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ORIGINAL RESEARCH

Circ_0002232 Acts as a Potential Biomarker for AML and Reveals a Potential ceRNA Network of *Circ_0002232/miR-92a-3p/PTEN*

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Xiao-Yu Su[1]
Qian Zhao[1]
Jin-Ming Ke[2]
De-Hong Wu[1]
Xin Zhu[1]
Jiang Lin[1]
Zhao-Qun Deng[1]

[1]Laboratory Center, The Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu, People's Republic of China; [2]Faculty of Forestry, Zhejiang A&F University, Hangzhou, Zhejiang, People's Republic of China

Purpose: Our research aimed to investigate the expression level of *circ_0002232*, which is transcribed from *PTEN*, and find out the association of *circ_0002232/miR-92a-3p/PTEN* network in acute myeloid leukemia (AML).

Methods: *Circ_0002232* expression in 115 AML patients and 48 controls was detected by using real-time quantitative PCR. The diagnostic value of *circ_0002232* expression was evaluated by receiver operating characteristic curve. Kaplan–Meier curves were used to analyse the impact of *circ_0002232* for overall survival. Associated network of *circ_0002232* was predicted by using interaction prediction websites.

Results: Compared with controls, *circ_0002232* was notably low-expressed in AML ($P<0.001$). According to the result of receiver operating characteristic curve, *circ_0002232* expression could distinguish AML patients from controls ($P<0.001$). There were significant differences in patients' age ($P=0.004$), FAB classifications ($P=0.036$), white blood cell count ($P=0.041$) and platelet count ($P=0.021$) between low-expressed *circ_0002232* group and high-expressed *circ_0002232* group. Moreover, there was a positive correlation between *circ_0002232* expression and patients' age (Pearson r=0.256, $P=0.0057$). Interestingly, we found that patients in low-expressed *circ_0002232* group had better overall survival both in whole AML ($P=0.030$) and non-APL AML ($P=0.014$). Remarkably, the expression of *circ_0002232* was positively correlated with *PTEN* (Spearman r=0.678, $P<0.001$). Furthermore, there was a negative correlation in AML between *circ_0002232* and *miR-92a-3p* (Spearman r=−0.301, $P=0.016$), *miR-92a-3p* and *PTEN* (Spearman r=−0.324, P=0.034). Interaction prediction websites revealed that *circ_0002232* might affect the expression of *PTEN* and the process of AML through sponging *miR-92a-3p*.

Conclusion: *Circ_0002232*, one of the circRNAs transcribed from *PTEN*, was remarkably down-regulated in AML and could act as a promising biomarker for the diagnosis of AML. In addition, there might be a potential association network of *circ_0002232/miR-92a-3p/PTEN* in AML.

Keywords: circular RNAs, *circ_0002232*, *miR-92a-3p*, *PTEN*, acute myeloid leukemia

Introduction

Acute myeloid leukemia (AML), the most common malignant myeloid disease in adults, is characterized by loss of differentiation of blasts (myeloid progenitor cell) and clonal amplification in the peripheral blood and bone marrow.[1,2] It had a poor prognosis in the past.[2] Cytogenetics analyses play a crucial role to identify subgroups of AML with different outcomes.[3] Meanwhile, identifying molecular genetic markers also helps to divide AML patients into different groups and refine their prognosis.[3]

Correspondence: Zhao-Qun Deng
Laboratory Center, The Affiliated People's Hospital of Jiangsu University, 8 Dianli Road, Zhenjiang, Jiangsu 212002, People's Republic of China
Fax +86 51185234387
Email zqdeng2002@163.com

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In recent years, non-coding RNAs have increasingly caught researchers' attention. A wide variety of studies have shown that non-coding RNAs participate in the process of controlling cell differentiation through regulating expression of the gene.[4]

Circular RNAs (circRNAs) are an emerging class of non-coding RNAs and are characterized by having covalent binding between the 3′ and 5′ ends which are generated by the mechanism of reverse splicing.[5] Due to the conserved characteristic across species and tissue, circRNAs have been found to be ideal diagnostic and prognostic biomarkers for disease, especially cancer.[6] For example, according to Xia et al, their study indicated that high-expressed of *circ_0067934* in esophageal cancer was related with poor proliferation. Up-regulated expression of *circ_0067934* was an unfavorable factor for esophageal squamous cell carcinoma.[7] Shao et al revealed that *circ_0014717* expression significantly decreased in gastric carcinoma. The level of its expression was related to tumor staging and distal metastasis. Due to the stable expression of *circ_0014717*, it had been regarded as an ideal biomarker for clinical detection of gastric cancer.[8]

Moreover, circular RNAs, which have also been named as competing endogenous RNAs (ceRNAs), could regulate cell biological function through acting as miRNA sponges.[5] Actually, circRNAs play an essential regulatory role in diseases through interacting with disease-related miRNAs.[9] For example, Weng et al illustrated that over-expressed *ciRs-7* acted as miRNA sponge to abolish the tumor suppressive effect of *miR-7* and promoted tumorgenesis in colorectal cancer.[10] *Circ_FBLIM1* had been found to serve as ceRNA and regulate the process of hepatocellular cancer through binding with *miR-346*. This process promoted the progression of hepatocellular cancer.[11] But there are few studies focused on the diagnostic and prognostic value of circular RNAs or their function acting as ceRNA in malignant hematonosis.

Phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*) serves as a classic tumor suppressor.[12] It mainly participates the homeostasis of the phosphatidylinositol 3 kinase (*PI3K*)*/AKT* pathway.[12] And losing the suppressive function of *PTEN* plays an essential role in the occurrence of cancer. *PTEN* has been found to be down-expressed in several solid cancers, like prostate cancer and breast cancer.[13,14] Furthermore, some research illustrated that the expression of *PTEN* transcript was remarkably lower in AML than controls and inactivation of *PTEN* promoted AML progression.[15,16]

To our knowledge, *PTEN*-dependent circular RNAs have seldom been studied in cancer, let alone AML. *Circ_0002232* is one of circRNAs of *PTEN*. The purpose of this research was to analyse *circ_0002232* expression in AML and to investigate its clinical relevance. We wanted to find whether it could serve as a biomarker for diagnosis and prognosis of AML and reveal the potential ceRNA network behind it.

Materials and Methods

Patients and Samples

A total of 163 samples, including 48 controls and 115 de novo AML patients, were provided by the Affiliated People's Hospital of Jiangsu University. This study was approved by Human Research Ethics Committee of the Affiliated People's Hospital of Jiangsu University and was conducted in accordance with the Declaration of Helsinki. Patients involved in this study were clearly diagnosed and classified according to guidelines of World Health Organization (WHO) and French-American-British (FAB) criteria.[17,18] To refine the group, the total 115 AML samples we used included 98 non-acute promyelocytic leukemia AML (non-APL AML) samples and 51 normal cytogenetic AML (CN-AML) samples. All of the enrolled patients have to meet the following conditions: 1) age ≥ 18 years old; 2) patients were diagnosed with AML for the first time; 3) patients had not received any form of treatment; and 4) patients had complete pathological data in our hospital. 5) participatants signed informed consent and patients who failed to meet above criteria were excluded from the study. Bone marrow (BM) specimen was collected after every participant signed informed consent. Extraction of bone marrow mononuclear cells (BMNCs) was conducted by using Lymphocyte Separation Medium (TBD sciences corporation, Tianjin, China). The vital clinical and laboratory features of these patients are listed in Table 1 and demographic data of controls are shown in Supplemental File 2

RNA Isolation and Reverse Transcription

The process of isolating total RNA from BMNCs was conducted by using Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription mixture contains 2μg of total RNA from each sample, 10mM of dNTPs, 10μM of random hexamers, 80U of

Table 1 Comparison of clinical and laboratory characteristics between AML patients with low and high *circ_0002232* expression

Patient's Parameters	Low (n=87)	High (n=28)	*P* value
Sex, male/female	58/29	15/13	0.261
Median age, years (range)	54 (21–81)	64 (20–88)	0.004*
Median WBC, $\times 10^9$/L (range)	14.9 (0.3–528.0)	34.50 (1.1–207.5)	0.041*
Median hemoglobin, g/L (range)	78 (34–144)	82 (42–119)	0.618
Median platelets, $\times 10^9$/L (range)	34 (3–415)	52 (9–382)	0.021*
BM blasts, % (range)	48.50 (1.00–109.00)	30.00 (6.50–81.00)	0.566
CR(+/-)	38/37	8/15	0.235
FAB			0.036*
M0	0 (0%)	0 (0%)	
M1	4 (4.9%)	0 (0%)	
M2	39 (48.1%)	6 (26.1%)	
M3	14 (17.3%)	2 (8.7%)	
M4	17 (21%)	9 (39.1%)	
M5	7 (8.6%)	6 (26.1%)	
M6	0 (0%)	0 (0%)	
Cytogenetic abnormalities' classification			0.279
Favorable	24 (27.6%)	3 (11.1%)	
Intermediate	52 (59.8%)	20 (74.1%)	
Poor	9 (10.3%)	3 (11.1%)	
No data	2 (2.3%)	1 (3.7%)	
Cytogenetic abnormalities			0.350
Normal	40 (46%)	12 (44.4%)	
t (8;21)	9 (10.3%)	1 (3.7%)	
t (15;17)	14 (16.1%)	2 (7.4%)	
+8	2 (2.3%)	3 (11.1%)	
Complex	8 (9.2%)	3 (11.1%)	
Others	11 (12.6%)	5 (18.5%)	
No data	2 (2.3%)	1 (3.7%)	
Gene mutation			
CEBPA (+/-)	10/64	0/18	0.201
NPM1(+/-)	7/67	0/18	0.338
FLT3-ITD (+/-)	11/63	1/17	0.448
C-KIT (+/-)	4/70	1/17	1.000
N/K-RAS(+/-)	3/60	2/11	0.200
IDH1/2 (+/-)	0/74	1/17	0.196
DNMT3A (+/-)	5/69	1/17	1.000
U2AF1 (+/-)	1/73	1/17	0.355
SRSF2(+/-)	1/62	0/13	1.000

Notes: Prognostic group standard according to chromosomal abnormalities: Karyotype associated with favorable prognosis include t(8;21)(q22; q22), inv(16)(p13q22)/t(16;16)(p13;q22); Karyotype associated with intermediate prognosis include t(15;17)(q22;q12), normal cytogenesis, +8; Karyotype associated with adverse prognosis include complex karyotype consisting of ≥3 abnormalities, t(6;9)(p23;q34), abnormal 11q23 excluded t(9:11), del(5q), −5, del(7q),-7, t(9:22). *Indicated statistical significance (P<0.05).

