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Circular RNA of *vimentin* expression as a valuable predictor for acute myeloid leukemia development and prognosis

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Abstract

The focus of this study was to investigate the expression status of *Circ-vimentin* (*VIM*) and further analyze its pathogenesis and clinical significance in acute myeloid leukemia (AML) patients. Real-time quantitative polymerase chain reaction was carried on *Circ-VIM* in 113 AML patients and 42 healthy controls. *Circ-VIM* was significantly upregulated in AML compared with control and was positively correlated with white blood cells (WBC) count. Receiver operating characteristic curve analysis indicated that the performance of *Circ-VIM* expression could serve as a promising biomarker for differentiating AML patients from controls. Significant correlations of *Circ-VIM* expression were found with WBC and French–American–British classifications. Survival analyses further showed that over-expressed *Circ-VIM* were associated with markedly shorter overall survival (OS) and leukemia-free survival (LFS) in whole-cohort AML, nonacute promyelocytic leukemia AML and cytogenetically normal-AML patients. Multivariate analysis also disclosed that *Circ-VIM* over-expression was an independent poor prognostic factor for OS and LFS in AML patients. Remarkably, Pearson correlation analysis evidenced that the expression of *Circ-VIM* was positively correlated with *VIM* expression in all AML patients. These results indicated that overexpression *Circ-VIM* could serve as a significant biomarker.

KEYWORDS
acute myeloid leukemia, circular RNA, *Circ-VIM*, tumor marker

1 | INTRODUCTION

Acute myeloid leukemia (AML) is a group of highly heterogeneous disease derived from bone marrow hematopoietic stem or progenitor cell, characterized by the rapid growth of abnormal white blood cells (WBC) that enhance in the bone marrow and inhibit the production of normal hematopoietic cells (Estey & Döhner, 2006; de Kouchkovsky & Abdulhay, 2016). AML is the most common acute leukemia in adults; nevertheless, the prognosis remains very poor in clinical practice due to the poor diagnostic approach and therapeutic schedule (Deschler & Lübbert, 2006). Cytogenetic abnormalities play crucial roles in deciphering the pathogenesis of AML, epigenetic alteration and genetic abnormalities are proved to be intervened in leukemogenesis as well as the leading factors of prognosis and therapy (Esteller, 2008; Odenike et al., 2011). Recently, the genetic or molecular markers not merely contribute to the pathogenesis of AML, but provided the important gist to predict the diagnosis, prognosis, and therapy of these patients (Yohe, 2015).

The last decade, noncoding RNAs (ncRNAs), including piwi-associated RNAs, endogenous short-interfering RNAs, microRNAs (miRNAs) and long ncRNAs, have revolutionized understanding of regulation and function (Hombach & Kretz, 2016). Recently, the accumulated research indicates that ncRNAs play critical roles in a wide variety of biological processes (Beermann, Piccoli, Viereck, & Thum, 2016). Circular RNAs (circRNAs) are a class of new endogenous ncRNAs that are formed by back-splicing events and have covalently closed loops of the 3′ and 5′ ends without poly-adenylated tails (Barrett & Salzman, 2016). The distinct properties of

