International Journal of General Medicine

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ORIGINAL RESEARCH

Serum circRNA_100199 is a Prognostic Biomarker in Acute Myeloid Leukemia

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Background: An aberrant level of serum microRNA expression has been demonstrated to be a prognostic marker for acute myeloid leukemia (AML). The therapeutic relevance of serum circRNA 100199 remained unknown, however. This research aimed to investigate the probable prognostic significance of serum circRNA_100199 for AML.

Methods: This study included a total of 200 participants consisting of 114 AML-diagnosed patients and 86 healthy people. Blood samples were taken, and the level of circRNA_100199 in the serum was measured using quantitative reverse transcription polymerase chain reaction (qRT-PCR) to explore its potential clinical significance.

Results: Our study demonstrated that circRNA_100199 expression in the serum was substantially higher in AML subjects than in healthy persons. This increase in serum circRNA_100199 levels was particularly noticeable in M4/M5 subtype AML patients, and those with poor cytogenetic risk or higher white blood cell counts. Using receiver operating characteristic (ROC) analysis, AML cases were effectively differentiated from healthy persons based on the level of serum circRNA_100199. Furthermore, it was found that high serum circRNA_100199 expression was strongly linked with shorter survival times and more severe clinical features. Our study also confirmed that high serum circRNA_100199 expression was an independent predictor of relapse-free survival (RFS) and overall survival (OS) in AML patients. Interestingly, the serum expression level of circRNA_100199 was significantly reduced following treatment, and its levels were substantially lower in AML patients who achieved complete remission (CR) than those who did not.

Conclusion: Overall, these findings suggest that serum circRNA_100199 has the potential to be a favorable prognostic biomarker for AML.

Keywords: circRNA_100199, prognostic biomarker, acute myeloid leukemia

Introduction

Blood cancer, AML, develops from the aberrant proliferation and differentiation of myeloid cells in the bone marrow. Due to the wide range of genetic and molecular abnormalities that cause it, patients with the same diagnosis will have vastly different clinical outcomes and responses to therapy.[1] In AML, immature myeloid cells accumulate in the bone marrow and peripheral blood, causing symptoms including anemia, infections, and bleeding.[2] Treating older or ineligible patients who cannot receive intensive chemotherapy can be particularly challenging,[3,4] as can treating relapsed or refractory AML patients.[5–7] Despite the significant progress made in AML treatment, the 5-year OS rate for patients with AML remains poor, ranging from 11% to 55%.[8,9] The diagnosis of AML usually involves bone marrow biopsy and genetic testing to determine the subtype and risk classification of the disease, which guides treatment decisions. Therefore, there is a critical need to find new and reliable prognostic biomarkers to help stratify the risk of AML patients and select optimal therapeutic strategies to improve clinical outcomes.[10,11]

Circular RNAs (circRNAs) are produced through the precursor RNA alternative splicing and formed through a covalent bond between the 3' and 5' ends of RNA molecules, resulting in a circular loop structure. It has drawn significant attention due to its distinct features, such as its cellular abundance, biological function, and high stability, attributed to its covalently closed-loop structure.[12,13] CircRNAs have emerged as important regulators of gene expression

Received: 4 July 2023
Accepted: 13 September 2023
Published: 16 October 2023

and have been involved in various biological processes, including differentiation, proliferation, and apoptosis.[14,15] For example, Circ-UBR1 regulates the miR-1299/CCND1 axis in breast cancer, promoting proliferation and metastasis while inhibiting apoptosis.[16] Circ-METTL15 regulates the miR-1299/PDL1 axis in lung cancer, leading to increased immune escape, metastasis, proliferation, and inhibition of apoptosis.[17] Additionally, circRNAs can be detected with high stability in blood, making them attractive biomarkers for screening and potential therapeutic targets for multiple types of cancer.[18,19] Overall, circRNAs represent a promising avenue for developing new biomarkers and therapeutic targets for cancer.

Our study focused on circRNA_100199, a circular RNA with its gene located at chr1:44877652-44878394 and relative gene symbol RNF220. We selected circRNA_100199 for investigation due to its association with gastric cancer, as determined by our previous microarray screening results (GEO No. GSE94591). Our objective was to explore the possible prognostic significance of serum circRNA_100199 in AML.

