its expression at the time of initial diagnosis.**
Methods: **Bone marrow samples from 60 AML patients were collected for the observation group, with 20 normal human bone marrow samples serving as controls. Clinical and pathological data were gathered from the AML patients. Real-time quantitative PCR (RT-qPCR) was employed to measure CDK9 expression levels in both groups, and the association between CDK9 expression, clinical characteristics, and prognosis in AML patients was analyzed. Kaplan-Meier curves were used to assess the impact of CDK9 on overall survival (OS) in AML, while Cox regression analysis was performed to identify prognostic factors in AML patients.**
Results: **The expression of CDK9 was significantly elevated in AML patients, compared to the control group ($p < 0.05$). High CDK9 expression was associated with increased white blood cell (WBC) count, poor treatment response, and worse prognosis compared to low expression ($p < 0.05$). Additionally, patients with high CDK9 expression exhibited significantly shortened OS compared to those with low expression ($p < 0.05$). High CDK9 expression emerged as an independent risk factor influencing prognosis in AML.**
Conclusions: **CDK9 is markedly upregulated in AML patients, suggesting its potential utility as both a prognostic indicator and a therapeutic target, particularly for patients with unfavorable clinical and pathological characteristics and poor prognosis.**
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INTRODUCTION

Acute myeloid leukemia (AML) presents a diverse array of genetic and molecular characteristics, rendering it challenging to treat. Up to 40% of newly diagnosed AML patients fail to achieve complete remission (CR) following initial induction chemotherapy [2]. Moreover, approximately 50% of the patients who initially achieve CR experience relapse, often leading to poor prognosis [3], with long-term survival remaining elusive even with hematopoietic stem cell transplantation. Thus, identifying novel therapeutic targets is crucial to en-

hancing treatment efficacy and prolonging survival. Protein kinases are frequently overexpressed in tumor cells and play pivotal roles in various biological processes underlying tumor initiation and progression, making them promising molecular targets for therapy. Cyclin-dependent kinase 9 (CDK9) belongs to the cyclin-dependent kinase family, participating in the regulation of gene transcriptional activity [6]. Research has demonstrated the significance of CDK9 in RNA transcription and elongation, making it a compelling target for cancer treatment [7]. CDK9 has been implicated in the pathogenesis of several malignancies, including leukemia, liver carcinoma, colorectal cancer, endometrial cancer, and breast cancer [8-12]. However, the precise role of CDK9 in the development of acute myeloid leukemia remains unclear. Therefore, this study aimed to investigate the expression of CDK9 in 60 patients with newly diagnosed AML compared to 20 healthy volunteers, analyzing its association with clinical characteristics and prognosis. The findings seek to elucidate whether CDK9 could serve as a potential monitoring and treatment target in AML management.

MATERIALS AND METHODS

General information

Bone marrow specimens were obtained from 60 patients diagnosed with acute myeloid leukemia (AML) and 20 healthy volunteers at the First Affiliated Hospital of Bengbu Medical University between 2019 and 2023. Additional bone marrow samples were retained and stored at -80°C for future use. Detailed clinical data were collected for each AML patient at the time of initial diagnosis, including gender, age, white blood cell count, hemoglobin level, platelet count, bone marrow blast percentage, French-American-British (FAB) classification, cytogenetics, gene mutations, chemotherapy regimens, treatment response assessment, and prognosis evaluation. Informed consent was obtained from all participants, and the study was approved by the Ethical Review Committee of the First Affiliated People's Hospital of Bengbu Medical University.

Reagent and instrument

Trizol reagent was procured from Shanghai Biological Engineering Company, while the reverse transcription kit and Novostart SyBR QPCR Supermix Plus kit were obtained from Shanghai Nearshore Protein Company and Er Technology Company, respectively.

RNA extraction

Bone marrow specimens (2 mL) were collected in EDTA-K2 tubes and treated with red blood cell lysis buffer. Following centrifugation at 3,000 rpm for 2 - 3 times, single nucleocytes were extracted and stored at -80°C. Thawed cells were centrifuged and total RNA was extracted, using 200 μL of Trizol reagent, 40 μL of chloroform, and 100 μL of isopropyl alcohol. The purity and concentration of the extracted RNA were measured by using an ultramicrospectrophotometer SMA1000, ensuring an OD260/280 ratio between 1.8 and 2.0 and a total RNA concentration of 30 - 70 ng/μL.