circRNAs make it ubiquitous in biological processes, such as transcription, messenger RNA (mRNA) splicing, RNA decay and translation (Y. Chen, Li, Tan, & Liu, 2016). Especially, circRNAs can function as sponges of miRNA to remove miRNA from its mRNA targets and consequently alter gene expression (Jeck et al., 2013). For example, research show that *CDR1as* has more than 60 binding sites for *miR-7* and are highly expressed in many tissues, *CDR1as* can affects the occurrence of Parkinson's disease, Alzheimer's disease, renal cell carcinoma, lung cancer, and cervical cancer by regulating the expression of *miR-7* (Dropcho, Chen, Posner, & Old, 1987; Hansen et al., 2013; J. Li, Zheng, Sun, & Xiong, 2014; Memczak et al., 2013; W. T. Yang & Zheng, 2014). Other circRNAs as sponges of miRNA include *ciR-SRY*, *ciR-ITCH*, *circ-001569*, and *circRNA-CER* (Huang et al., 2015; Q. Liu et al., 2016; Wang, Tang, Yin, & Dong, 2013; Xie et al., 2016). In addition to, as a major gene regulator, multiple studies have shown that circRNAs contribute to the development of many different human disorders, one of which is cancer development (Abu & Jamal, 2016; Hansen, Kjems, & Damgaard, 2013). *Hsa-circ-002059*, a typical circRNA, was significantly downregulated in gastric cancer (GC) tissues than in the adjacent normal tissues, and its downregulation was significantly associated with distal metastasis, TNM stage, gender, and age, suggesting that circRNAs may be used as an emerging diagnostic biomarkers for GC (Guo et al., 2015; Meng & Xiang, 2016). In lung cancer, *circ-ITCH* played an inhibitory role by enhancing its parental gene expression (Wan et al., 2016). Besides, evidence suggest that *circ-ITCH* inhabits the action of the canonical Wnt/β-catenin pathway to produce antineoplastic effect in many cancer subtypes including hepatocellular carcinoma, pancreatic cancer, ovarian carcinoma, and esophageal squamous cell carcinoma and so forth (F. Li, Zhang, et al., 2015). *Hsa-circRNA-100855*, *circ-CDY*, and *Hsa-circ-0001649* were associated with the progress of laryngeal squamous cell cancer, colorectal cancer, and hepatocellular carcinoma (tissue), respectively (Abu & Jamal, 2016). Previous studies have strongly suggested that circRNAs did not just decipher the pathogenesis of cancer, but might serve as stable and useful biomarkers for cancer. However, there are few studies of circRNAs in AML.

Vimentin (VIM), a major component of type III intermediate filament (IF) protein, is encoded by the *VIM* gene and is ubiquitously expressed in endothelial and other mesenchymal cells (Ivaska, Pallari, Nevo, & Eriksson, 2007). *VIM* in fact is instrumental in vital physiological and pathological processes, such as adhesion, migration, cell signaling, inflammation, neurite extension, and vascularization (Kidd, Shumaker, & Ridge, 2014; Nijkamp et al., 2011). Increased *VIM* expression has been found in various epithelial tumors including lung cancer, hepatocellular carcinoma, breast cancer, gastrointestinal tumors, central nervous system tumors, malignant melanoma, and hematopoietic malignances, which may be concerned with tumor growth, invasion, and poor prognosis (Satelli & Li, 2011). *Circ-VIM*, a circRNA of *VIM* genes, has rarely been studied in any cancer, let alone in AML. The aim of this study was to investigate and analyze the expression of *Circ-VIM* in de novo AML patients and to explore the clinical relevance and the correlation with gene *VIM*.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

A total of 155 samples, including 113 patients with a diagnosis of de novo AML and 42 healthy donors as normal control, were offered by the Affiliated People's Hospital of Jiangsu University in this study. All the samples were separated by lymphocyte separation medium (TBD Sciences, Tianjin, China) for bone marrow mononuclear cells (BMNCs) and all the patients provided written informed consent. According to the French–American–British (FAB) and World Health Organization criteria combined to the results of immunophenotyping and cytogenetic analysis, AML patients were diagnosed and classified normatively (Bennett et al., 1985; Campo et al., 2011). The treatment protocol was described as reported previously (Y. Li et al., 2013). The main clinical and laboratory features of the patient cohort were summarized in Table 1.

2.2 | RNA isolation and reverse transcription

BMNCs were extracted by Ficoll–Hypaque gradient centrifugation from bone marrow specimens. Total RNA was isolated by using TRIzol reagent (Invitrogen, Carlsbad, CA) on the basis of the manufacturer's instructions. Reverse transcription reaction with 2 μg of total RNA in a total volume of 40 μl including 10 mM of dNTPs, 80 U of RNase inhibitor, 200 U of Moloney Murine leukemia virus reverse transcriptase (MBI Fermentas, Hanover, MD), and 10 μM of random hexamers was executed on iCycler Thermal Cycler (Eppendorf, Hamburg, Germany) to synthesize complementary DNA (cDNA). The system of reverse transcription was incubated for 10 min at 25°C, 60 min at 42°C, and then stored at −20°C .

