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

CURRENT STATUS: UNDER REVIEW

Zhao-qun Deng
Affiliated People's hospital of Jiangsu University

zqdeng2002@163.com
Corresponding Author
ORCID: https://orcid.org/0000-0003-0037-4697

Xiao-yu Su
The affiliated People's Hospital of Jiangsu University

Xin Zhu
Affiliated people's hospital of Jiangsu university

Qian Zhao
Affiliated people's hospital of Jiangsu university

Jin-ming Ke
Zhejiang A&F university

De-long Wu
Affiliated people's hospital of Jiangsu university

Yun-yun Yi
Affiliated people's hospital of Jiangsu university

Jing Yi
Affiliated people's hospital of Jiangsu university

Jiang Lin
affiliated people's hospital of Jiangsu university

Jun Qian
Affiliated people's hospital of Jiangsu university

10.21203/rs.3.rs-25574/v1
SUBJECT AREAS
  Cancer Biology  Oncology

KEYWORDS
  circular RNAs, circ_0002232, miR-92a-3p, PTEN, acute myeloid leukemia
Abstract

**Background:** PTEN, known as a classical tumor suppressor, has been reported to be down-expressed in acute myeloid leukemia (AML) and affected the progression of AML patients. Our research was aimed to investigate the expression level of circ_0002232, one of circular RNAs of PTEN, reveal the clinical significance and potential ceRNA interaction network in AML of it.

**Methods:** Circ_0002232 expression in 117 AML patients and 48 controls was detected by using Real-time quantitative PCR.

**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 = 0.002), FAB classifications (P = 0.025), white blood cell count (P = 0.034) and platelet count (P = 0.047) between low-expressed circ_0002232 group and high-expressed circ_00022332 group. Moreover, there was a positive correlation between circ_0002232 expression and patients’ age (Pearson r = 0.256, P = 0.0053). Interestingly, we found that patients in low-expressed circ_0002232 group had better overall survival both in whole AML (P = 0.019) and non-APL AML (P = 0.044). Remarkably, the expression of circ_0002232 was positively correlated with PTEN (Pearson r = 0.769, P < 0.001). Furthermore, there was a negative correlation in AML between circ_0002232 and miR-92a-3p (Pearson r=-0.262, P = 0.032), miR-92a-3p and PTEN (Pearson r=-0.358, P = 0.019). Interaction prediction websites revealed that circ_0002232 might regulate the expression of PTEN through sponging miR-92a-3p and affect the process of AML.

**Conclusions:** 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.

1. 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 poor prognosis in the past\(^2\). Cytogenetics analyses
play a crucial role to identify subgroups of AML with different outcomes\textsuperscript{3}. Meanwhile, identifying molecular genetic markers also help to divide AML patients into different groups and refine their prognosis\textsuperscript{3}.

In recent years, non-coding RNAs have increasingly caught researchers’ attention. A wide variety of studies have showed that non-coding RNAs participate the process of controlling cell differentiation through regulating expression of the gene\textsuperscript{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\textsuperscript{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\textsuperscript{6}. For example, according to Xia et al., their study indicated that high-expressed of \textit{circ_0067934} in esophageal cancer was related with poor proliferation. Up-regulated expression of \textit{circ_0067934} was an unfavorable factor for esophageal squamous cell carcinoma\textsuperscript{7}. Shao et al. revealed that \textit{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 \textit{circ_0014717}, it had been regarded as ideal biomarker for clinical detection of gastric cancer\textsuperscript{8}.

Moreover, circular RNAs, which have also been named as competing endogenous RNAs (ceRNAs), could participate the process of regulating gene expression by acting as miRNA sponges\textsuperscript{5}. Actually, circRNAs play an essential regulatory role in diseases through interacting with disease-related miRNAs\textsuperscript{9}. For example, Weng W et al. illustrated that over-expressed \textit{ciRs-7} acted as miRNA sponge to abolish the tumor suppressive effect of \textit{miR-7} and promoted tumorigenesis in colorectal cancer\textsuperscript{10}. \textit{Circ_FBLIM1} had been found to function as ceRNA and regulate \textit{FBLIM1} expression through binding with \textit{miR-346}. This process promoted the progression of hepatocellular cancer\textsuperscript{11}. But there are few studies focused on the diagnostic and prognostic value of circular RNAs or their function acting as ceRNA in malignant hematosis.
Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) serves as a classic tumor suppressor\textsuperscript{12}. It mainly participates the homeostasis of the phosphatidylinositol 3 kinase (PI3K)/AKT pathway\textsuperscript{12}. And losing the suppressive function of PTEN plays an essential role in the occurrence of cancer. PTEN have been found to be down-expressed in several solid cancers, like prostate cancer and breast cancer\textsuperscript{13,14}. Furthermore, some researches illustrated that the expression of PTEN transcript was remarkably lower in AML than controls and inactivation of PTEN promoted AML progression\textsuperscript{15,16}.