Materials and Methods

Patients and Sample Collection

This study involved 114 patients who were recently diagnosed with AML and 86 healthy individuals who served as controls. Diagnosis and classification of patients with AML were performed using the World Health Organization and French-American-British (FAB) criteria. All of these patients have been informed of the purpose of this study, and all subjects provided written consent per the Declaration of Helsinki. The First People's Hospital of Wenling approved the study. The patients' characteristics are given in Table 1, and patients were divided into three cytogenetic risk groups (favorable, intermediate, and poor). Less than 5% blast cells in the bone marrow, normal peripheral blood counts four

Table 1 The Association Between Serum circRNA_100199 and the Clinicopathological Parameters of AML

Characteristics	circRNA_100199		P
	Low	High	
Age (years)			0.351
<60	32	35	
≥60	25	22	
Gender			0.285
Male	30	32	
Female	27	25	
BM Blasts (%)			0.194
<50	33	31	
≥50	24	26	
WBC counts ($\times 10^9$/L)			< 0.05
<10	38	15	
≥10	19	42	
FAB subtype			< 0.05
M0	19	6	
M1/M2	29	20	
M4/M5	9	31	

(*Continued*)

Table 1 (Continued).

Characteristics	circRNA_100199		P
	Low	High	
Platelet counts (× 10^9/L)			0.102
<50	41	17	
≥50	16	40	
Cytogenetics			< 0.05
Favorable	20	10	
Intermediate	25	31	
Poor	2	16	
Complete Remission			0.118
Yes	28	26	
No	29	31	

weeks after induction treatment, and no residual extramedullary disease were the criteria for CR. RFS was calculated from obtaining CR to relapse or the final follow-up, whereas OS was calculated from the time of diagnosis until death from any cause or the last follow-up. The median follow-up duration was 23 months (5.4–60 months), and no patients were lost. Following the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for AML, all patients were treated with chemotherapy similarly based on their clinical condition. The study assessed the expression level of serum circRNA_100199 both before and after treatment in AML patients.

Reverse Transcribed Quantitative PCR (RT-qPCR)

The serums were processed to isolate total RNA with the RNAprep Pure Cell Kit (Solarbio, Beijing, China). Subsequently, it was reverse transcribed into cDNA utilizing the Hifair II 1st Strand cDNA Synthesis Kit (YESEN, Shanghai, China). Hieff qPCR SYBR Green Master Mix (No Rox) (YESEN) was utilized to do qRT-PCR. The $2^{-\Delta\Delta Ct}$ technique was employed to quantify the relative expression of circRNAs to GAPDH (internal reference gene).

Statistical Analysis

A Student's *t*-test or one-way ANOVA was used to compare serum circRNA_100199 levels in two or three groups respectively. Possible correlations between clinical variables and serum circRNA_100199 expression were assessed using the chi-square test. ROC curve analysis was performed, and the area under the ROC curve (AUC) was analyzed to determine the discriminatory ability of serum circRNA_100199. Survival curves for AML patients were generated using the Kaplan-Meier technique, and differences in survival curves were compared using the Log rank test. Multivariate Cox regression analysis was employed to recognize independent prognostic factors. GraphPad Prism 7 (GraphPad Software, USA) was employed for all statistical analyses, and a p-value < 0.05 was deemed statistically significant.

Results

Serum circRNA_100199 was Downregulated in AML Patients

In this study, 1137 differentially expressed circRNAs were examined in AML patient samples using a previously published GEO dataset (GSE94591), and it was found that these circRNAs could distinguish AML patients from healthy controls (Figure 1A–C), but circRNA_103254 is no difference between 114 AML patients and 86 healthy persons. CircRNA_100199 was highly upregulated in AML patients than in healthy persons (Figure 1D). The serum

