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Circular RNA SPI1 expression before and after induction therapy and its correlation with clinical features, treatment response, and survival of acute myeloid leukemia patients

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Abstract

Background: Circular RNA spi-1 proto-oncogene (circ-SPI1) regulates cell proliferation, apoptosis, and bone marrow differentiation in acute myeloid leukemia (AML). This study aimed to assess the relationship of circ-SPI1 expression with the clinical features, induction therapy response, and survival of AML patients.

Methods: In total, 80 AML patients were included with bone marrow (BM) samples collected at baseline and after induction therapy. Additionally, 20 healthy donors (HDs) and 20 disease controls (DCs) were enrolled with BM samples collected after enrollment. BM circ-SPI1 expression was detected by reverse-transcription quantitative polymerase chain reaction assay.

Results: Circ-SPI1 expression was highest in AML patients, moderate in DCs, and lowest in HDs (median (interquartile range): 3.01 [2.02–4.14] versus 1.71 [1.01–2.85] versus 0.98 [0.74–1.71]) ($p < 0.001$). Moreover, lower circ-SPI1 expression was related to its decreased located gene SPI1 expression ($p = 0.029$), white blood cells (WBC) $< 18.8 \times 10^9$/L ($p = 0.010$), trisomy 8 ($p = 0.025$), and more favorable risk stratification ($p = 0.014$) in AML patients. Additionally, circ-SPI1 expression was reduced in AML patients after induction therapy ($p < 0.001$), and its low expression after induction therapy was correlated with the achievement of complete remission ($p < 0.001$). Furthermore, circ-SPI1 decline ≥30% during therapy (versus <30%) was independently related to longer event-free survival (EFS) (hazard ratio (HR): 0.445, $p = 0.028$) and overall survival (OS) (HR: 0.319, $p = 0.025$) in AML patients.

Conclusion: Decreased circ-SPI1 expression is related to lower WBC, favorable risk stratification, and better therapy response; moreover, its decline during therapy is an independent factor to predict longer EFS and OS in AML patients.

KEYWORDS
acute myeloid leukemia, circular RNA spi-1 proto-oncogene, event-free survival, induction therapy response, overall survival

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1 | INTRODUCTION

Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults,[1] which is a genetically heterogeneous malignant tumor characterized by excessive clonal proliferation of myeloid precursor cells.[1,2] It has been reported that 119,570 individuals are diagnosed with de novo AML in 2017 around the world, and its incidence continues to increase.[3] Although some therapeutic progress in AML has been made in recent years, the five-year survival rate of AML patients remains low[4–7]; moreover, recurrence also commonly occurs after treatment of AML patients,[8] suggesting that the prognosis of those patients remains unfavorable. Therefore, it is critical to explore potential biomarkers for predicting the prognosis of AML, which may contribute to the management of AML patients.

Circular RNAs (circRNAs) are endogenous RNAs that are divided into noncoding circRNAs and coding circRNAs, which play key roles in various biological functions, such as sponging microRNA, regulating gene transcription, and binding to RNA-binding proteins.[9–13] CircRNA spi-1 proto-oncogene (circ-SPI1; also named hsa_circ_0000303) is a novel identified circRNA that is abnormally expressed in AML patients, which controls cell proliferation, apoptosis, and bone marrow differentiation in AML.[14] In addition, the host gene of circ-SPI1, SPI1, is one of the hematopoietic transcription factors of the E-twenty-six (Ets) family and is considered to have a potential prognostic role in AML patients.[15–17] For example, a previous study shows that SPI1 is overexpressed and that its high expression is related to poorer prognosis in AML patients.[16] Furthermore, another study also elaborates that low SPI1 expression is related to longer disease-free survival and overall survival (OS) in AML patients.[17] However, the potential of circ-SPI1 as a biomarker for the management of AML is still unclear.

Therefore, this study aimed to evaluate circ-SPI1 expression and its correlation with the clinical features and induction therapy response of AML patients, as well as its ability to predict the occurrence and survival of AML.

2 | METHODS

2.1 | Subjects

From July 2016 to June 2021, a total of 80 patients with the first diagnosed AML were recruited in this research. The inclusion criteria were as follows: (i) first diagnosed with AML by morphology, immunology, cytogenetics, and molecular biology of bone marrow (MCIM); (ii) >18 years old; (iii) willing to provide bone marrow (BM). Patients complicated with BM failure syndromes or other cancers were excluded. Besides, a total of 20 patients who were diagnosed with non-myelodysplasia hematologic malignancies and needed BM examination were enrolled as disease controls (DCs). Furthermore, a total of 20 healthy donors (HDs) were enrolled during the same period when they were examined for eligibility for bone marrow transplantation. This study received the approval of the Ethics Committee. Written informed consent was obtained from all subjects or their guardians.