2.3 | Real-time quantitative polymerase chain reaction

Real-time quantitative polymerase chain reaction (qRT-PCR) was performed on a 7500 Thermocycler (Applied Biosystems, Foster City, CA). *Circ-VIM* was amplified using the specific primers 5′-GCAAGTATC CAACCAACTTGGTTCT-3′ (forward) and 5′-GGCACTTGAAAGCT GTTTCTTTAAG-3′ (reverse) with expected PCR products of 185 bp. The PCR reaction system with 20 μl volume consisted of 20 ng of cDNA, 0.8 μM of primers, 10 μM of SYBR Premix Ex Taq II, 0.4 μM ROX Reference Dye (TaKaRa, Tokyo, Japan) and 6 μl of H_2O. The amplification conditions of qRT-PCR reaction was carried out at 95°C for 30 s, followed by 40 cycles at 95°C for 5 s, 66.9°C for 30 s, 72°C for 30 s, and 70°C for 31 s to collect fluorescence, and finally followed by the melting program at 95°C for 15 s, 60°C for 1 min , 99°C for 15 s, and 60°C for 15 s. Both positive and negative controls were included in each assay. The abundance of *Circ-VIM* mRNA was calculated by the housekeeping gene (ABL). Relative *Circ-VIM* expression values were calculated using the following equation: $N_{Circ\text{-}VIM} = (E_{Circ\text{-}VIM})^{\Delta CT\ Circ\text{-}VIM\ (control-sample)} / (EABL)^{\Delta CT\ ABL\ (control-sample)}$. The parameter efficiency (E) derived from

TABLE 1 Comparison of clinical manifestations and laboratory features between AML patients with low and high expression

Patient's parameters	Status of *Circ-VIM* expression		
	Low ($n = 90$)	High ($n = 23$)	p Value
Sex (male/female)	52/38	17/6	0.231
Median age (range), years	55 (10–93)	56 (17–79)	0.806
Median WBC (range), $\times 10^9$/L	16.6 (0.3–197.7)	37.7 (1.1–528.0)	0.031
Median hemoglobin (range), g/L	78 (34–144)	79 (42–126)	0.961
Median platelets (range), $\times 10^9$/L	34 (3–415)	42 (12–190)	0.358
BM blasts (range), %	43.0 (1.0–94.5)	40.3 (10.5–97.5)	0.531
CR (+/−)	41/43	10/13	0.814
FAB			0.005
M0	1 (1%)	0 (0%)	
M1	5 (6%)	1 (4%)	
M2	40 (44%)	3 (13%)	
M3	20 (22%)	3 (13%)	
M4	15 (17%)	11 (48%)	
M5	7 (8%)	5 (22%)	
M6	2 (2%)	0 (0%)	
Karyotype classification			0.154
Favorable	29 (32%)	3 (13%)	
Intermediate	51 (57%)	15 (65%)	
Poor	8 (9%)	4 (17%)	
No data	2 (2%)	1 (4%)	
Karyotype			0.234
normal	38 (42%)	11 (48%)	
t(8;21)	8 (9%)	1 (4%)	
t(15;17)	20 (22%)	2 (9%)	
+8	5 (6%)	0 (0%)	
Others	11(12%)	3 (13%)	
Complex	6 (7%)	5 (22%)	
No data	2 (2%)	1 (4%)	
Gene mutation			
CEBPA (+/−)	10/65	1/18	0.452
NPM1 (+/−)	9/66	1/18	0.681
FLT3-ITD (+/−)	11/64	3/16	1.000
c-KIT (+/−)	4/71	0/19	0.579
NRAS or K-RAS (+/−)	6/69	1/18	1.000
IDH1/2 (+/−)	1/74	1/18	0.365
DNMT3A (+/−)	7/68	1/18	1.000
U2AF1 (+/−)	3/72	0/19	1.000
SRSF-2 (+/−)	2/76	0/19	1.000

Note. AML: acute myeloid leukemia; BM: bone marrow; CR: complete remission; FAB: French–American–British classification; WBC: white blood cells.

the formula: $E = 10^{(-1/\text{slope})}$ (the slope referred to CT vs. cDNA concentration plot).

2.4 | Gene mutation detection

CEBPA, *N/K-RAS*, *DNMT3A*, *IDH1/2*, *C-KIT*, *U2AF1*, *SRSF-2*, and *NPM1* mutations were detected by HRMA (Lin et al., 2011, 2012; Qian et al., 2012; X. Yang et al., 2013). All positive samples were confirmed by DNA direct sequencing. *FLT3-ITD* and *CEBPA* mutations were detected by direct DNA sequencing (BGI Tech Solutions Co., Shanghai, China).