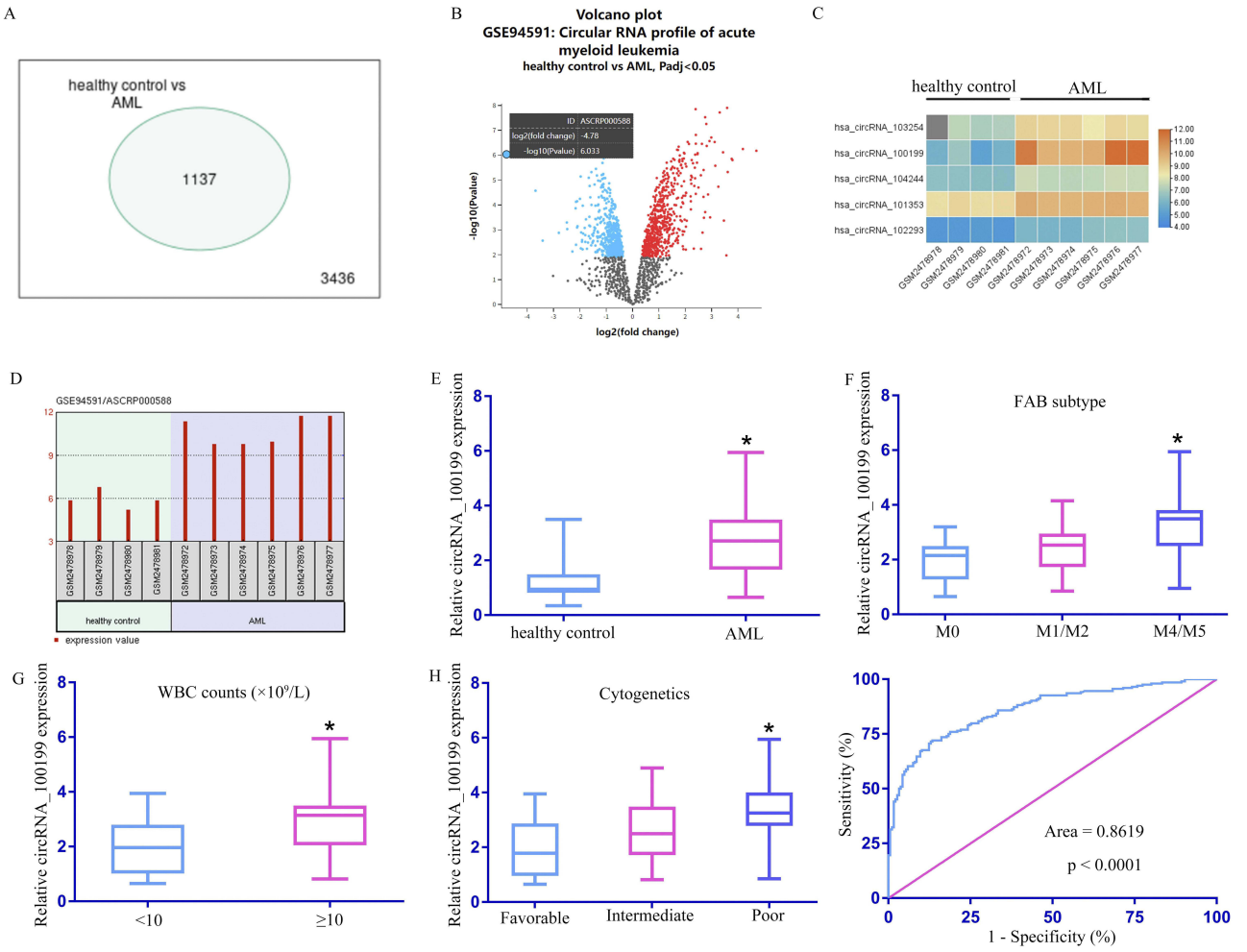

Figure 1 Downregulation of Serum circRNA_100199 in AML patients. (**A**) Differentially expressed circRNAs. (**B**) Differentially expressed genes in the GSE94591 dataset, presented using a Volcano plot. C. Genes upregulated in the GSE94591 dataset, subjected to clustering analysis. (**D**) circRNA_100199 was the highly upregulated in AML relative to healthy control levels. (**E**) serum circRNA_100199 levels were significantly higher in AML patients than in the healthy controls. (**F**) Serum circRNA_100199 levels were significantly upnregulated in M4/M5 subtypes compared to M1/M2 subtypes. (**G**) Serum circRNA_100199 levels were significantly upregulated in AML patients with higher WBC counts. (**H**) Serum circRNA_100199 levels were gradually upregulated in AML patients with favorable risk cytogenetic, intermediate risk cytogenetic, and poor risk cytogenetic groups. *p < 0.05.