2.2 | Data and sample collection

The clinical characteristics of all AML patients were recorded for analysis, in which risk stratification was referring to a criterion published by the national comprehensive cancer network (NCCN).[18] For circ-SPI1 expression detection, BM samples from all subjects were collected after enrollment. Besides, BM samples were only collected again after induction therapy in AML patients. For SPI1 gene expression detection, 20 BM samples from AML patients at baseline were selected randomly.

2.3 | Sample detection

Circ-SPI1 expression and SPI1 gene expression were detected by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) assay. Total RNA from BM samples was extracted with RNeasy Protect Mini Kit (Qiagen). For circ-SPI1 expression only, complementary DNA was synthesized using iScript™ cDNA Synthesis Kit (with random primer; Bio-Rad). Subsequently, qPCR was conducted via QuantiNova SYBR Green PCR Kit (Qiagen). The relative expressions were calculated as $2^{-\triangle\triangle Ct}$. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control. The primers were referenced to a previous study.[14]

2.4 | Follow-ups

All AML patients underwent routine follow-ups until March 31, 2022. Complete remission (CR) after induction therapy was assessed by referring to an existing guideline.[19] Event-free survival (EFS) and OS were calculated. The circ-SPI1 expression at baseline and expression after induction therapy were divided into high and low expression by their own median values. The circ-SPI1 expression decline was cut by 30%, which was defined as: (the expression at baseline—the expression after induction therapy)/ the expression at baseline.

2.5 | Statistics

SPSS v.26.0 and GraphPad Prism v.8.01 were used for data analysis and figure plotting. Comparison of circ-SPI1 expressions between two groups or expressions among multi-groups was conducted by the Mann–Whitney U test or Kruskal–Wallis test, respectively.

The correlation between the circ-SPI1 expression and SPI1 gene expression was determined by Spearman's rank correlation test. Comparison of the circ-SPI1 expression at baseline and after induction therapy was assessed by Wilcoxon signed-rank test. The visualization of EFS and OS was shown by the Kaplan–Meier curves, and the differences were analyzed by the log-rank test. The factors related to EFS or OS were determined via univariable and enter-multivariable Cox regression analyses, in which the factors with $p < 0.05$ from the univariable regression analysis were selected for multivariable regression analysis. $p < 0.05$ indicated significance.

3 | RESULTS

3.1 | Baseline characteristics of AML patients

The AML patients included 49 (61.2%) males and 31 (38.8%) females with a mean age of 58.1 ± 10.0 years. The median (interquartile range (IQR)) values of white blood cell (WBC) and BM blasts were 18.8 $(8.9–28.1) \times 10^9$/L and 69.5 (55.3–78.0) %, respectively. In terms of the French-American-Britain classification (FAB classification), the number of AML patients classified as M1, M2, M4, M5, and M6 were 6 (7.5%), 21 (26.2%), 20 (25.0%), 27 (33.8%), and 6 (7.5%),
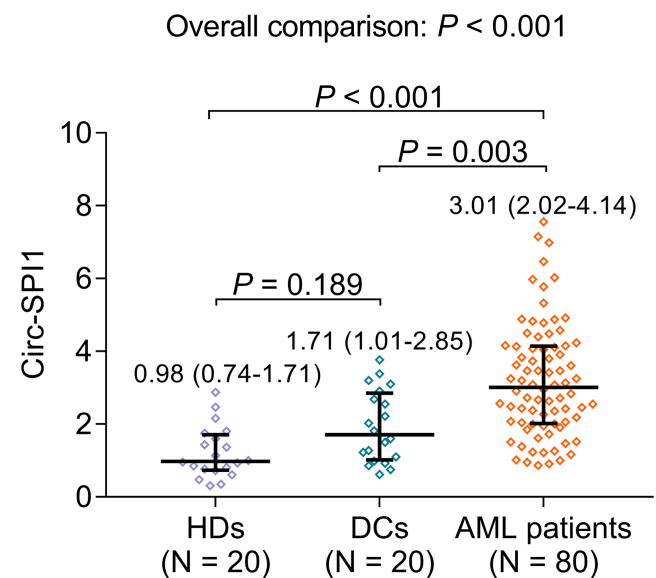
TABLE 1 Clinical characteristics of AML patients.