Abbreviations: WBC, white blood cell; BM blast, bone marrow blast; FAB, French-American-British criteria.

RNase inhibitor, and 200U of reverse transcriptase (Thermo Fisher Scientific, Waltham, MA, USA). The reverse transcript system was incubated at 25°C for 10 min, at 42°C for 60 min and stored at −20°C.

Real-Time Quantitative PCR

The expression of *circ_0002232, miR-92a-3p* and *PTEN* was detected by real-time quantitative PCR (RQ-PCR) with specific primers listed in supplemental File

1. The PCR reaction systems of detecting *circ_0002232* and *PTEN* were SYBR Premix Ex Taq II (TaKaRa, Japan) and the reaction system of detecting the expression of *miR-92a-3p* was miScript SYBR green PCR kit (Qiagen, Duesseldorf, Germany). 7500 Thermocycler (Thermo Fisher Scientific, Waltham, MA, USA) was used to perform reaction system. A housekeeping gene (*ABL*) was used to calculate the quantity of *circ_0002232* and *PTEN*. And the quantity of *miR-92a-3p* was valued by *U6*. Relative expression level of *circ_0002232, miR-92a −3p* and *PTEN* were calculated by using $2^{-\Delta\Delta CT}$ formula.

Gene Mutation Detection

Mutations of gene *NPM1, N/K-RAS, DNMT3A, c-KIT, U2AF1, IDH1/2* and *SRSF2* were detected by High Resolution Melting analysis.[19–22] Direct DNA sequencing was used to detect mutations of gene *CEBPA* and *FLT3-ITD*.

Bioinformatics and Statistical Analysis

Micro RNAs which might bind with *circ_0002232* were predicted by circRNA-miRNA interaction prediction websites, miRanda (http://miranda.org.uk) and RNAhybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/submis