2.5 | Statistical analyses

Statistical analyses were performed using SPSS 20.0 software package (SPSS, Chicago, IL). For comparing the difference of continuous variables between two groups, Mann–Whitney U test (two groups) was used. Pearson's χ^2 analysis or Fisher's exact test was used to compare the difference of categorical variables between patients group. Receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) were carried out to assess the diagnostic value of *Circ-VIM* expression in discriminating AML patients from normal controls. The prognostic value of *Circ-VIM* expression for overall survival (OS) and leukemia-free survival (LFS) was analyzed by Kaplan–Meier analysis and Cox regression analysis (univariate and multivariate analysis). Pearson's correlation test examined the correlation between *Circ-VIM* and *VIM*, *Circ-VIM*, and WBC count, respectively. For all analyses, a $p < 0.05$ (two-tailed) was considered statistically significant.

3 | RESULTS

3.1 | *Circ-VIM* expression in normal controls and de novo AML

The levels of *Circ-VIM* expression in whole-cohort AML patients (range: 0.001–107.829, median: 0.579) was significantly upregulated as compared with healthy controls (range: 0.003–2.845, median: 0.074; $p < 0.001$; Figure 1). Moreover, significant upregulation of *Circ-VIM* expression was shown in non-acute promyelocytic leukemia (APL) AML and cytogenetically normal-AML patients ($p < 0.001$; Figure 1).

3.2 | Discriminative capacity of *Circ-VIM* expression

ROC curve analysis indicated that the performance of *Circ-VIM* expression could serve as a promising biomarker for differentiating whole AML patients from controls (AUC = 0.741, 95% confidence interval (CI): 0.657–0.825, $p < 0.001$; Figure 2a). Moreover, significant distinguishing value was also revealed in non-APL AML (AUC = 0.740, 95% CI: 0.652–0.828; $p < 0.001$; Figure 2b), CN-AML patients (AUC = 0.749,

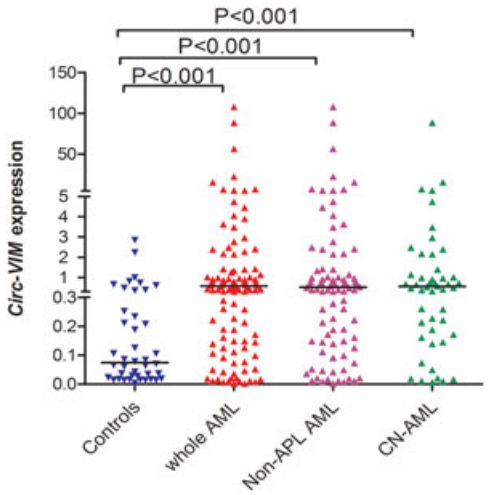

FIGURE 1 Relative expression levels of *Circ-VIM* in controls and AML patients. AML: acute myeloid leukemia; APL: acute promyelocytic leukemia; Circ-VIM: circ-vimentin; CN: cytogenetically normal [Color figure can be viewed at wileyonlinelibrary.com]

95% CI: 0.645–0.852; $p < 0.001$; Figure 2c) according to ROC curve analysis.

3.3 | Clinical and laboratory characteristics of AML patients

According to the cut-off value of 2.003 (defined as mean ± 3*SD* in controls), the cohort of 113 AML patients was divided into two groups: low *Circ-VIM* expression (*Circ-VIM*^{low}) group and high *Circ-VIM* expression (*Circ-VIM*^{high}) group (Table 1). There were no significant differences in sex, age, hemoglobin (Hb), platelets (PLTs) BM blasts, karyotypy classifications and nine gene mutations between the *Circ-VIM*^{low} and *Circ-VIM*^{high} groups ($p > 0.05$). While, significant differences were found between the two groups in the distribution of WBC count and FAB subtypes ($p < 0.05$).

3.4 | Correlation between Circ-*VIM* expression and clinical outcome

To explore the prognostic impact of *Circ-VIM* expression in AML, survival data were obtained for 113 AML patients with median follow-up time of 10 months (range: 1–90 months). After induction therapy, there was no significant difference in complete remission rate between *Circ-VIM*^{low} patients and *Circ-VIM*^{high} patients ($p = 0.814$; Table 1). Kaplan–Meier manifested that patients with *Circ-VIM*^{high} had significantly shorter OS ($p < 0.001$) and LFS ($p < 0.001$) than *Circ-VIM*^{low} patients in the whole cohort of AML patients (Figure 3a,b). This poor prognosis associated with *Circ-VIM* over-expression was also observed in the Non-APL cohort of AML patients (OS: $p < 0.001$; LFS: $p < 0.001$) and CN-AML patients (OS: $p = 0.002$; LFS: $p = 0.011$; Figure 3c–f).