Characteristics	AML patients (N = 80)
Age, mean ± SD	58.1 ± 10.0
Gender, No. (%)	
Male	49 (61.2)
Female	31 (38.8)
WBC (10^9/L), median (IQR)	18.8 (8.9–28.1)
BM blasts (%), median (IQR)	69.5 (55.3–78.0)
FAB Classification, No. (%)	
M1	6 (7.5)
M2	21 (26.2)
M4	20 (25.0)
M5	27 (33.8)
M6	6 (7.5)
Cytogenetics, No. (%)	
NK	42 (52.4)
CK	8 (10.0)
inv (16) or t(16;16)	6 (7.5)
t(9;11)	4 (5.0)
−7 or 7q-	4 (5.0)
+8	2 (2.5)
−5 or 5q-	1 (1.3)
Others (not included in better or poor risk)	13 (16.3)
MK, No. (%)	7 (8.8)
FLT3-ITD mutation, No. (%)	24 (30.0)
Isolated biallelic CEBPA mutation, No. (%)	6 (7.5)
NPM1 mutation, No. (%)	18 (22.5)
WT1 mutation, No. (%)	5 (6.3)
Risk stratification (NCCN), No. (%)	
Favorable	15 (18.8)
Intermediate	42 (52.5)
Poor	23 (28.7)

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; CEBPA, CCAAT/enhancer-binding protein α; CK, complex karyotype; FAB classification, French-American-Britain classification; FLT3-ITD, internal tandem duplications in the FMS-like tyrosine kinase 3; IQR, interquartile range; MK, monosomal karyotype; NCCN, national comprehensive cancer network; NK, normal karyotype; NPM1, nucleophosmin 1; SD, standard deviation; WBC, white blood cell; WT1, Wilms' Tumor 1.

FIGURE 1 Circ-SPI1 expression among AML patients, DCs, and HDs. Circ-SPI1 expression was highest in AML patients, followed by in DCs, and lowest in HDs.
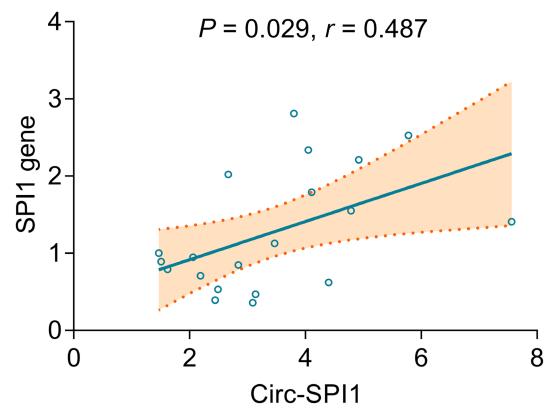

FIGURE 2 Correlation between circ-SPI1 expression and SPI1. Circ-SPI1 expression was positively associated with SPI1 in AML patients.

TABLE 2 Correlation of circ-SPI1 with clinical characteristics in AML patients.

Items	Circ-SPI1, median (IQR)	p-Value
Age		
≥60 years	3.54 (2.04–4.50)	0.138
<60 years	2.78 (1.99–3.68)	
Gender		
Male	2.95 (1.88–4.11)	0.474
Female	3.14 (2.08–4.24)	
WBC (cut by median)		
High	3.69 (2.41–4.56)	0.010
Low	2.48 (1.87–3.37)	
BM blasts (cut by median)		
High	3.25 (2.38–4.72)	0.106
Low	2.70 (1.96–3.97)	
FAB classification		
M1	3.76 (3.40–5.42)	0.186
M2	2.84 (2.03–4.33)	
M4	2.44 (1.48–3.66)	
M5	3.25 (2.00–4.50)	
M6	3.03 (2.17–3.54)	
Cytogenetics		
NK		
Yes	3.09 (2.08–4.14)	0.461
No	2.78 (1.70–4.18)	
CK		
Yes	3.69 (1.92–4.50)	0.418
No	2.88 (2.02–4.13)	
inv (16) or t(16;16)		
Yes	2.56 (1.69–3.30)	0.273
No	3.09 (2.04–4.18)	
t (9;11)		
Yes	3.48 (1.33–4.72)	0.912
No	3.02 (2.02–4.13)	
−7 or 7q-		
Yes	2.82 (2.16–4.15)	0.965
No	3.02 (2.00–4.15)	
+8		
Yes	1.31 (1.00-NR)	0.025
No	3.09 (2.07–4.16)	
−5 or 5q-		
Yes	1.73 (NR-NR)	0.269
No	3.08 (2.05–4.15)	
Others (not included in better or poor risk)		
Yes	2.91 (1.95–4.28)	0.809
No	3.08 (2.01–4.14)	
MK		
Yes	3.25 (2.49–4.49)	0.540
No	2.91 (2.01–4.13)	