Real-time quantitative reverse transcription PCR (qRT-PCR) detection method of CDK9 expression level

CDNA synthesis was performed by using Novoscript® Plus All-in-One 1st Strand cDNA Synthesis Supermix (Novoprotein, Shanghai, China), followed by PCR amplification using Novostart® SYBR QPCR Supermix Plus (Shanghai, China) to quantify CDK9 expression levels and eliminate specific DNA fragments. The reaction mixture comprised 10 μL of 2 × Novostart SYBR QPCR Supermix Plus, 1 μL of forward primer, 1 μL of reverse primer, 1.5 μL of template cDNA, 0.4 μL of ROXII, and 6.1 μL of RNase-free water. The extension conditions included an initial denaturation at 95°C for 1 minute, followed by 40 cycles of denaturation at 95°C for 20 seconds and annealing/extension at 60°C for 1 minute. Each sample was run in triplicate, and the average value was calculated. GAPDH was used as the internal reference gene for both the observation and control groups. The relative expression level of the CDK9 gene was calculated by using the $2^{-\Delta\Delta CT}$ method. The genetic primer sequences are provided in Table 1.

Table 1. Primer sequence of the gene of interest.

CDK9	**Forward: GATGGCTCTGTGGATGTGGT**
CDK9	**Reverse: GCTCTCTCTGGTCAGGTTGC**
GAPDH	**Forward: CCCATCACCATCTTCCAGG**
GAPDH	**Reverse: CATCACGCCACAGTTTCCC**

Genetic mutation detection method

Bone marrow specimens from patients were stored at low temperatures and sent to Jinyu Medical Inspection Labs for second-generation sequencing of AML. This comprehensive analysis includes detection of monocytic variations and mutations in 30 genes associated with AML using general exogenous sub-sequences. The sequencing was performed with an average depth of 2,000 × by using the Illumina Hiseq X TEN sequencer (ILLUMINA). The procedure involved DNA extraction followed by library preparation of target genes using the Rapid DNA Lib Prep Kit for Illumina (Wuhan Aibaitik). Targeted NGS sequencing was conducted on the Illumina Hiseq X TEN sequencer, and the results were analyzed accordingly.

Chromosome examination method

Bone marrow specimens from patients were sent to the Hefei Golden Domain Medical Testing Laboratory for

Table 2. The relationship between CDK9 expression and clinical characteristics of AML patients.

Clinical characteristics	CDK9 expression		F/χ^2	p
	low (n = 30)	high (n = 30)		
Age (years)			1.071	0.301
< 60	18	14		
≥ 60	12	16		
Gender			0.071	0.791
Male	18	19		
Female	12	11		
BM blast cells (%)			1.017	0.313
< 20	1	0		
≥ 20	29	30		
WBC count ($\times 10^9$/L)			4.286	**0.038**
< 10	18	10		
≥ 10	12	20		
HB count (g/L)			1.763	0.184
< 70	14	9		
≥ 70	16	21		
PLT count ($\times 10^9$/L)			0.067	0.795
< 30	14	13		
≥ 30	16	17		
FAB classification			8.687	0.070
M0	1	0		
M1	4	2		
M2	17	9		
M4	3	6		
M5	5	13		

Table 3. The relationship between CDK9 expression and chemotherapy regimen and efficacy of AML patients.

Chemotherapy regimen and efficacy	CDK9 expression		F/χ^2	R
	low (n = 30)	high (n = 30)		
Curative effect of chemotherapy			11.507	**0.003**
CR	9	11		
PR	13	2		
NR	8	17		
Chemotherapy regimen			0.601	0.438
IA	16	13		
HMA ± Ara-C	14	17		

chromosome examination using the cell culture G-banding method. The procedure involved culturing bone marrow cells for 24 hours, followed by harvesting of conventional cell production. Prepared chromosomal specimens were baked and then digested in pancreatin solution. Subsequently, they were stained with GIEMSA dye solution and rinsed with tap water before air-drying the slides. Chromosomes were examined under a

Table 4. The relationship between CDK9 expression and karyotype, gene mutation, and risk grouping of AML patients.