To our knowledge, circular RNAs of PTEN have seldom been studied in cancer, let alone AML. Circ\textsubscript{0002232} is one of circRNAs of PTEN. The purpose of this research was to analyse circ\textsubscript{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.

2. Materials And Methods
2.1 Patients and samples
A total of 165 samples, including 48 controls and 117 de novo AML patients, were provided by the Affiliated People’s Hospital of Jiangsu University. Patients involved in this study were clearly diagnosed and classified according to guidelines of World Health Organization (WHO) and French-American-British (FAB) criteria\textsuperscript{17,18}. Bone marrow (BM) specimen was collected after every participator signed informed consent. Extraction of bone marrow mononuclear cells (BMNCs) were conducted by using Lymphocyte Separation Medium (TBD sciences corporation, Tianjin, China). The vital clinical and laboratory features of these patients were listed in Table 2.

2.2 RNA isolation and reverse transcription
The process of isolating total RNA from BMNCs was conducted by using Trizol reagent (Invitrogen, Carlsbad, USA). Reverse transcription mixture contains 2 µg of total RNA from each sample, 10 mM of dNTPs, 10 µM of random hexamers, 80U of RNase inhibitor, and 200U of reverse transcriptase (MBI Fermentas corporation, Hanover, USA). The reverse transcript system was incubated at 25°C for 10 min, at 42°C for 60 min and store at -20°C.

2.3 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 Table 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 (Applied Biosystems, CA, 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.

| Primers      | Sequence (5’ to 3’)          |
|--------------|------------------------------|
| circ-0002232 Forward | CTGAAAAGGACGACTGGAATGT      |
| circ-0002232 Reverse  | TCTCTGACTGATGCTGTCAT        |
| miR-92a-3p Forward    | TATTCACCCCTCCGGCCGCTGTCGTA |
| miR-92a-3p Reverse    | Manufacturer-provided miScript Universal primer |
| PTEN Forward       | ACCCACCACAGCTAGAACCCTTG     |
| PTEN Reverse       | CGCCTCTGACTGGAAATGT         |
| ABL Forward        | TCCTCCAGCTTATCTGGAAGA       |
| ABL Reverse        | TCAAACGACGGCTTACCA          |
| U6 Forward         | GTCGTCGCTTCGGCGCAACTATAC   |
| U6 Reverse         | AAAATATGGAAACGCTCAGCAATTGT |

### 2.4 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 were used to detect mutation of gene CEBPA and FLT3-ITD.

### 2.5 Bioinformatics and statistical analysis

Micro RNAs which might bind with circ_0002232 were predicted by circRNA-miRNA interation prediction websites, miRanda (http://miranda.org.uk) and RNAhybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/submission.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 were used to assess its independent prognostic value. Pearson correlation analysis was used respectively to examine the correlation relationship among the expression of circ_0002232, miR-92a-3p, platelet count and the patients’ age. P value less than or equal to 0.05 (two-sided) was considered statistically significant in all analyses.

3. Results
3.1 Circ_0002232 expression in AML and controls
In our experiment, the expression level of circ_0002232 in de novo AML (median 0.0492, range 0.000215-1.066) was notably decreased compared with that in controls (median 0.468, range 0.00693–40.518) (P < 0.001, Fig. 1). In addition, circ_0002232 expression level was remarkably down-regulated in non-acute promyelocytic leukemia AML (non-APL AML) patients (P = 0.0021, Fig. 1) and in normal karyotype AML (CN-AML) patients (P = 0.027, Fig. 1).

3.2 Differentiating ability of circ_0002232 expression
The capacity of circ_0002232 expression to distinguish AML patients from controls was analysed by ROC curve (AUC:0.846, 95% CI:0.782–0.910, P < 0.001, Fig. 2A). It indicated that circ_0002232 could act as a significant marker in differentiating between AML patients and controls. In addition, the remarkable significance was found in non-APL AML patients (AUC:0.841, 95% CI:0.774–0.909, P < 0.001, Fig. 2B).