TABLE 2 (Continued)

Items	Circ-SPI1, median (IQR)	p-Value
FLT3-ITD mutation		
Yes	3.47 (2.40–4.74)	0.137
No	2.73 (2.00–3.89)	
Isolated biallelic CEBPA mutation		
Yes	3.05 (2.00–4.10)	0.971
No	3.02 (2.04–4.15)	
NPM1 mutation		
Yes	2.56 (1.93–4.21)	0.388
No	3.20 (2.16–4.15)	
WT1 mutation		
Yes	4.05 (1.19–4.13)	0.788
No	2.95 (2.05–4.16)	
Risk stratification (NCCN)		
Favorable	2.27 (1.39–2.84)	0.014
Intermediate	3.02 (2.05–4.22)	
Poor	3.47 (2.49–4.58)	

Abbreviations: BM, bone marrow; CEBPA, CCAAT/enhancer-binding protein α; CK, complex karyotype; FAB classification, French-American-Britain classification; FLT3-ITD, internal tandem duplications in the FMS-like tyrosine kinase 3; IQR, interquartile range; MK, monosomal karyotype; NCCN, national comprehensive cancer network; NK, normal karyotype; NPM1, nucleophosmin 1; NR, not reach; WBC, white blood cell; WT1, Wilms' Tumor 1.

respectively. In addition, there were 15 (18.8%), 42 (52.5%), and 23 (28.7%) AML patients classified as favorable, intermediate, and poor according to risk stratification (NCCN). More detailed information about the clinical features of AML patients was shown in Table 1.

3.2 | Comparison of circ-SPI1 expression among AML patients, DCs, and HDs

Circ-SPI1 expression was highest in AML patients, moderate in DCs, and lowest in HDs (median [IQR]: 3.01 [2.02–4.14] vs. 1.71 [1.01–2.85] vs. 0.98 [0.74–1.71]) ($p < 0.001$). Further comparisons suggested that circ-SPI1 expression was elevated in AML patients compared to DCs ($p = 0.003$) and HDs ($p < 0.001$); however, it did not differ between DCs and HDs ($p = 0.189$) (Figure 1).

3.3 | Relationship of circ-SPI1 expression with SPI1 and clinical features in AML patients

Circ-SPI1 expression was positively related to SPI1 in AML patients ($p = 0.029$, $r = 0.487$; Figure 2). Additionally, lower circ-SPI1 expression was correlated with WBC low (below median; $p = 0.010$), trisomy 8 ($p = 0.025$), and more favorable risk stratification (NCCN;

$p = 0.014$) in AML patients. However, there was no relationship of circ-SPI1 expression with other clinical features in AML patients, including age, gender, BM blasts, FAB classification, other cytogenetics, MK, FLT3-ITD mutation, isolated biallelic CEBPA mutation, NPM1 mutation, or WT1 mutation (all $p > 0.05$; Table 2).

3.4 | Association of circ-SPI1 expression with treatment response in AML patients

Circ-SPI1 expression was reduced in AML patients after induction therapy compared with baseline (median (IQR): 1.99 [1.12–2.77] vs. 3.02 [2.02–4.15]) ($p < 0.001$) (Figure 3A). Low circ-SPI1 expression at

baseline showed a correlation trend with CR ($p = 0.057$; Figure 3B). Whereas reduced circ-SPI1 expression after induction therapy was associated with CR ($p < 0.001$; Figure 3C).

3.5 | Relationship of circ-SPI1 expression with EFS and OS in AML patients

There was no correlation between EFS and circ-SPI1 expression at baseline ($p = 0.073$; Figure 4A), while low circ-SPI1 expression after induction therapy was linked with longer EFS in AML patients ($p = 0.019$; Figure 4B). Meanwhile, circ-SPI1 expression decline ≥30% during therapy was related to better EFS in AML patients ($p = 0.029$; Figure 4C).
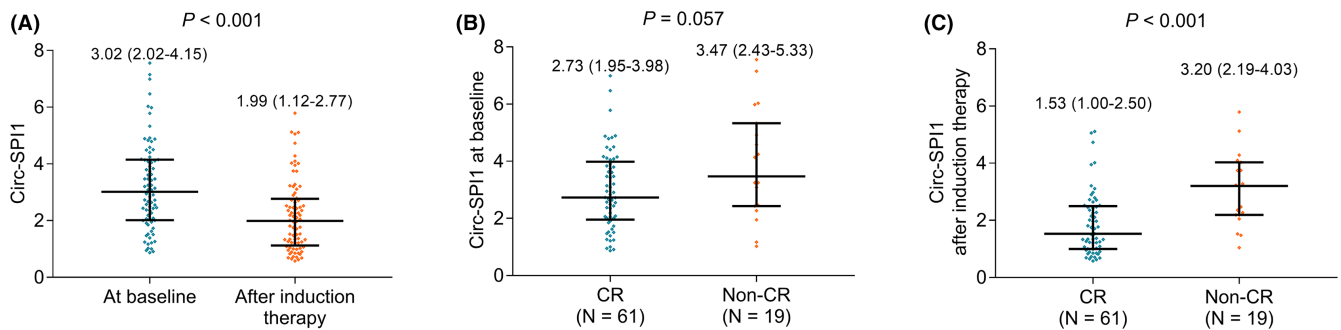