Multivariate analysis, including age (≤60 vs. >60 years), WBC (≥30 × 10^9/L vs. <30 × 10^9/L), karyotype classifications (favorable vs. intermediate vs. poor), gene mutations (mutant vs. wild-type) and *Circ-VIM* expression status (high vs. low) with $p < 0.20$ in univariate analysis, also disclosed that high *Circ-VIM* expression was an independent prognostic factor in both OS and LFS among all AML patients (Tables 2,3). However, there was no significant difference for *Circ-VIM* expression in OS and LFS analyses between CN-AML and non-APL AML patients, respectively (data not shown). There was positive correlation between *Circ-VIM* expression and WBC count by using Pearson's correlation analysis ($r = 0.259$; $p < 0.001$; Figure 4; http://www.youdao.com/example/oral/positive_correlation/).

3.5 | Correlation between *Circ-VIM* and *VIM* expression in AML

Pearson correlation analysis was applied to investigate the relationship between *Circ-VIM* expression and the parental gene *VIM* expression in AML patients and demonstrated that there was a positive correlation between *Circ-VIM* expression and *VIM* expression ($r = 0.439$; $p < 0.001$; Figure 5). The data of *VIM* (the gene of *VIM*) expression in AML ($n = 82$,

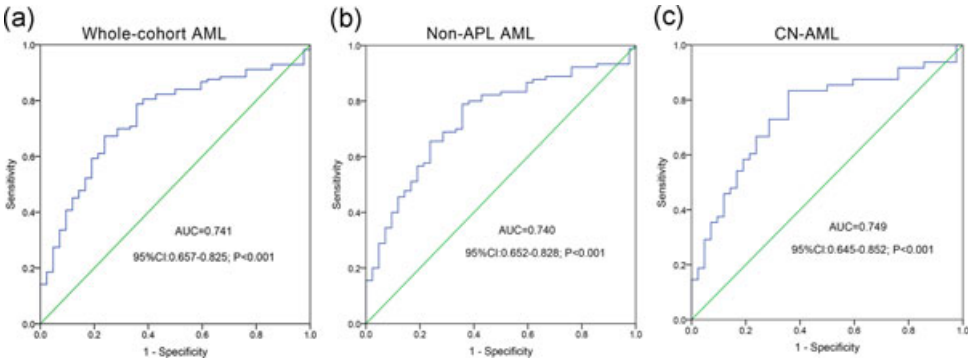

FIGURE 2 ROC curve analysis of *Circ-VIM* expression for discriminating acute myeloid leukemia patients from controls. (a) Whole-cohort AML; (b) Non-APL AML; (c) CN-AML. AML: acute myeloid leukemia; APL: acute promyelocytic leukemia; AUC: area under the ROC curve; Circ-VIM: circ-vimentin; CN: cytogenetically normal [Color figure can be viewed at wileyonlinelibrary.com]