3.3 Clinical and laboratory characteristics of AML patients
For the purpose of exploring the relationship between clinical parameters and circ_0002232 expression, 117 AML patients were divided into low-expressed group (circ_0002232low) and high-expressed group (circ_0002232high) by the cut-off value of 0.165 (Table 2). There were no significant discrepancies between the two groups in sex, hemoglobin, BM blasts, complete remission (CR), karyotypes and nine gene mutations (P > 0.05).
| Patient's parameters | Low (n = 88) | High (n = 29) | P value |
|----------------------|-------------|--------------|--------|
| Sex, male/female     | 59/29       | 15/14        | 0.183  |
| Median age, years (range) | 54(21–81)   | 64(20–88)    | 0.002* |
| Median WBC, ×10^9/L (range) | 14.25(0.3–528.0) | 35.35(1.1-207.5) | 0.034* |
| Median hemoglobin, g/L (range) | 78(34–144)  | 82(42–119)   | 0.578  |
| Median platelets, ×10^9/L (range) | 35.5(3-415) | 51.5(9-382)  | 0.047* |
| BM blasts, % (range)  | 47.75(1.00-109.00) | 30.50(6.50-92.00) | 0.776  |
| CR (+/-)              | 39/37       | 16/8         | 0.241  |
| FAB                   |             |              | 0.025* |
| M0                    | 0(0%)       | 1(4.2%)      |        |
| M1                    | 4(4.9%)     | 0(0%)        |        |
| M2                    | 39(47.6%)   | 6(25%)       |        |
| M3                    | 14(17.1%)   | 2(8.3%)      |        |
| M4                    | 17(20.7%)   | 9(37.5%)     |        |
| M5                    | 7(8.5%)     | 6(25%)       |        |
| M6                    | 1(1.2%)     | 0(0%)        |        |
| Karyotype classification |           |              | 0.286  |
| Favorable             | 24(27.3%)   | 4(13.8%)     |        |
| Intermediate          | 53(60.2%)   | 20(69.0%)    |        |
| Poor                  | 9(10.2%)    | 3(10.3%)     |        |
| No data               | 2(2.3%)     | 2(6.9%)      |        |
| Karyotype             |             |              | 0.289  |
| Normal                | 40(40.5%)   | 12(41.4%)    |        |
| t(8;21)               | 9(10.2%)    | 1(3.4%)      |        |
| t(15;17)              | 14(15.9%)   | 2(6.9%)      |        |
| +8                    | 2(2.3%)     | 3(10.3%)     |        |
| complex               | 8(9.1%)     | 3(10.3%)     |        |
| others                | 13(14.7%)   | 6(20.6%)     |        |
| No data               | 2(2.3%)     | 2(6.9%)      |        |
| Gene mutation         |             |              |        |
| CEBPA (+/-)           | 10/65       | 0/18         | 0.200  |
| NPM1 (+/-)            | 8/67        | 0/18         | 0.347  |
| FLT3-ITD (+/-)        | 11/64       | 1/17         | 0.450  |
| C-KIT(+/-)            | 4/71        | 1/17         | 0.533  |
| N/K-RAS (+/-)         | 3/61        | 2/11         | 0.196  |
| IDH1/2 (+/-)          | 0/75        | 1/17         | 0.194  |
| DNMT3A (+/-)          | 5/70        | 1/17         | 1.000  |
| U2AF1 (+/-)           | 1/74        | 1/17         | 0.351  |
| SRSF2(+/+)            | 1/63        | 0/13         | 1.000  |

WBC, white blood cell; BM blast, bone marrow blast; FAB, French-American-British criteria. *indicated statistical significance (P < 0.05).

Table 2

Comparison of clinical and laboratory characteristics between AML patients with low and high circ_0002232 expression.

However, remarkable differences were observed in FAB classifications (P = 0.025), white blood cell (WBC) count (P = 0.034) and platelet count (P = 0.047) between circ_0002232^low and circ_0002232^high groups. Age of the patients in circ_0002232^low group were notably younger than those in circ_0002232^high group (P = 0.002). Moreover, we found that there was a positive correlation between circ_0002232 expression and patients’ age (Pearson r = 0.256, P = 0.0053, Fig. 3A). The expression of circ_0002232 also had a weak correlation with platelet count (Pearson r = 0.176, P =
3.4 Correlation between circ_0002232 expression and patients’ clinical outcome

Survival analysis included 90 AML patients and excluded 27 patients who were failed to follow up.

Median follow-up time of included patients was 8 months, which range from 1 months to 90 months.