FIGURE 3 Circ-SPI1 expression change after induction therapy and its correlation with CR. Circ-SPI1 expression declined after induction therapy compared with baseline (A); low circ-SPI1 expression at baseline exhibited a correlation trend with CR (without statistical significance) (B); low circ-SPI1 expression after induction therapy was correlated with CR (C) in AML patients.
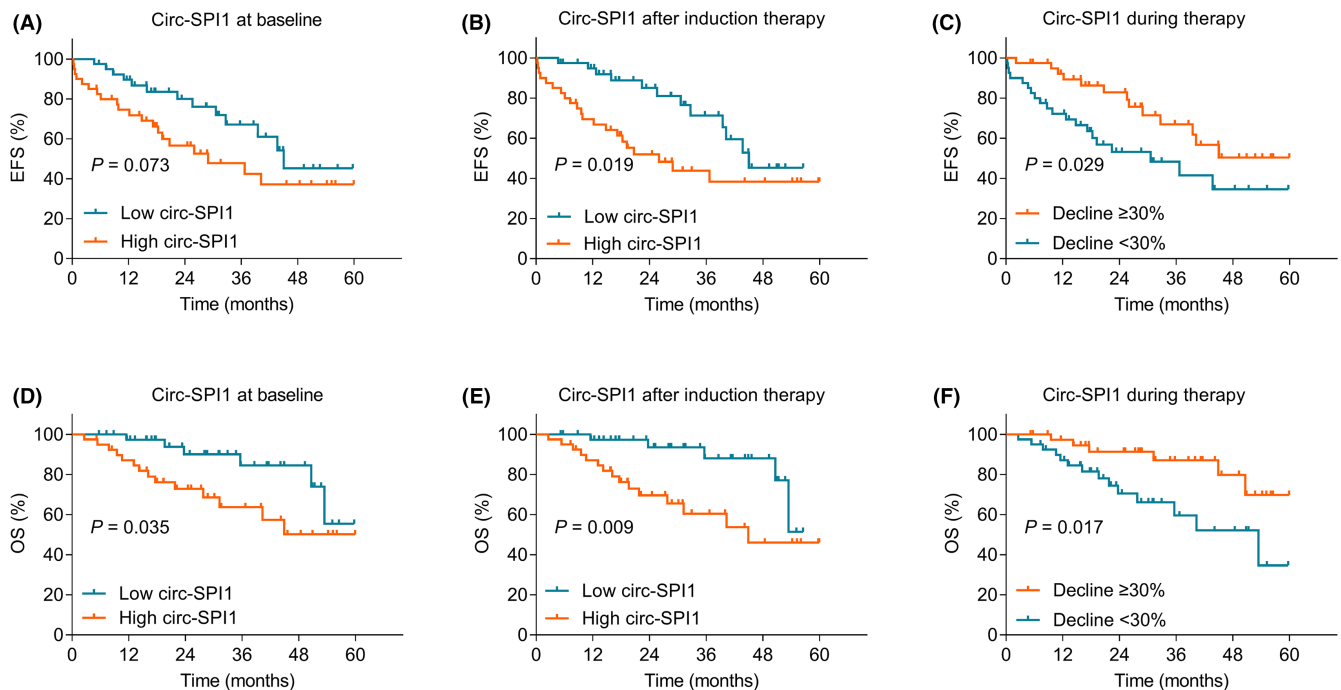

FIGURE 4 Correlation of circ-SPI1 expression with EFS and OS. No relationship of circ-SPI1 expression at baseline with EFS (A); low circ-SPI1 expression after induction therapy (B) and circ-SPI1 expression decline ≥30% during therapy (C) were related to longer EFS; low circ-SPI1 expression at baseline (D) and after induction therapy (E) were linked with longer OS; circ-SPI1 expression decline ≥30% during therapy was associated with longer OS (F) in AML patients.