circRNA_100199 expression levels in 114 AML patients and 86 healthy persons were measured using qRT-PCR, and serum circRNA_100199 levels were considerably greater in AML patients than in healthy persons (Figure 1E). It was also observed that low serum circRNA_100199 expression was more frequent in AML patients with M1/M2 subtypes compared to those with M4/M5 subtypes (Figure 1F) and in AML patients with WBC <10 × 10^9/L compared to those with WBC ≥ 10 × 10^9/L (Figure 1G). Moreover, serum circRNA_100199 levels were considerably increased in the poor cytogenetic risk group than the intermediate and favorable groups and in the intermediate group than the favorable group (Figure 1H). ROC analysis revealed that serum circRNA_100199 effectively discerned AML patients from normal persons with an Area of 0.8619 (Figure 1I).

Correlation Between Clinical Characteristics and Serum circRNA_100199 Expression

All AML patients were divided into the low- (n = 57), and high serum circRNA_100199 expression groups (n = 57) based on the serum circRNA_100199 level mean value. Table 1 shows that there is a significant association between high serum circRNA_100199 expression and cytogenetics and WBC counts, while no substantial association was found between serum circRNA_100199 and CR, platelet counts, FAB subtype, bone marrow blasts, gender, and age. The Log rank test and Kaplan-Meier method were employed to determine the relationship between survival and serum circRNA_100199

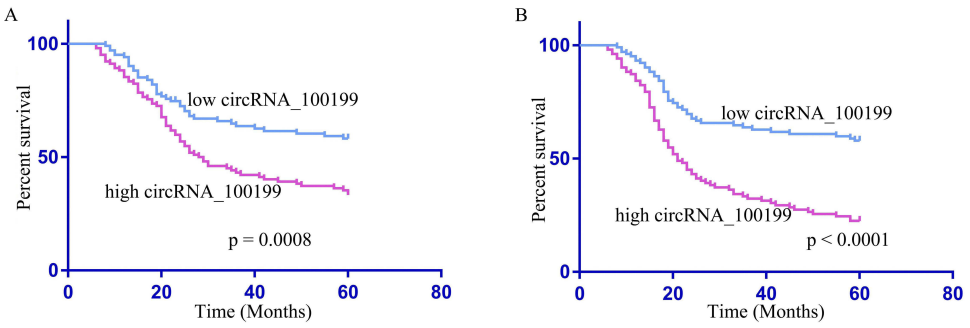

Figure 2 Correlation between serum circRNA_100199 expression and clinical characteristics. (**A**) The AML patients in the high serum circRNA_100199 group had worse OS than those in the low serum circRNA_100199 group. (**B**) The AML patients in the high serum circRNA_100199 group had worse RFS than those in the low serum circRNA_100199 group.

expression. The results indicated that OS (Figure 2A) and the RFS (Figure 2B) rates were considerably elevated in AML patients with low serum circRNA_100199 expression than those with high serum circRNA_100199 expression.

Serum circRNA_100199, as an Independent Prognostic Factor for RFS and OS

Univariate analysis and multivariable analysis revealed that serum circRNA_100199 expression, cytogenetics, and WBC counts, were significant indicators of poorer OS (Table 2). Similarly, serum circRNA_100199 expression, cytogenetics, and WBC counts, were recognized as independent prognostic markers for RFS in AML patients (Table 3).

The Changes in Expression Levels of Serum circRNA_100199 in AML Patients Following Treatment

Blood samples were collected on day 28 of the first chemotherapy cycle from all AML patients to determine the levels of serum circRNA_100199 and monitor therapeutic response. 54 out of 114 AML patients achieved CR, while 60 did not. The results showed that serum circRNA_100199 levels significantly decreased in both the CR and non-CR subgroups (Figure 3A and B). Furthermore, AML patients who achieved CR had significantly lower serum circRNA_100199 expression levels than those who did not before and after treatment (Figure 3C and D). These findings suggest that serum circRNA_100199 may be a valuable marker for monitoring the therapeutic response in patients with AML.