According to Kaplan-Meier analysis, circ_0002232<sub>low</sub> group had significantly longer OS (P = 0.019) compared with circ_0002232<sub>high</sub> group in whole AML (Fig. 4A). In non-APL AML, patients in low-expressed circ_0002232 group tended to have better prognosis (P = 0.044, Fig. 4B). However, in low age group (age < 40y), we found that patients with high circ_0002232 expression tended to have better OS (P = 0.287, Fig. 4C).

Univariate analysis, including age (≤ 60y or > 60y), WBC count (≥ 30 × 10<sup>9</sup>/L or < 30 × 10<sup>9</sup>/L), karyotype classification, circ_0002232 expression with P < 0.20, 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 = 0.609) among AML patients (Table 3).

| Variables                  | Overall survival |
|----------------------------|------------------|
|                            | Univariate analysis | Multivariate analysis |
|                            | HR (95% CI) | P value | HR (95% CI) | P value |
| Age                        | 2.544 (1.553–4.168) | < 0.001 | 1.242 (0.690–2.235) | 0.470 |
| WBC                        | 3.016 (1.840–4.943) | < 0.001 | 2.217 (1.274–3.858) | 0.005* |
| Karyotype classifications   | 2.026 (1.468–2.796) | < 0.001 | 1.975 (1.318–2.959) | 0.001* |
| Circ_0002232 expression     | 1.875 (1.071–3.280) | 0.028 | 0.815 (0.373–1.783) | 0.609 |
| FLT3-ITD mutation           | 0.876 (0.395–1.941) | 0.745 | - | - |
| NPM1 mutation               | 1.693 (0.720–3.979) | 0.228 | - | - |
| CEBPA mutation              | 0.885 (0.377–2.075) | 0.778 | - | - |
| c-KIT mutation              | 0.581 (0.141–2.391) | 0.452 | - | - |
| N/K-RAS mutation            | 2.752 (1.072–7.067) | 0.035 | 2.981 (1.150–7.730) | 0.025* |
| IDH1/2 mutation             | 5.405 (0.707–41.327) | 0.104 | 4.023 (0.512–31.599) | 0.186 |
| DNMT3A mutation             | 1.635 (0.649–4.122) | 0.297 | - | - |
| U2AF1 mutation              | 4.679 (1.089–20.102) | 0.038 | 1.774 (0.221–14.206) | 0.589 |
| SRSF-2 mutation             | 2.652 (0.359–19.616) | 0.339 | - | - |

HR, hazard ratio; CI, confidence interval; WBC, white blood cell. Prognostic variables included WBC (≥ 30 × 10<sup>9</sup>/L or < 30 × 10<sup>9</sup>/L), patients’ age (≤ 60 vs. >60 years), Karyotype classifications (favorable vs. intermediate vs. poor), circ_0002232 expression level (Low vs. High), and gene mutations (mutant vs. wild-type). Variables with P < 0.200 in univariate analysis were included into multivariate analysis.

*indicated statistical significance (P < 0.05).

Table 3

Univariate and multivariate analyses of prognostic variables for overall survival in whole AML patients...
3.5 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 compared with controls (median 3.330, range 0.842-103.788) \((P = 0.0057, \text{Fig. 5A})\). Furthermore, the expression of circ_0002232 was positively correlated with its parental gene PTEN (Pearson \(r = 0.769, P < 0.001\), \(\text{Fig. 5B})\).

3.6 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 (Fig. 6A, 6B). The expression of miR-92a-3p were 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) \((P = 0.0087, \text{Fig. 6E})\). Pearson correlation analysis revealed that circ_0002232 expression was negatively correlated with miR-92a-3p expression in AML (Pearson \(r = -0.262, P = 0.032\), \(\text{Fig. 6F})\). Moreover, prediction websites showed the potential binding sites between miR-92a-3p and PTEN (Fig. 6C, 6D). According to result of Pearson analysis, miR-92a-3p had negative correlation with PTEN (Pearson \(r = -0.358, P = 0.019\), \(\text{Fig. 6G})\).

4. 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 up-regulated when the patients got complete remission and down-regulated again when they got relapsed. Circ_0004277 expression changed dynamically with process of AML, which proved that it could be used as AML biological marker\(^24\).

According to what we know, this is the first report focused on the expression of circular RNA of 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 relation between the expression of circ_0002232 and clinical characteristic, 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 already have several reports focused on the abnormal platelet count and dysfunction in AML\textsuperscript{25}. Low platelet count was associated with poor prognosis and recovery of platelet was concerned with relapse-free survival rate after chemotherapy in AML\textsuperscript{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.