Karyotype/Gene mutation/Risk grouping	CDK9 expression		F/χ^2	p
	low (n = 30)	high (n = 30)		
Karyotype			**0.069**	**0.793**
Normal	**17**	**18**		
Abnormal	**13**	**12**		
Gene mutation			**4.326**	**0.742**
CEBPA	**4**	**6**		
NPM1	**7**	**6**		
FLT3-ITD	**5**	**8**		
DNMT3A	**7**	**7**		
IDH1/2	**8**	**4**		
TET2	**3**	**7**		
ASXL1	**2**	**1**		
TP53	**1**	**1**		
Risk grouping			**4.286**	**0.038**
Favorable/Intermediate	**18**	**10**		
Poor	**12**	**20**		

Table 5. Independent prognosis factors of patients with AML by COX multivariate analysis.

Factors	HR (95% CI)	p
CDK9 level (low/high)	**2.438 (1.300 - 4.571)**	**0.005**
Age (years) (≥ 60/< 60)	**2.410 (1.321 - 4.400)**	**0.004**
WBC count (≥ 10/< 10 × 10^9/L)	**0.554 (0.311 - 0.986)**	**0.045**
PLT count (≥ 30/< 30 × 10^9/L)	**2.483 (1.324 - 4.655)**	**0.005**
Risk grouping		
Poor/Intermediate	**7.982 (2.593 - 24.589)**	**< 0.001**
Poor/Favorable	**3.395 (1.192 - 9.665)**	**0.022**

high-power microscope to assess their structure and identify abnormalities. The quality of the specimens was deemed satisfactory based on clear banding patterns observed on the chromosomes.

Clinical efficacy evaluation and follow-up

The diagnosis, classification, treatment plan, and efficacy evaluation of AML patients were conducted by following the 2023 Chinese AML Treatment Guidelines [13]. All 60 AML patients were followed up from the date of diagnosis until March 31, 2024, to determine their overall survival time (OS).

Statistical analysis

Data processing and statistical analysis were performed by using SPSS, version 22.0, software. The single-sample *t*-test was utilized to assess differences in CDK9 expression between AML patients and healthy controls. AML patients were divided into CDK9 high expression and CDK9 low expression groups based on the median relative expression of CDK9, and the chi-squared test or Fisher's exact test was employed to compare the clinical features between these groups. The Kaplan-Meier method was used to analyze the effect of CDK9 on overall survival time (OS), and Cox proportional hazards regression model was applied to identify prognostic factors influencing AML. A significance level of $p < 0.05$ was considered statistically significant.

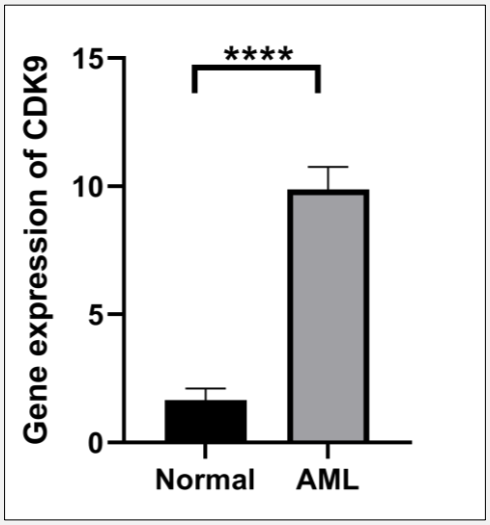

Figure 1. Expression of CDK9 in patients with AML and healthy person by qRT-PCR.