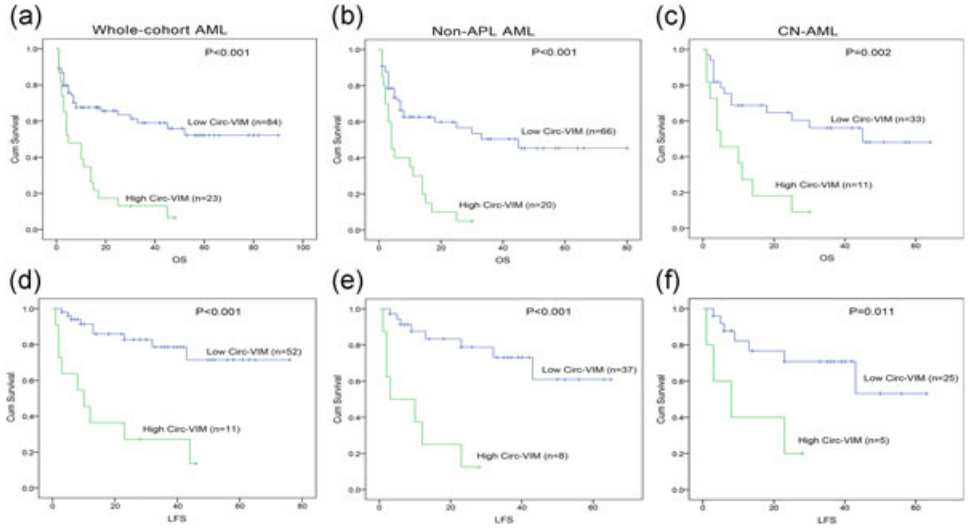

FIGURE 3 The impact of *Circ-VIM* expression on survival in AML patients. (a) OS among whole-cohort AML; (b) LFS among whole-cohort AML; (c) OS among non-APL AML; (d) LFS among non-APL AML; (e) OS among CN-AML; (f) LFS among CN-AML. AML: acute myeloid leukemia; APL: acute promyelocytic leukemia; Circ-VIM: circ-vimentin; CN: cytogenetically normal; LFS: leukemia-free survival; OS: overall survival [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Univariate and multivariate analyses of prognostic factors for overall survival in all AML patients

	Univariate analysis		Multivariate analysis	
Prognostic factors	**Hazard ratio (95% CI)**	***p* Value**	**Hazard ratio (95% CI)**	***p* Value**
Age	2.241 (1.303–3.855)	0.004	1.196 (0.629–2.273)	0.584
WBC	5.597 (3.085–10.152)	<0.001	3.136 (1.471–6.683)	0.003
Karyotypic classifications	2.349 (1.597–3.454)	<0.001	1.622 (0.970–2.712)	0.065
Circ-VIM expression	3.206 (1.832–5.609)	<0.001	2.034 (1.016–4.069)	0.045
FLT3-ITD mutation	1.677 (0.771–3.603)	0.194	1.108 (0.476–2.581)	0.811
NPM1 mutation	1.293 (0.460–3.631)	0.626	–	–
CEBPA mutation	0.889 (0.316–2.497)	0.823	–	–
c-KIT mutation	0.046 (0.001–23.411)	0.333	–	–
N/K-RAS mutation	1.415 (0.504–3.972)	0.510	–	–
IDH1/2 mutation	1.135 (0.155–8.285)	0.901	–	–
DNMT3A mutation	1.333 (0.523–3.397)	0.547	–	–
U2AF1 mutation	8.947 (2.556–31.326)	0.001	5.221 (1.336–20.404)	0.017
SRSF-2 mutation	1.823 (0.247–13.471)	0.556	–	–

Note. AML: acute myeloid leukemia; CI: confidence interval; WBC: white blood cells.

range: 0.014–1256.706, median: 16.605) was provided by our laboratory and the *VIM* expression was significantly upregulated compared with healthy controls ($p < 0.05$; data not yet published).

4 | DISCUSSION

Sanger, Klotz, Riesner, Gross, and Kleinschmidt (1976) discovered that the viroids, single-stranded covalently closed circRNA molecules, cause the disease in some higher plants and first proposed the concept of "circulating RNA" in 1976. Since then, some scholars have been keen on studying circRNAs, especially after describing in some papers the presence of circRNA species from back-spliced exons in mammals and determining that they are abundantly and distinctively expressed (Jeck & Sharpless, 2014; Vicens & Westhof, 2014). In recent years, there have seen a surge reports of circRNAs abundantly expressed in human body fluids such as plasma, saliva, and hematopoietic compartment indicating circRNAs as potential disease biomarkers (Bahn et al., 2015; Bonizzato, Gaffo, Te kronnie, & Bortoluzzi, 2016; P. Li, Chen, et al., 2015). Studies by W. Li et al. (2017) revealed that a large number of circRNAs expression in a leukemia specific manner in AML

TABLE 3 Univariate and multivariate analyses of prognostic factors for leukemia-free survival in all AML patients