Unexpectedly, results of Kaplan-Meier analysis revealed that OS of patients with low-expressed circ_0002232 were longer than that of patients with high-expressed circ_0002232 in whole AML.

\textit{PTEN}, parental gene of circ_0002232, plays a role of tumor suppressor in many diseases. At the beginning of our experiment, we proposed that patients with low-expressed circ_0002232 might have shorter overall survival time, which was obviously contract with current results.

However, our study indicated that patients in circ_0002232 low group were significantly younger than those in circ_0002232 high group. In other words, old patients were liable to have high expression of circ_0002232. 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\textsuperscript{28,29}. We suppose that it may help us to understand this conflicting result. The correlation between age and circ_0002232 expression led to this reverse result.

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 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 wasn't statistically significant. In the
future, additional experiments are needed to enlarge sample size and identify the relationship between \textit{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\textsuperscript{5}. In this research, prediction websites revealed that there were potential binding sites among \textit{circ\_0002232}, \textit{miR-92a-3p}, and \textit{PTEN}. \textit{MiR-92a-3p} expression has been revealed to be up-regulated in several solid cancers including breast cancer and brain glioma\textsuperscript{30,31}. According to our experiment, \textit{miR-92a-3p} expression of AML patients was obviously up-regulated compared with controls and was negatively correlated with the expression of \textit{circ\_0002232}.

Furthermore, high-expressed \textit{miR-92a} have been found to regulate colorectal cell migration and invasion by reducing the expression level of \textit{PTEN}\textsuperscript{32}. Alteration of \textit{miR-92a} also promoted its effect on metastatic behavior of nasopharyngeal carcinoma cell by targeting \textit{PTEN}\textsuperscript{33}. Notably, we found that the expression level of \textit{miR-92a-3p} was also negatively correlated with \textit{PTEN} in AML. Hence, we proposed that \textit{circ\_0002232} might regulate \textit{PTEN} expression through sponging \textit{miR-92a-3p} and affect the process of AML. In the future, we plan to design more experiments like knock-out and over-expressed experiments to explore the mechanism of this pathway in AML.

Conclusion

Our experiment revealed \textit{circ\_0002232}, one of circRNAs of \textit{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 \textit{circ\_0002232/miR-92a-3p/PTEN} in AML.

Abbreviations

\textit{PTEN}: phosphatase and tensin homolog; \textit{circRNAs}: circular RNAs; \textit{ceRNA}: competing endogenous RNAs; \textit{AML}: acute myeloid leukemia; \textit{APL}: acute promyelocytic leukemia; \textit{CN-AML}: normal karyotype AML; \textit{RQ-PCR}: real-time quantitative PCR; \textit{BM}: bone marrow; \textit{BMNCs}: BM mononuclear cells; \textit{FAB classification}: French-American-British classification; \textit{WHO criteria}: World Health Organization criteria; \textit{ROC}: receiver operating characteristic; \textit{AUC}: area under the ROC curve; \textit{CI}: confidence interval; \textit{OS}: overall survival; \textit{CR}: complete remission; \textit{WBC}: white blood cell.
Declarations
Ethics approval and consent to participate
This study was approved by Human Research Ethics Committee of the Affiliated People’s Hospital of Jiangsu University. All patients signed informed consents to participate in our research.

Consent for publication
Not applicable.

Availability of data and material
The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

Funding
This study was supported by National Natural Science foundation of China (81970156, 81970118), Medical Innovation Team of Jiangsu Province (CXTDB2017002), Zhenjiang Clinical Research Center of Hematology (AA2018009), Jiangsu Provincial “innovative & entrepreneurial talent team” Program.

Authors’ contributions
XYS leaded whole process of experiments, analyzed data and written manuscript. XZ, QZ, JMK, DLW, YYY, and JY were contributed to collect patients’ data and revised the manuscript. JL and JQ were involved in acquiring data and providing useful suggestion during the experiments. ZQD mainly took charge for experiments design and the manuscript revision. All authors read and approved the final manuscript.

Acknowledgements
Not applicable.

References
1. Short NJ, Rytting ME, Cortes JE. Acute myeloid leukaemia. Lancet. 2018;392(10147):593–606. doi: 10.1016/S0140-6736(18)31041-9.