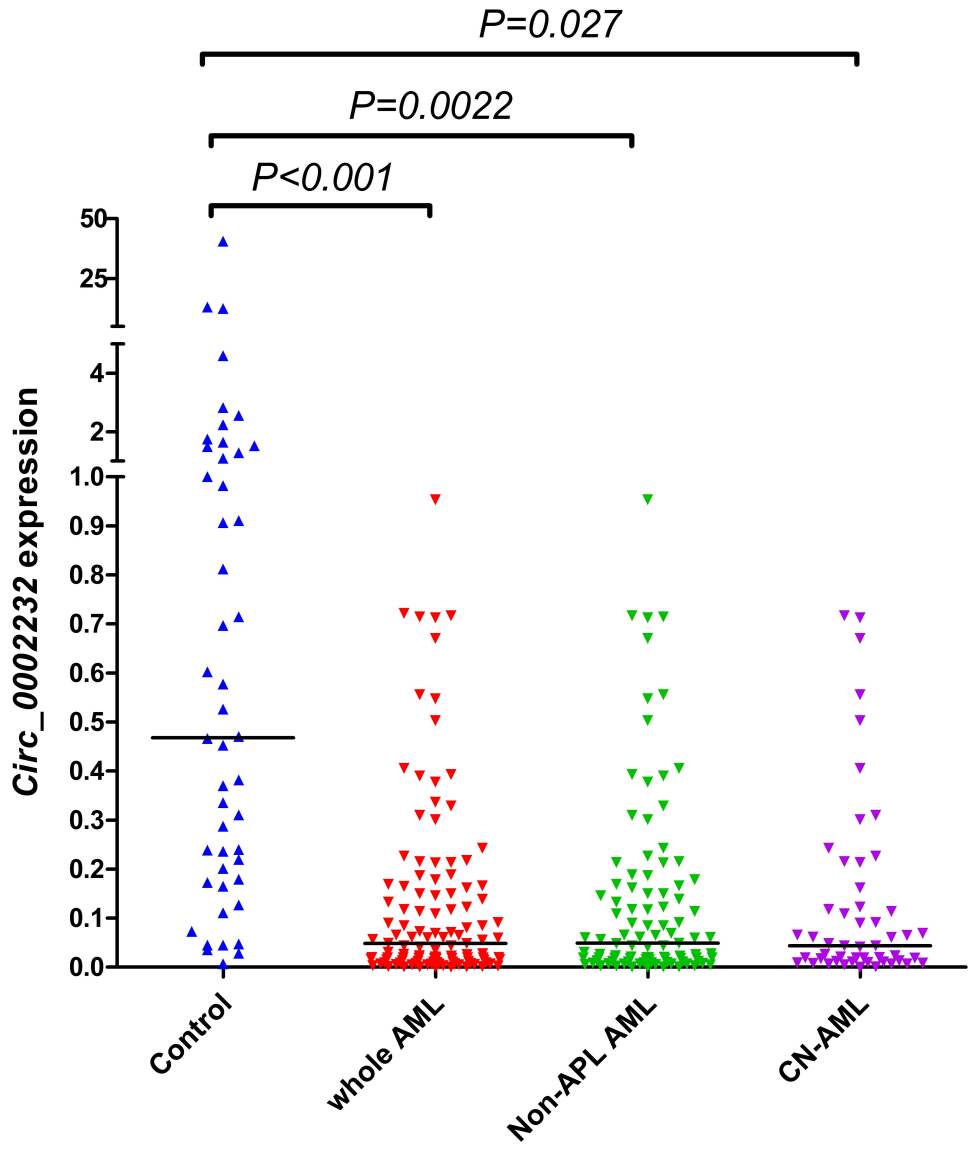

Figure 1 Relative expression level of *circ_0002232* in controls and AML. The expression of *circ_0002232* in controls, whole AML, non-APL AML and CN-AML patients was measured by using RQ-PCR. Each dot represents a single sample and horizontal line represents the median level of expression.

sion.html). Target genes of *miR-92a-3p* were predicted by miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/php/index.php), miRDB (http://www.mirdb.org) and TargetScan (http://www.targetscan.org/vert_72/).

Statistical analysis was conducted by using spss software version 22.0. The diagnostic value of *circ_0002232* expression was evaluated by receiver operating characteristic (ROC) curve and area under the ROC curve (AUC). The differences of categorical variables between the two groups were analysed by using Pearson Chi-square analysis or Fisher exact test and the differences of continuous variables were evaluated by using Mann–Whitney *U*-test. To explore prognostic potential of *circ_0002232*, Kaplan-Meier curves were used to analyse the impact of *circ_0002232* for overall survival (OS) and Cox regression analysis was used to assess its independent prognostic value. Spearman correlation analysis was used respectively to examine the correlationship among the expression of *circ_0002232, miR-92a-3p* and *PTEN*. *P* value less than or equal to 0.05 (two-sided) was considered statistically significant in all analyses.

Results

Circ_0002232 Expression in AML and Controls

In our experiment, the expression level of *circ_0002232* in de novo AML (median 0.0486, range 0.000215–0.953) was notably decreased compared with that in controls (median 0.468, range 0.00693–40.518*;* $P<0.001$, Figure 1). In addition, compared with controls, *circ_0002232* expression level was remarkably down-regulated in non-APL AML patients ($P=0.0022$, Figure 1) and was low-expressed in CN-AML patients ($P=0.027$, Figure 1).

Differentiating Ability of *Circ_0002232* Expression

The capacity of *circ_0002232* expression to distinguish AML patients from controls was analysed by ROC curve (AUC:0.851, 95% CI:0.788–0.915, $P<0.001$, Figure 2A). It indicated that *cric_00022232* could act as a significant marker in differentiating between AML patients and controls. In addition, the remarkable significance was found in distinguishing non-APL AML patients from controls (AUC:0.848, 95% CI:0.781–0.914, $P<0.001$, Figure 2B).

Clinical and Laboratory Characteristics of AML Patients

For the purpose of exploring the relationship between clinical parameters and *circ_0002232* expression, we obtained the cut-off value, which had the maximum sum of sensitivity and specificity according to ROC curve analysis, and divided 115 AML patients into low-expressed group (*circ_0002232*^{low}) and high-expressed group (*circ_0002232*^{high}). Hence, we used 0.165 as the cut-off value, whose sensitivity was 0.813 and specificity was 0.757. There were no significant discrepancies between the two groups in sex, hemoglobin, BM blasts, complete remission (CR), cytogenetic abnormalities and nine gene mutations ($P>0.05$, Table 1).

However, remarkable differences were observed in FAB classifications ($P=0.036$), white blood cell (WBC) count ($P=0.041$) and platelet count ($P=0.021$) between *circ_0002232*^{low} and *circ_0002232*^{high} groups. Age of the