****** p < 0.001, compared with normal group.**

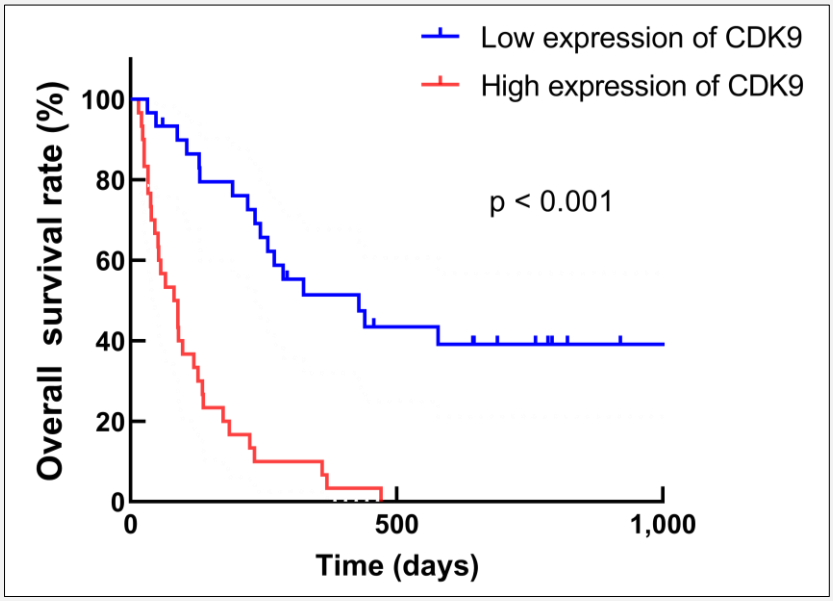

Figure 2. Overall survival rate in AML patients with low and high CDK9 expression.

RESULTS

Expression of CDK9 in patients with AML

The expression levels of CDK9 in AML patients and healthy volunteers were determined by using qRT-PCR. The analysis revealed a significantly higher expression of CDK9 in AML patients compared to the control group, with a statistically significant difference ($t = 5.268$, $p < 0.001$) (see Figure 1).

Relationship between CDK9 expression and clinicopathological features of AML patients

The correlation between CDK9 expression and various clinicopathological features of AML, including gender, age, white blood cell count at initial diagnosis, hemoglobin count, platelet count, bone marrow blast percentage, and FAB classification, was investigated. The results indicated a significant association between high WBC count and elevated CDK9 expression ($p = 0.038$). However, no significant differences were observed in age, gender, bone marrow blast percentage, hemoglobin level, platelet count, or FAB classification between the high and low CDK9 expression groups ($p > 0.05$) (refer to Table 2).

AML patients younger than 60 years were treated with the idarubicin + cytarabine (IA) regimen, while those over 50 and less than 60 years with poor physical status (ECOG >2 points) were administered the demethylation drug (HMA) + Ara-C combination regimen. Patients aged 60 or older received a combination regimen of demethylated drugs (HMA) with or without Ara-C, including decitabine (D) and azacitidine (A). Ara-C-containing regimens included D + HAG (harringtonine + Ara-C + granulocyte stimulating factor), A + HAG, D + Ara-C, and A + Ara-C. Among them, 29 cases received the IA regimen, and 31 cases received the HMA ± Ara-C combination regimen. According to the clinical efficacy evaluation criteria, 20 cases achieved complete response (CR), 15 cases achieved partial response (PR), and 25 cases showed no response (NR). Statistical analysis revealed a significantly higher number of NR cases in the high CDK9 expression group compared to the low expression group, demonstrating statistical significance ($p < 0.05$). However, there was no statistical significance observed regarding the choice of chemotherapy regimen ($p > 0.05$) (see Table 3).

Among the AML patients, 35 individuals exhibited a normal karyotype, while 25 patients presented with abnormal karyotypes, including anomalies in both number and structure. Additionally, 48 patients showed mutations in genes such as CEBPA, NPM1, FLT3-ITD, NARS, KAR, DNMT3A, IDH1/2, ASXL1, SRSF2, TP53, SMC3, RAS, and others. Among these, 34 patients had concurrent mutations in three or more genes, while 8 patients had simultaneous mutations in two genes. Moreover, 6 patients had single gene mutations, while 12 patients exhibited no gene mutations. Based on the guidelines for the diagnosis and treatment of acute myeloid leukemia, the patients were categorized into a good prognosis group (n = 28), a moderate prognosis group (n = 28), and a poor prognosis group (n = 32). Analysis revealed a notable increase in the number of patients with poor prognosis in the high CDK9 expression group, with a statistically significant difference observed ($p < 0.05$). Conversely, there was no statistically significant difference observed in chromosome karyotype and gene mutation distribution between the high and low CDK9 expression groups ($p > 0.05$) (refer to Table 4).

Relationship between CDK9 expression level and prognosis of patients with AML

To elucidate the association between CDK9 expression level and prognosis in AML patients, Kaplan-Meier survival curve analysis was conducted on a cohort of 60 AML patients. The analysis revealed that patients with high CDK9 expression exhibited significantly shorter overall survival (OS) compared to those with low expression ($p < 0.05$) (see Figure 2). These findings suggest that high CDK9 expression may serve as a potential indicator of poor prognosis in AML patients.