	Univariate analysis		Multivariate analysis	
Prognostic factors	**Hazard ratio (95% CI)**	***p* Value**	**Hazard ratio (95% CI)**	***p* Value**
Age	2.605 (0.984–6.899)	0.054	1.581 (0.520–4.808)	0.420
WBC	5.167 (1.963–13.602)	0.001	0.793 (0.141–4.455)	0.792
Karyotypic classifications	5.737 (1.837–17.919)	0.003	4.935 (1.427–17.066)	0.012
Circ-VIM expression	7.023 (2.697–18.288)	<0.001	17.186 (4.306–68.567)	<0.001
FLT3-ITD mutation	1.369 (0.308–6.077)	0.680	–	–
NPM1 mutation	0.045 (0.001–648.649)	0.525	–	–
CEBPA mutation	1.239 (0.278–5.516)	0.778	–	–
c-KIT mutation	0.046 (0.001–15497.162)	0.635	–	–
N/K-RAS mutation	1.152 (0.151–8.808)	0.891	–	–
IDH1/2 mutation	0.047 (0.001–16590.547)	0.683	–	–
DNMT3A mutation	2.506 (0.689–9.119)	0.163	6.449 (1.215–34.228)	0.029

Note. AML: acute myeloid leukemia; CI: confidence interval; WBC: white blood cells.

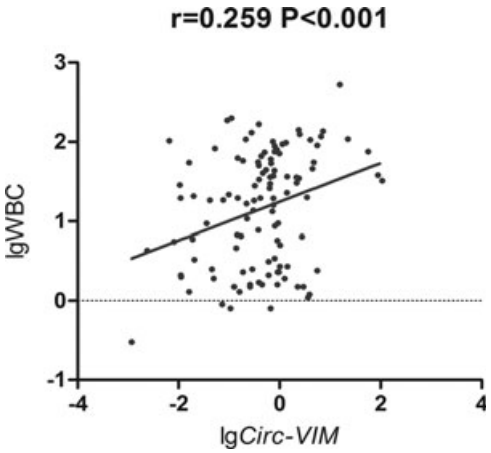

FIGURE 4 Pearson's correlation analysis between WBC count and *Circ-VIM* expression in AML patients. AML: acute myeloid leukemia; Circ-VIM: circ-vimentin; WBC: white blood cells

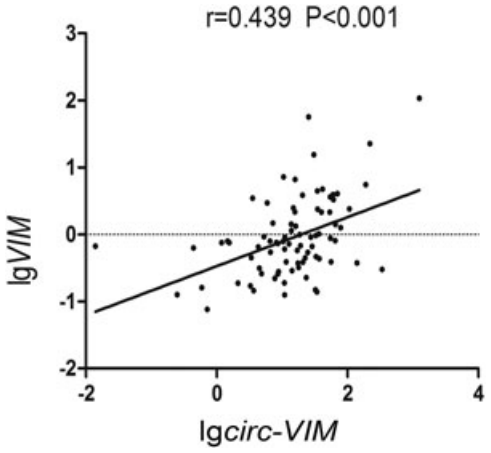

FIGURE 5 Pearson correlation analysis between the expression of *Circ-VIM* and *VIM* in whole-cohort AML patients. AML: acute myeloid leukemia; Circ-VIM: circ-vimentin

patients by using circRNA microarray and demonstrated that a downregulated circRNA, *hsa_circ_0004277*, could significantly restore the expression after chemotherapy, implying *hsa_circ_0004277* could serve as a potential diagnostic marker and treatment target in AML. ĹAbbate et al. (2017) have demonstrated that chromosome band 8q24 amplicons in AML were plastic DNA structures with an unexpected association to circRNAs, for example, *circPVT1* was strongly upregulated in AML-amp (8q24 in 23 cases of AML). In this study, we observed that the level of *Circ-VIM* was significantly upregulated in AML patients compared with healthy controls. ROC curve analysis indicated that high *Circ-VIM* expression might be a promising diagnostic biomarker for screening all AML patients from healthy controls, also including CN-AML and non-APL AML. To the best of our knowledge, this is the first case of *Circ-VIM* expression reported in cancer, especially in AML. Through further research, we predicted that *Circ-VIM* might be expected to be used in the diagnosis and treatment target of AML patients in the near future.