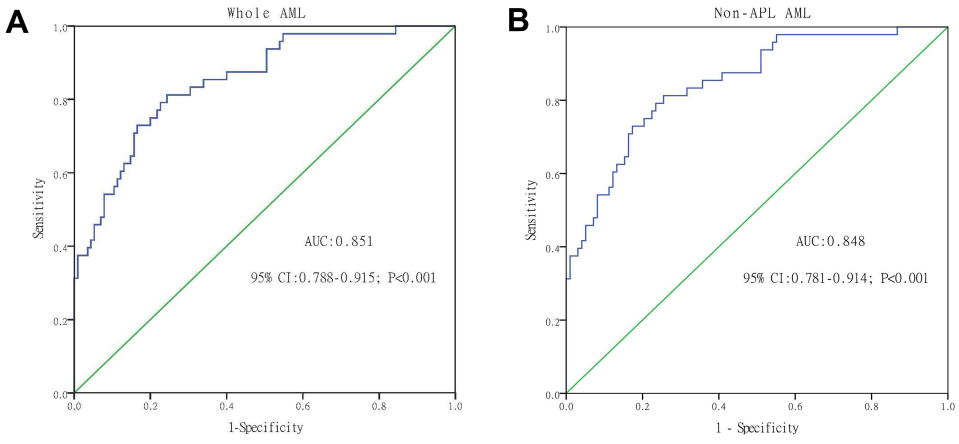

Figure 2 ROC curve analysis of *circ_0002232* for distinguishing AML patients from controls: (**A**) Whole AML; and (**B**) non-APL AML.

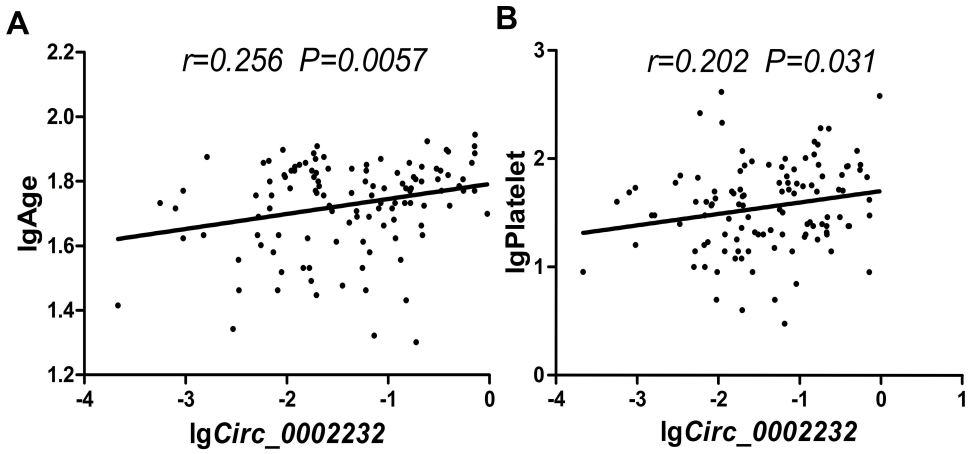

Figure 3 Pearson correlation analysis: (**A**) correlationship between patients' age and *circ_0002232* expression in AML; and (**B**) correlationship between platelet count and *circ_0002232* expression in AML.

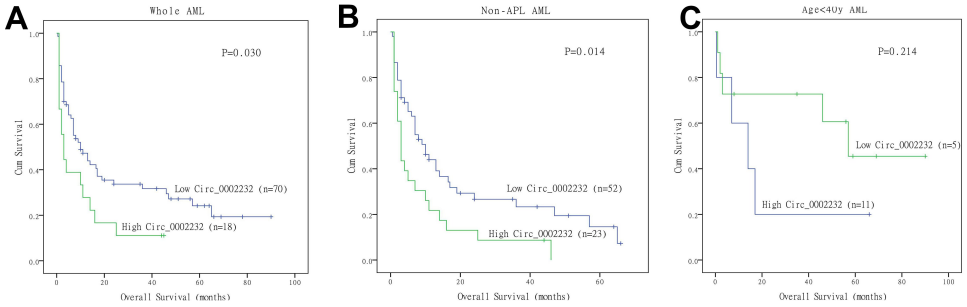

Figure 4 Kaplan-Meier analysis showed the differences in overall survival between *circ_0002232*^{low} and *circ_0002232*^{high} group: (**A**) overall survival among whole AML; (**B**) overall survival among non-APL AML; and (**C**) overall survival among AML (age<40y).

patients in *circ_0002232*^{low} group was notably younger than those in *circ_0002232*^{high} group (P=0.004). Moreover, we found that there was a positive correlation between *circ_0002232* expression and patients' age (Pearson r=0.256, P=0.0057, Figure 3A). And a positive correlation between the expression of *circ_0002232* and platelet count was also found (Pearson r=0.202, P=0.031, Figure 3B).

Correlation Between *Circ_0002232* Expression and Patients' Clinical Outcome

Survival analysis included 88 AML patients and excluded 27 patients who failed to follow up. Median follow-up time of included patients was 8 months, which ranged from 1 month to 90 months. According to Kaplan-Meier analysis, *circ_0002232*^{low} group had significantly longer OS compared with *circ_0002232*^{high} group in whole AML (P=0.030, Figure 4A). In non-APL AML, patients in low-expressed *circ_0002232* group tended to have better prognosis (P=0.014, Figure 4B). However, in low age group (age<40y), we found that patients with high *circ_0002232* expression tended to have better OS, but it was not statistically significant (P=0.214, Figure 4C).