TABLE 3 Cox regression analysis of factors related to EFS.

Items	*p*-Value	HR (95% CI)
Univariable regression		
Circ-SPI1 at baseline, high vs. low	0.078	1.878 (0.933–3.783)
Circ-SPI1 after induction therapy, high vs. low	0.023	2.281 (1.120–4.645)
Circ-SPI1 decline, ≥30% vs. <30%	0.033	0.465 (0.230–0.938)
Age, ≥60 years vs. <60 years	0.023	2.280 (1.119–4.648)
Gender, male vs. female	0.372	1.383 (0.678–2.818)
WBC, high vs. low	0.004	2.993 (1.419–6.309)
BM blasts, high vs. low	0.214	1.549 (0.776–3.093)
FAB Classification		
M1	Reference	(–)
M2	0.178	4.156 (0.522–33.067)
M4	0.400	2.463 (0.302–20.061)
M5	0.233	3.437 (0.451–26.177)
M6	0.562	2.037 (0.184–22.545)
Cytogenetics		
NK, yes vs. no	0.886	0.951 (0.479–1.887)
CK, yes vs. no	0.309	1.728 (0.602–4.957)
inv (16) or t (16;16), yes vs. no	0.210	0.042 (0.000–5.944)
t (9;11), yes vs. no	0.401	1.858 (0.437–7.905)
−7 or 7q-, yes vs. no	0.456	0.469 (0.064–3.438)
+8, yes vs. no	0.994	0.995 (0.237–4.181)
−5 or 5q-, yes vs. no	0.497	2.001 (0.270–14.815)
Others, yes vs. no	0.225	1.685 (0.726–3.914)
MK, yes vs. no	0.762	1.202 (0.366–3.950)
FLT3-ITD mutation, yes vs. no	<0.001	4.048 (1.987–8.245)
Isolated biallelic CEBPA mutation, yes vs. no	0.721	0.770 (0.184–3.225)
NPM1 mutation, yes vs. no	0.797	1.117 (0.482–2.587)
WT1 mutation, yes vs. no	0.211	1.951 (0.684–5.567)
Poorer risk stratification (NCCN)	<0.001	2.806 (1.593–4.944)
Enter-multivariable regression		
Circ-SPI1 decline, ≥30% vs. <30%	0.028	0.445 (0.216–0.916)
Age, ≥60 years vs. <60 years	0.002	3.359 (1.544–7.308)
WBC, high vs. low	0.009	2.778 (1.288–5.994)
FLT3-ITD mutation, yes vs. no	0.010	2.982 (1.294–6.875)

TABLE 3 (Continued)

Items	*p*-Value	HR (95% CI)
Poorer risk stratification (NCCN)	0.025	2.124 (1.098–4.109)

Abbreviations: BM, bone marrow; CEBPA, CCAAT/enhancer-binding protein α; CI, confidence interval; CK, complex karyotype; EFS, event-free survival; FAB classification, French-American-Britain classification; FLT3-ITD, internal tandem duplications in the FMS-like tyrosine kinase 3; HR, hazard ratio; MK, monosomal karyotype; NCCN, national comprehensive cancer network; NK, normal karyotype; NPM1, nucleophosmin 1; WBC, white blood cell; WT1, Wilms' Tumor 1.

In terms of OS, low circ-SPI1 expression at baseline ($p = 0.035$; Figure 4D) and after induction therapy ($p = 0.009$; Figure 4E) were associated with prolonged OS in AML patients. Meanwhile, circ-SPI1 expression decline ≥30% during therapy was also correlated with longer OS in AML patients ($p = 0.017$; Figure 4F).

3.6 | Factors related to EFS in AML patients

Factors influencing EFS in AML patients were assessed by univariable Cox regression analysis, which indicated that circ-SPI1 decline (≥30% vs. <30%; hazard ratio (HR): 0.465, $p = 0.033$) was correlated with longer EFS; while circ-SPI1 after induction therapy (high vs. low; HR: 2.281, $p = 0.023$), age (≥60 years vs. <60 years; HR: 2.280, $p = 0.023$), WBC (high vs. low; HR: 2.993, $p = 0.004$), FLT3-ITD mutation (yes vs. no; HR: 4.048, $p < 0.001$), and poorer risk stratification (NCCN; HR: 2.806, $p < 0.001$) were related to worse EFS in AML patients. Moreover, enter-multivariable Cox regression analysis displayed that circ-SPI1 decline (≥30% vs. <30%; HR: 0.445, $p = 0.028$) was independently associated with longer EFS; while age (≥60 years vs. <60 years; HR: 3.359, $p = 0.002$), WBC (high vs. low; HR: 2.778, $p = 0.009$), FLT3-ITD mutation (yes vs. no; HR: 2.982, $p = 0.010$), and poorer risk stratification (NCCN; HR: 2.124, $p = 0.025$) were independently associated with shorter EFS in AML patients (Table 3).