Multivariate analysis of influencing prognosis factors of patients with AML

Clinical data of AML patients, including gender, age, FAB classification, bone marrow blast percentage, WBC count, HB level, PLT count, chemotherapy regimen, chromosome karyotype, prognostic risk stratification, and CDK9 expression, were included in a multivariate Cox regression analysis. The results indicated that age, WBC count, PLT count, prognostic grouping, and CDK9 expression were independent risk factors affecting the prognosis of AML patients (refer to Table 5).

DISCUSSION

Acute myeloid leukemia (AML) represents a malignant clonal disorder originating from myeloid stem/progenitor cells, characterized by the uncontrolled proliferation of leukemia cells within the bone marrow, thereby impeding the differentiation and maturation of normal hematopoietic cells. AML stands as the predominant form of leukemia in adults, constituting approximately 80% of all leukemia cases [14]. Cyclin-dependent kinases (CDKs) are serine/threonine (Ser/Thr) protein kinases that function through heterodimer complexes composed of catalytic subunits (kinases) and regulatory subunits (cyclins) [15,16]. Recent studies have underscored the role of CDKs in driving and sustaining tumor cell growth, particularly in tumors propelled by aberrant transcription factor regulation [17]. Notably, CDK9 has been implicated in various tumors, correlating with tumor metastasis and poor prognosis. In lung cancer, CDK9 fosters cell proliferation and metastasis, while its inhibitors curb cell proliferation, colony formation, and cell cycle progression, ultimately inducing apoptosis

[18]. Likewise, in ovarian cancer and osteosarcoma, small interfering RNA or CDK9 inhibitors effectively restrain proliferation and metastasis, eliciting apoptosis [4,19]. In cervical cancer, CDK9 expression levels significantly correlate with disease stage, pathological grade, depth of infiltration, tumor size, and lymph node metastasis. Moreover, specific siRNA-mediated CDK9 elimination suppresses cervical cancer cell proliferation *in vitro* and tumorigenesis in vivo [20]. Hence, CDK9 expression tightly correlates with tumor cell proliferation and metastasis. However, there remains a paucity of research elucidating the development and mechanisms of CDK9 in AML.

The results of this study demonstrated a significant upregulation of CDK9 expression in AML patients ($p < 0.001$). Among the clinicopathological features analyzed, patients with high WBC counts in the high CDK9 expression group outnumbered those in the low expression group ($p < 0.05$). However, no significant differences were observed in gender, age, bone marrow blast percentage, hemoglobin level, platelet count, or FAB classification, potentially attributable to the small sample size. Notably, a higher proportion of patients with high CDK9 expression failed to respond to treatment compared to those with low expression, indicating a significant association ($p = 0.003$). This resistance to traditional chemotherapy suggests a potential need for alternative targeted therapies or allogeneic hematopoietic stem cell transplantation. Moreover, patients with high CDK9 expression exhibited a strong correlation with poor prognostic stratification ($p = 0.038$), with a majority falling into the poor prognosis group. Furthermore, the overall survival (OS) of patients with high CDK9 expression was significantly shortened, suggesting an increased propensity for chemotherapy resistance, poor treatment efficacy, and shorter survival times. Multivariate Cox regression analysis identified old age, high WBC count at onset, low platelet count, prognostic grouping, and CDK9 expression level as independent risk factors affecting the prognosis of AML patients. These findings suggest that CDK9 expression levels can serve as a prognostic indicator for AML. However, the study has certain limitations. Firstly, the incidence of specific AML subtypes, such as M6 and M7, was extremely low and not included in the study. Future research should collect more cases to enable comparison and include a broader range of AML subtypes to provide more comprehensive insights into CDK9 expression across different subtypes. Secondly, while this study identified a potential role for CDK9 in the pathogenesis of AML based on bone marrow samples from clinical AML patients, further validation and mechanistic investigation through in vivo and *in vitro* experiments are warranted to elucidate its specific mechanisms.

CONCLUSION

In conclusion, our study highlights the significant upregulation of CDK9 expression in AML patients, with elevated levels correlating with shorter survival times. The high expression of CDK9 emerges as a notable risk factor for poor prognosis in AML patients, serving as a valuable clinical indicator for prognostic assessment. Moreover, these findings underscore the potential for exploring targeted therapies aimed at CDK9 in the management of AML.

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Declaration of Interest:
The authors have no conflicts of interest to declare.

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