Table 2 Univariate and Multivariate Cox Analysis of Factors Related to AML Patient OS

Characteristics	Univariate			Multivariate		
	Risk Ratio	95% CI	p	Risk Ratio	95% CI	p
Age	0.854	0.451–1.845	0.645	–	–	–
Gender	0.719	0.365–1.754	0.554	–	–	–
BM Blasts (%)	0.794	0.395–2.021	0.342	–	–	–
WBC counts ($\times 10^9$/L)	2.054	0.519–2.845	0.019	2.354	0.625–3.415	0.015
FAB subtype	1.794	0.432–2.675	0.025	2.015	0.485–3.015	0.019
Platelet counts ($\times 10^9$/L)	0.556	0.584–1.955	0.451	–	–	–
Cytogenetics	1.882	0.619–2.965	0.021	2.215	0.549–3.254	0.017
Complete Remission	0.846	0.387–1.642	0.394	–	–	–
circRNA_100199	2.856	0.746–3.559	0.011	3.254	1.245–3.954	0.009

Table 3 Univariate and Multivariate Cox Analysis of Factors Related to AML Patient RFS

Characteristics	Univariate			Multivariate		
	Risk Ratio	95% CI	p	Risk Ratio	95% CI	p
Age	0.954	0.354–1.654	0.124	–	–	–
Gender	0.845	0.495–1.954	0.218	–	–	–
BM Blasts (%)	0.805	0.451–1.855	0.299	–	–	–
WBC counts ($\times 10^9$/L)	2.565	0.674–3.415	0.015	2.845	0.518–3.415	0.013
FAB subtype	2.322	0.385–3.025	0.018	2.594	0.548–3.154	0.015
Platelet counts ($\times 10^9$/L)	0.685	0.484–2.065	0.354	–	–	–
Cytogenetics	2.491	0.674	0.017	2.784	0.745–3.254	0.014
Complete Remission	0.915	0.542–2.022	0.195	–	–	–
circRNA_100199	3.019	1.201–3.854	0.009	3.451	1.308–3.988	0.005

Discussion

Identifying early-stage cancers in asymptomatic individuals and distinguishing between benign and malignant diseases are two major challenges in ensuring the long-term survival of cancer patients. Another obstacle is the lack of effective means to monitor and address the dynamic changes during and after therapy.

In recent years, circRNAs have gained recognition as potent gene expression regulators. These non-coding RNAs have excellent stability and conservation across species because they are generated by the reverse splicing of exons or introns, giving them a covalently closed loop structure impenetrable by exonucleases. Many disorders, including cancer, have been linked to the dysregulation of circRNAs because of their critical roles in biological processes, including