Univariate analysis, including age (≤60y or >60y), WBC count (≥30×10^9/L or <30×10^9/L), cytogenetic abnormalities' classification, *circ_0002232* expression, *RAS* mutation, *U2AF1* mutation with P<0.05, showed that expression of *circ_0002232* could be used as a valuable factor for AML patients' prognosis. However, according to multivariate analysis, expression of *circ_0002232* could not act as an independent factor for OS (P=0.727) among AML patients (Table 2).

Correlation Between Expression of *Circ_0002232* and *PTEN* in AML

The expression of *PTEN* in AML (median 1.984, range 0.00701–88.0896) was remarkably down-regulated

Table 2 Univariate and multivariate analyses of prognostic variables for overall survival in whole AML patients

Variables	Overall Survival			
	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age	2.532 (1.534–4.179)	<0.001	1.310 (0.731–2.346)	0.364
WBC	3.021 (1.829–4.990)	<0.001	2.277 (1.302–3.981)	0.004*
Cytogenetic abnormalities' classifications	2.076 (1.503–2.867)	<0.001	1.962 (1.306–2.948)	0.001*
Circ_0002232 expression	1.817 (1.024–3.223)	0.041	0.874 (0.409–1.866)	0.727
FLT3-ITD mutation	0.881 (0.397–1.954)	0.755	–	–
NPM1 mutation	1.697 (0.672–4.287)	0.263	–	–
CEBPA mutation	0.891 (0.380–2.093)	0.791	–	–
c-KIT mutation	0.586 (0.142–2.416)	0.460	–	–
N/K-RAS mutation	2.753 (1.071–7.081)	0.036	3.000 (1.155–7.795)	0.024*
IDH1/2 mutation	5.328 (0.697–40.737)	0.107	–	–
DNMT3A mutation	1.644 (0.652–4.147)	0.292	–	–
U2AF1 mutation	4.609 (1.073–19.801)	0.040	1.593 (0.202–12.584)	0.659
SRSF-2 mutation	2.610 (0.353–19.303)	0.347	–	–

Notes: Prognostic variables included WBC (≥30×10^9 vs <30×10^9/L), patients' age (≤60 vs >60 years), cytogenetic abnormalities' classifications (favorable vs intermediate vs poor), *circ_0002232* expression level (Low vs High), and gene mutations (mutant vs wild-type). Variables with *P*<0.05 in univariate analysis were included into multivariate analysis. *Indicated statistical significance (P<0.05).

Abbreviations: HR, hazard ratio; CI, confidence interval; WBC, white blood cell.

compared with controls (median 3.330, range 0.842–103.788) (*P*=0.0057, Figure 5A). Furthermore, the expression of *circ_0002232* was positively correlated with its parental gene *PTEN* (Spearman r=0.678, *P*<0.001, Figure 5B).

Potential Interaction Network of *Circ_0002232/miR-92a-3p/PTEN*

CircRNA-miRNA interaction prediction websites were used to predict miRNAs which might bind with *circ_0002232*. Through searching literature, we finally choose *miR-92a-3p* (Figure 6A and B). The expression of *miR-92a-3p* was detected in controls and AML patients. *MiR-92a-3p* was notably up-expressed in AML (median 6.215, range 0.0610–218.199) compared with controls (median 0.472, range 0.00815–2.964) (*P*=0.0087, Figure 6E). Spearman correlation analysis revealed that *circ_0002232* expression was negatively correlated with *miR-92a-3p* expression in AML (Spearman r=−0.301, *P*=0.016, Figure 6F). Moreover, prediction websites

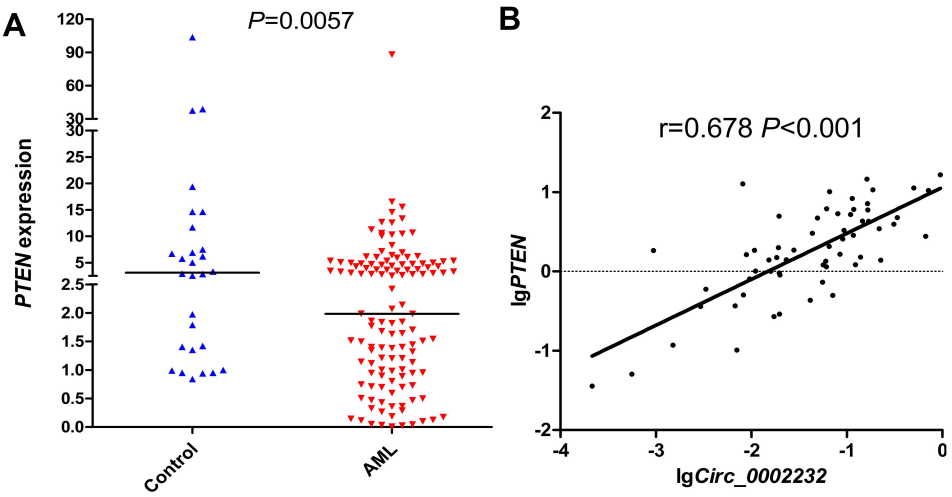

Figure 5 (**A**) Relative expression level of *PTEN* in controls and whole AML. (**B**) Spearman correlation analysis between the expression of *PTEN* and *circ_0002232* in AML.