Furthermore, our research disclosed that patients with elevated *Circ-VIM* expression achieved significantly negative OS, LFS in whole AML, CN-AML, and non-APL AML. We also found that the expression of *Circ-VIM* was an independent prognostic factor for OS and LFS in the entire AML cohort in light of univariate and multivariate analyses, and it could be conductive to predicting inferior survival and more to assessing treatment outcome, and might serve as assessment criteria for the therapy in AML. The previous study have shown that high WBC count was important prognostic factors for AML patients with the genotypic combination "*NPMc+* with *FLT3-ITD*" (de Jonge et al., 2011). Our study revealed a positive correlation between WBC count and *Circ-VIM* expression. Based on the above study results, we can come to the conclusion that the expression level of *Circ-VIM* could be used as an important indicator of prognosis and evaluation of curative effect. Obviously, more in-depth studies on large quantities of AML patients are needed to confirm our findings.

Notably, our study further demonstrated that a significant correlation was observed between the level of *Circ-VIM* expression and *VIM* expression in all AML patients. As the parental gene of *Circ-VIM*, *VIM* over-expression has been found in a variety of solid tumors except AML. In some metastatic tumor cells, upregulated *VIM* gene can be used as a marker of oncogenic progression (L. K. Liu, Jiang, Wang, & Song, 2010). *VIM*, as a direct target of PU.1 inhibition in leukemia cells, plays an important role in various hematological malignancies (Imoto et al., 2010). Previous works suggested that *VIM* can form an IF network in peripheral blood lymphocytes and display anomalous function in chronic lymphocytic leukemia lymphocytes, promyelocytic leukemia cells, and human monocytic leukemia cells (Bruel et al., 2001; Honke & Wada, 1997; Stark, Liebes, Shelanski, & Silber, 1984). The exonic circRNAs, such as *CDR1as* and *SRY*, have been proved to bind miRNAs without being degraded, making them outstanding candidates for competing endogenous RNA activity to increase the expression of same coding RNAs (Jeck & Sharpless, 2014). On the other side, Knockdown of *CDR1as* decreased the expression of known *miR-7* target genes, indicating that circRNAs can act as miRNA sponges to regulate the parental genes (Jeck & Sharpless, 2014). Li et al. have provided evidence that some of circRNAs tend to be localized in the nucleus and interact with the Pol II transcription complexes at the promoters of their parental genes in *cis* to enhance parental genes expression (I. Chen, Chen, & Chuang, 2015; Z. Li, Huang, et al., 2015). Knockdown of circRNAs that can regulate Pol II (e.g., *circEIF3J* and *circPAIP2*) may result in a significant decrease of theirs parental gene (Bonizzato et al., 2016). In our study, we found that the levels of *Circ-VIM* was upregulated in AML patients compared with the control group, suggesting that high-expression *Circ-VIM* may play a role in the pathogenesis of AML. The expression of *VIM* was also upregulated in AML patients. We boldly speculate that *Circ-VIM*, as a tumor promoter, may accelerate the progression of AML by upregulating the expression of *VIM* genes through certain miRNAs and the Pol II transcription complexes, which clearly needs further functional research to confirm and expand our prediction.

Unfortunately, partly because of our limited sample size, no significant association was observed between *Circ-VIM* expression and laboratory features (including sex, age, Hb, PLT, BM blasts, karyotypy classifications, and gene mutations) by analyzing the *Circ-VIM* expression with clinical and laboratory characteristics. While, in the two groups of high and low expression of *Circ-VIM*, there was a statistically significant difference in WBC count and FAB classifications. *Circ-VIM*, never reported as a tumor promoter in any human cancer, limits our description and confirmation of *Circ-VIM* functions. Next, we plan to design and overcome more additional studies, including in vitro and in vivo functional assays, expression detection after chemotherapy and the relationship between *Circ-VIM* and its parental coding gene, to assess and confirm mechanisms for potential effects of *Circ-VIM* in AML. There are great differences in circRNAs, but more research should be done to find out if these differences between *Circ-VIM* and other circRNA are biologically relevant to develop circRNA into biomarkers of diseases. In the future, prospective screening of *Circ-VIM* expression and targeted intervention with *Circ-VIM* may open new pathway for the diagnosis and treatment of AML. Either reducing the circularization of functional transcripts or isolating exons contributing to dysfunctional transcripts through an mRNA-trap might be a future target for the therapies of AML.

In summary, our findings suggested that *Circ-VIM* overexpression was a frequent event in de novo AML patients and might serve as promising diagnosis, prognostic, and initial treatment marker for AML. The expression of *Circ-VIM* was positively correlated with *VIM* expression in all AML patients, suggesting that *Circ-VIM* might promote the occurrence and progress of AML by regulating *VIM*.

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