3.7 | Factors related to OS in AML patients

Univariable Cox regression analysis exhibited that circ-SPI1 decline (≥30% vs. <30%; HR: 0.327, $p = 0.023$) was related to favorable OS; while circ-SPI1 at baseline (high vs. low) (HR: 2.698, $p = 0.042$), circ-SPI1 after induction therapy (high vs. low) (HR: 3.554, $p = 0.014$), gender (male vs. female; HR: 4.526, $p = 0.016$), BM blasts (high vs. low; HR: 2.717, $p = 0.041$), FLT3-ITD mutation (yes vs. no; HR: 6.500, $p < 0.001$), and poorer risk stratification (NCCN; HR: 5.392, $p < 0.001$) were related to poorer OS in AML patients. Next, enter-multivariable Cox regression analysis suggested that circ-SPI1 decline (≥30% vs. <30%; HR: 0.319, $p = 0.025$) was independently correlated with longer OS; while BM blasts (high vs. low) (HR: 4.741, $p = 0.004$), FLT3-ITD mutation (yes vs. no; HR: 4.208, $p = 0.019$), and

TABLE 4 Cox regression analysis of factors related to OS.

Items	*p* Value	HR (95% CI)
Univariable regression		
Circ-SPI1 at baseline, high vs. low	0.042	2.698 (1.035–7.032)
Circ-SPI1 after induction therapy, high vs. low	0.014	3.554 (1.286–9.822)
Circ-SPI1 decline, ≥30% vs. <30%	0.023	0.327 (0.125–0.857)
Age, ≥60 years vs. <60 years	0.135	2.020 (0.803–5.078)
Gender, male vs. female	0.016	4.526 (1.322–15.489)
WBC, high vs. low	0.056	2.541 (0.975–6.620)
BM blasts, high vs. low	0.041	2.717 (1.039–7.101)
FAB classification		
M1	Reference	(–)
M2	0.348	2.855 (0.319–25.530)
M4	0.501	2.110 (0.240–18.586)
M5	0.551	1.884 (0.235–15.105)
M6	0.874	1.252 (0.077–20.439)
Cytogenetics		
NK, yes vs. no	0.507	1.356 (0.551–3.335)
CK, yes vs. no	0.636	1.430 (0.325–6.291)
inv (16) or t (16;16), yes vs. no	0.351	0.043 (0.000–32.478)
t (9;11), yes vs. no	0.602	1.714 (0.226–13.029)
−7 or 7q-, yes vs. no	0.507	0.046 (0.000–416.832)
+8, yes vs. no	0.433	1.806 (0.413–7.900)
−5 or 5q-, yes vs. no	0.224	3.529 (0.463–26.914)
Others, yes vs. no	0.798	0.852 (0.249–2.916)
MK, yes vs. no	0.560	1.549 (0.355–6.753)
FLT3-ITD mutation, yes vs. no	<0.001	6.500 (2.599–16.258)
Isolated biallelic CEBPA mutation, yes vs. no	0.712	0.684 (0.091–5.149)
NPM1 mutation, yes vs. no	0.398	1.557 (0.558–4.350)
WT1 mutation, yes vs. no	0.881	1.119 (0.255–4.919)
Poorer risk stratification (NCCN)	<0.001	5.392 (2.217–13.112)
Enter-multivariable regression		
Circ-SPI1 decline, ≥30% vs. <30%	0.025	0.319 (0.117–0.868)
Gender, male vs. female	0.428	1.690 (0.462–6.186)
BM blasts, high vs. low	0.004	4.741 (1.667–13.485)
FLT3-ITD mutation, yes vs. no	0.019	4.208 (1.273–13.911)
Poorer risk stratification (NCCN)	0.027	3.430 (1.147–10.261)

Abbreviations: BM, bone marrow; CEBPA, CCAAT/enhancer-binding protein α; CI, confidence interval; CK, complex karyotype; FAB classification, French-American-Britain classification; FLT3-ITD, internal tandem duplications in the FMS-like tyrosine kinase 3; HR, hazard ratio; MK, monosomal karyotype; NCCN, national comprehensive cancer network; NK, normal karyotype; NPM1, nucleophosmin 1; OS, overall survival; WBC, white blood cell; WT1, Wilms' Tumor 1.

poorer risk stratification (NCCN; HR: 3.430, *p* = 0.027) were independently related to worse OS in AML patients (Table 4).