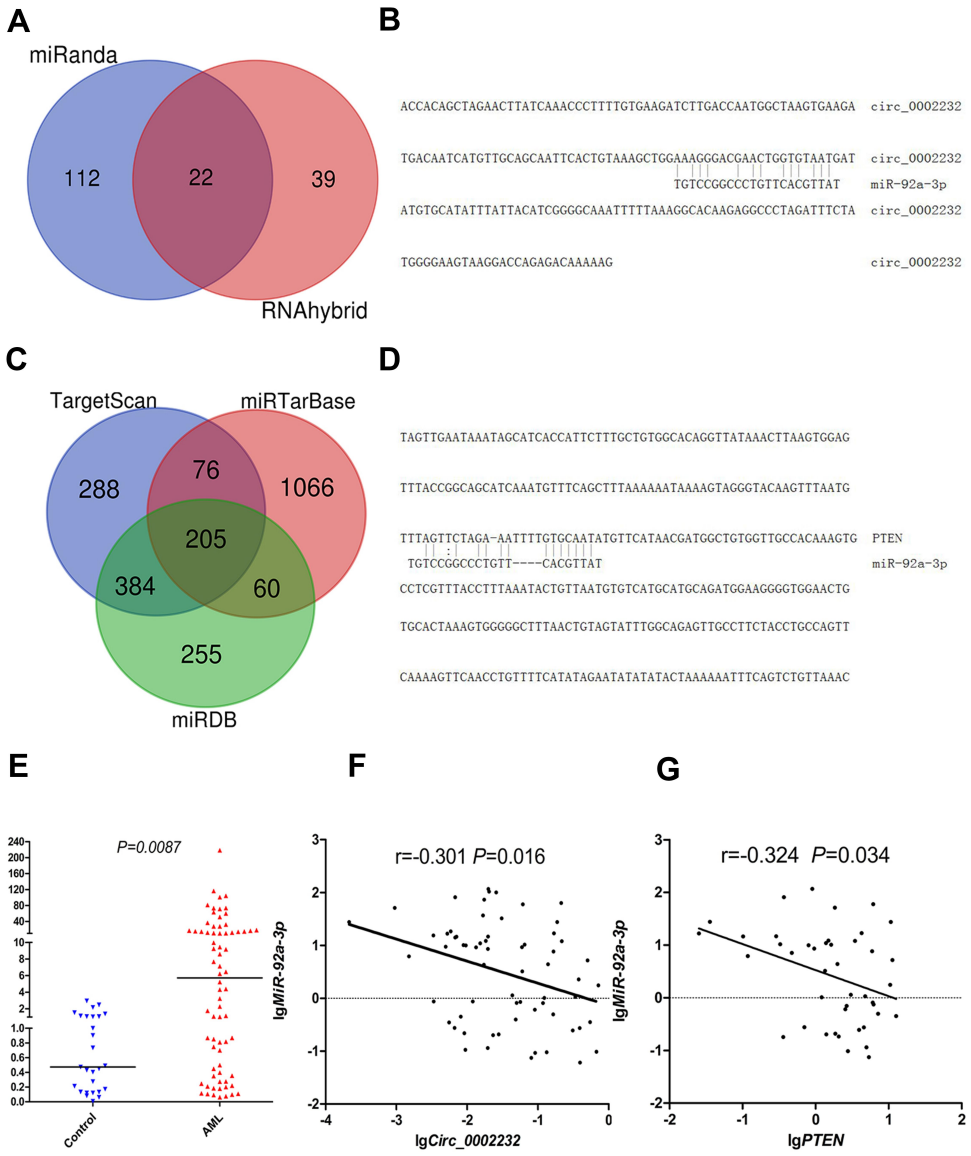

Figure 6 (**A**) Venn results of microRNAs which could bind with *circ_0002232* predicted by miRanda and RNAhybrid. (**B**) Binding sites between *circ_0002232* and *miR-92a-3p* predicted by miRanda. (**C**) Venn results of genes which could bind with *miR-92a-3p* predicted by TargetScan, miRTarBase and miRDB. (**D**) Binding sites between *miR-92a-3p* and *PTEN* predicted by miRTarBase. (**E**) Relative expression level of *miR-92a-3p* in controls and AML. (**F**) Spearman correlation analysis between the expression of *circ_0002232* and *miR-92a-3p* in AML. (**G**) Spearman correlation analysis between the expression of *miR-92a-3p* and *PTEN* in AML.

showed the potential binding sites between *miR-92a-3p* and *PTEN* (Figure 6C and D). According to the result of Spearman analysis, *miR-92a-3p* had negative correlation with *PTEN* (Spearman r=−0.324, *P*=0.034, Figure 6G).