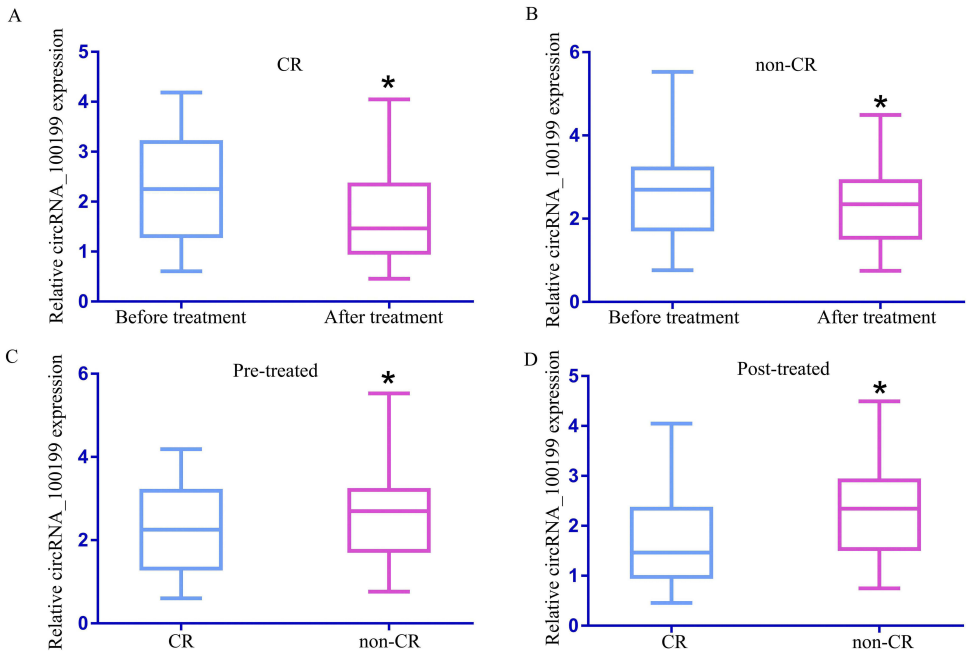

Figure 3 The changes in serum circRNA_100199 expression in AML patients following treatment. (**A**) For the AML cases achieving CR, serum circRNA_100199 levels were markedly deseased in the post-treated blood samples compared to the pre-treated blood samples. (**B**) For the AML cases not achieving CR, serum circRNA_100199 levels were decreased in the post-treated blood samples compared to the pre-treated blood samples. (**C** and **D**). For both pre-treated and post-treated blood samples, serum circRNA_100199 levels were significantly lower in AML achieving CR than in those not achieving CR. *p < 0.05.

apoptosis, differentiation, proliferation, and carcinogenesis. As such, they have gained increasing attention as potential diagnostic and prognostic biomarkers for cancer due to their stable, conserved nature and specificity for different tissues, times, and diseases.[20]

Multiple clonal expansions of myeloid progenitor cells in the bone marrow define AML, a heterogeneous hematological cancer. While significant therapeutic breakthroughs have been made in treating AML, the disease still has a dismal 5-year survival rate of 11–55%.[8,9] This highlights the critical need for new biomarkers that may help in AML patient screening, risk assessment, and follow-up. CircRNAs have shown promise as potential biomarkers for AML due to their tissue-specific expression patterns and association with clinical characteristics. Several studies have reported aberrant expression of circRNAs in AML.[21] For example, circ_POLA2, a circRNA derived from the POLA2 gene, was considerably upregulated in AML samples compared to normal controls. Functional analysis revealed that circ_POLA2 promoted AML cell proliferation by suppressing the production of miR-34a, a tumor suppressor miRNA that regulates various oncogenic pathways.[22] Other studies have shown strong correlations between circRNAs and various clinical characteristics of AML patients.[23] In addition to their potential as diagnostic and prognostic biomarkers, circRNAs also hold promise as therapeutic targets for AML. Recent studies have demonstrated that circRNAs can be targeted by various strategies, including small interfering RNAs, antisense oligonucleotides, and CRISPR-Cas9 gene editing. These findings highlight the potential of circRNAs as druggable targets for AML therapy.

In this research, AML patients had significantly higher levels of serum circRNA_100199 compared to healthy individuals, indicating that this circRNA may be produced or released in higher amounts by cancer cells into the bloodstream. ROC analysis was utilized to confirm that serum circRNA_100199 can effectively distinguish AML patients from healthy individuals. Furthermore, higher serum circRNA_100199 levels were associated with more severe clinicopathological features and poor patient survival outcomes, suggesting that patients with higher serum levels of circRNA_100199 may require more intensive therapy. Additionally, the increased serum circRNA_100199 was an independent prognosticator of poor OS and RFS in AML patients, indicating that this biomarker has great potential as a prognostic biomarker for this disease. Moreover, the levels of circRNA_100199 were lower among AML patients who received treatment, particularly those who achieved CR, indicating that serum circRNA_100199 is a sensitive biomarker for monitoring patient response to therapy. Collectively, these findings suggest that serum circRNA_100199 has the potential to serve as a useful biomarker for detecting and monitoring AML, which is consistent with previous studies that have highlighted its oncogenic role in this cancer type.

In conclusion, this study explored the prognostic importance of serum circRNA_100199 in AML patients. The findings suggest that serum circRNA_100199 may be a useful prognostic marker for AML patients, and the serum circRNA_100199 was considerably upregulated in AML patients than in healthy persons, and high serum circRNA_100199 levels were linked with poor RFS and OS in AML patients. Moreover, the serum circRNA_100199 can predict the risk of relapse and mortality and potentially serve as an independent prognostic factor for AML. This study provides valuable insights into the potential clinical applications of circRNA as a prognostic biomarker in AML. Further investigation is required to establish the biological mechanisms underlying the association between serum circRNA_100199 levels and AML prognosis. Nonetheless, the study suggests that circRNA represents a promising avenue for developing new biomarkers and therapeutic targets for cancer.

Funding

There is no funding to report.

Disclosure

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

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