4 | DISCUSSION

Circ-SPI1 promotes the myeloid differentiation of AML cells through interaction with the translation initiation factor eIF4AIII and induces AML cell proliferation and apoptosis by interacting with several miRNAs, such as miR-1307-3p and miR-382-5p.[14] However, there are few studies on the expression of circ-SPI1 in AML patients, and only one previous study indicates that circ-SPI1 expression is up-regulated in AML patients compared with healthy normal subjects.[14] This was similar to our findings: Our study revealed that circ-SPI1 expression was elevated in AML patients compared with DCs and HDs. The possible explanations for this were as follows: Circ-SPI1 might reflect the proliferation rate of cells to some extent.[14] Meanwhile, the malignant proliferation rate of cells was higher in AML patients than in DCs and HDs; thus, circ-SPI1 expression was high in AML patients.

Meanwhile, the correlation of circ-SPI1 with the clinical features of AML patients is also noteworthy. Our research indicated that lower circ-SPI1 expression was related to decreased circ-SPI1 located gene SPI1, WBC < 18.8×10^9/L, trisomy 8, and more beneficial risk stratification (NCCN) in AML patients. These findings could be interpreted as follows: (1) Circ-SPI1 could sponge with eIF4AIII[14]; meanwhile, the latter reduced the risk of pathogen infection.[20] Thereby, the circ-SPI1 expression had the potential to induce pathogen infection. Consequently, low circ-SPI1 expression was linked with WBC < 18.8×10^9/L. (2) Low circ-SPI1 expression was related to reduced WBC and cytogenetics, where the two features were classified as factors related to more favorable risk stratification in AML patients[21,22] and thus was correlated with more favorable risk stratification. In addition, we also found that circ-SPI1 expression decreased after induction treatment, and its low expression after induction treatment was related to CR. This might be because: (1) Induction therapy might alleviate the malignant proliferation of AML cells to some extent[23]; as we mentioned earlier, circ-SPI1 reflected the ability of malignant proliferation of AML cells. Therefore, circ-SPI1 expression after induction treatment was reduced in AML patients. (2) The decrease in circ-SPI1 during induction therapy inhibited the proliferation of AML cells[14]; thus, it was related to the better therapy response of AML patients. Furthermore, our study only selected 20 BM samples for SPI1 detection for the following reasons: (1) Consideration of research cost. (2) This study focused on the circ-SPI1 level rather than the SPI1 level, and the SPI1 level was only displayed as an auxiliary result; therefore, only 20 BM samples were randomly selected for testing.

In addition, in order to evaluate the prognostic role of circ-SPI1 in AML patients, our study analyzed the relationship of circ-SPI1 expression at baseline, after induction therapy, and its variation during therapy with EFS and OS in AML patients, respectively. The data suggested that low circ-SPI1 expression at baseline and after

induction treatment could both predict satisfactory survival of AML patients, and its posttreatment level exhibited a better prognostic effect; moreover, circ-SPI1 decline ≥30% during therapy was independently related to longer EFS and OS in AML patients. This might be because: (1) Circ-SPI1 decline indicated that AML patients were more likely to achieve CR after induction therapy, so those patients had better survival.[24–26] (2) Low circ-SPI1 expression could inhibit the proliferation of AML cells to alleviate the progression of AML,[14] so AML patients with low circ-SPI1 expression had better prognoses.

Our study existed some limitations: (1) There was a small sample size in this study and the potential of bone marrow circ-SPI1 as a biomarker of AML required to be verified with a large sample size in further research. (2) Our study only detected circ-SPI1 expression in the bone marrow and did not evaluate its expression in some body fluids such as plasma and serum.[27] (3) Our study only recruited adult AML patients; however, further study should be conducted to assess the prognostic ability of circ-SPI1 expression in children with AML. (4) Due to the small sample size of this study, the number of patients without the achievement of CR was small, and the statistical significance between circ-SPI1 expression and the achievement of CR was weak.

In conclusion, low circ-SPI1 expression is linked with lower WBC, favorable risk stratification, and more desirable induction therapy response, whose decline during therapy independently relates to longer EFS and OS in AML patients.

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CONFLICT OF INTEREST
None.

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

CONSENT TO PARTICIPATE
Written informed consents were obtained from all subjects or their guardians.

CONSENT FOR PUBLICATION
Not applicable.

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