Discussion

CircRNAs, known as a novel category of non-coding RNAs, exist widely in mammalian cells.[23] They have been considered as ideal biomarkers for disease because of their conservative feature across species. There are a few studies concentrated on the role of circRNAs in hematological malignancies. For instance, *circ_0004277* expression had been reported to be down-regulated in AML. And the expression of *circ_0004277* tended to be up-regulated when the patients had complete remission and down-regulated again when they relapsed. *Circ_0004277* expression changed dynamically with process of AML, which proved that it could be used as AML biological marker.[24]

As far as we know, this is the first report focused on the expression of the circular RNA transcribed from *PTEN* in AML. In this study, *circ_0002232* expression in AML was

notably down-regulated compared with that in controls. The same results were found in groups of non-APL AML and CN-AML. According to ROC curve analysis, *circ_0002232* could act as a valuable marker to identify AML patients and control groups.

Identifying the relationship between the expression of *circ_0002232* and clinical characteristics, we found that the expression level of *circ_0002232* was positively correlated with platelet count. *Circ_0002232*^{low} group tended to have lower platelet count. There have already been several reports focused on the abnormal platelet count and dysfunction in AML.[25] Low platelet count was associated with poor prognosis and recovery of platelet was concerned with relapse-free survival rate after chemotherapy in AML.[26,27] Moreover, *circ_0002232*^{low} group also tended to have lower hemoglobin and higher percentage of blast compared with *circ_0002232* high expression group. This means *circ_0002232*^{low} group have more severe myelosuppression and more serious infiltration in BM. Hence, low expression of *circ_0002232* is an adverse factor of AML.

Because *PTEN*, parental gene of *circ_0002232*, plays a role of tumor suppressor in many diseases, at the beginning of the experiment we proposed that the circular RNA transcribed from *PTEN* might also have tumor suppressive effect and down-regulation of *circ_0002232* might lead to shorter overall survival time. Unexpectedly, results of Kaplan-Meier analysis revealed that OS of patients with low-expressed *circ_00002232* were longer than that of patients with high-expressed *circ_00002232* in whole AML. This result was obviously in contrast with our initial prospect.

However, our study indicated that patients in *circ_0002232*^{high} group were significantly older than those in *circ_0002232*^{low} group. Pearson analysis was used to confirm this result, which revealed that the *circ_0002232* expression was positively correlated with patients' age. Age is an important risk factor for AML. Survival time of AML patients tends to decrease with increased age.[28,29] We suppose that it may help us to understand this conflicting result. High-expressed *circ_0002232* group tends to have poor prognosis because of the increase of age.

Then according to the expression level of *circ_0002232*, we divided the patients (age<40y) into two groups and compared the differences in survival time. The result in the subgroup under 40 showed that *circ_0002232*^{high} group tended to have better OS compared with *circ_0002232*^{low} group. This result confirmed our conjecture. But due to the limitation of our experiment size, this result was not statistically significant. In the future, additional experiments are needed to enlarge sample size and identify the relationship between *circ_0002232* expression and OS in different age subgroups.

Moreover, the phenomenon of circRNAs acting as miRNA sponges in regulating proliferation, metastasis and relapse of gastrointestinal cancer have been reported in some studies.[5] In this research, prediction websites revealed that there were potential binding sites among *circ_0002232, miR-92a-3p* and *PTEN*. *MiR-92a-3p* expression has been revealed to be up-regulated in several solid cancers including breast cancer and brain glioma.[30,31] According to our experiment, *miR-92a-3p* expression of AML patients was obviously up-regulated compared with controls and was negatively correlated with the expression of *circ_0002232*.

Furthermore, high-expressed *miR-92a* have been found to regulate colorectal cell migration and invasion by suppressing the expression of *PTEN*.[32] Alteration of *miR-92a* also promoted its effect on metastatic behavior of nasopharyngeal carcinoma cell by targeting *PTEN*.[33] Notably, we found that the expression level of *miR-92a-3p* was also negatively correlated with *PTEN* in AML. Hence, we proposed that *circ_0002232* might affect the process of AML and the expression of *PTEN* through sponging *miR-92a-3p*. In the future, we plan to design more experiments to explore the mechanism of this pathway in AML.

Conclusion

Our experiment revealed *circ_0002232*, one of circRNAs of *PTEN*, was remarkably down-regulated in AML and could act as a promising biomarker for the diagnosis of AML. In addition, there might be a potential ceRNA interaction network of *circ_0002232/miR-92a-3p/PTEN* in AML.

Abbreviations

PTEN, phosphatase and tensin homolog; circRNAs, circular RNAs; ceRNA, competing endogenous RNAs; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; CN-AML, cytogenetic normal AML; RQ-PCR, real-time quantitative PCR; BM, bone marrow; BMNCs, BM mononuclear cells; FAB classification, French–American–British classification; WHO criteria, World Health Organization criteria; ROC, receiver operating characteristic; AUC, area under the ROC curve; CI, confidence

interval; OS, overall survival; CR, complete remission; WBC, white blood cell; Lg, logarithm based on 10.

Data Sharing Statement

The datasets used during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consents

This study was approved by Human Research Ethics Committee of the Affiliated People's Hospital of Jiangsu University and was conducted in accordance with the Declaration of Helsinki. All patients signed informed consent to participate in our research.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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