2. Medinger M, Passweg JR. Acute myeloid leukaemia genomics. Br J Haematol. 2017;179(4):530–42. doi:10.1111/bjh.14823.

3. Valk PJ, Verhaak RG, Beijen MA, et al. Prognostically Useful Gene-Expression Profiles in Acute Myeloid Leukemia. N Engl J Med. 2004;350(16):1617–28.
10. Weng W, Wei Q, Toden S, Yoshida K, Nagasaka T, Fujiwara T, et al. Circular RNA ciRS-7-A Promising Prognostic Biomarker and a Potential Therapeutic Target in Colorectal Cancer. Clin Cancer Res. 2017;23(14):3918-28. doi:10.1158/1078-0432.CCR-16-2541.

11. Bai N, Peng E, Qiu X, Lyu N, Zhang ZJ, Tao YM, et al. circFBLIM1 act as a ceRNA to promote hepatocellular cancer progression by sponging miR-346. J Exp Clin Cancer Res. 2018;37(1):172. doi:10.1186/s13046-018-0838-8.

12. Chen L, Guo D. The functions of tumor suppressor PTEN in innate and adaptive immunity. Cell Mol Immunol. 2017;14(7):581-9. doi:10.1038/cmi.2017.30.

13. Wise HM, Hermida MA, Leslie NR. Prostate cancer, PI3K, PTEN and prognosis. Clin Sci
Comprehensive molecular portraits of human breast tumours. Nature. 2012;490(7418):61–70. doi: 10.1038/nature11412.

Li Y, Gao L, Luo X, Wang LL, Gao XN, Wang W, et al. Epigenetic silencing of microRNA-193a contributes to leukemogenesis in t(8;21) acute myeloid leukemia by activating the PTEN/PI3K signal pathway. Blood. 2013;121(3):499–509. doi:10.1182/blood-2012-07-444729.

Zayed R, Eltaweel M, Botros S, Zaki M. MN1 and PTEN Gene Expression in Acute Myeloid Leukemia. Cancer Biomark. 2017;18 (2):177–182. doi: 10.3233/CBM-160235.

Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med. 1985;103(4):620–5. doi:10.7326/0003-4819-103-4-620.

Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Beau M, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405. doi:10.1182/blood-2016-03-643544.

Lin J, Yao DM, Qian J, Chen Q, Qian W, Li Y, et al. Recurrent DNMT3A R882 mutations in Chinese patients with acute myeloid leukemia and myelodysplastic syndrome. PLoS One. 2011;6(10):e26906. doi:10.1371/journal.pone.0026906.

Lin J, Yao DM, Qian J, Chen Q, Qian W, Li Y, et al. IDH1 and IDH2 mutation analysis in Chinese patients with acute myeloid leukemia and myelodysplastic syndrome. Ann Hematol. 2012;91(4):519–25. doi:10.1007/s00277-011-1352-7.

Yang X, Qian J, Sun A, Lin J, Xiao GF, Yin J, et al. RAS mutation analysis in a large cohort of Chinese patients with acute myeloid leukemia. Clin Biochem. 2013;46(7-
22. Qian J, Yao DM, Lin J, Qian W, Wang CZ, Chai HY, et al. U2AF1 mutations in Chinese patients with acute myeloid leukemia and myelodysplastic syndrome. PLoS One. 2012;7(9):e45760. doi:10.1371/journal.pone.0045760.

23. Dong Y, He D, Peng Z, Peng W, Shi W, Wang J, et al. Circular RNAs in cancer: an emerging key player. J Hematol Oncol. 2017;10(1):2. doi:10.1186/s13045-016-0370-2.

24. Li W, Zhong C, Jiao J, Li P, Cui BX, Ji CY, et al. Characterization of hsa_circ_0004277 as a New Biomarker for Acute Myeloid Leukemia via Circular RNA Profile and Bioinformatics Analysis. Int J Mol Sci. 2017;18(3). doi:10.3390/ijms18030597.

25. Qian X, Wen-jun L. Platelet changes in acute leukemia. Cell Biochem Biophys. 2013;67:1473–9. doi:10.1007/s12013-013-9648-y.

26. Zhang QY, Dai KC, Bi LX, Jiang SF, Han YX, Yu K, et al. Pretreatment platelet count predicts survival outcome of patients with de novo non-M3 acute myeloid leukemia. PeerJ. 2017;5:e4139. doi:10.7717/peerj.4139.

27. Yamazaki E, Kanamori H, Itabashi M, Ogusa E, Numata A, Yamamoto W, et al. Hyper-recovery of platelets after induction therapy is a predictor of relapse free survival in acute myeloid leukemia. Leuk Lymphoma. 2017;58(1):104–9. doi:10.1080/10428194.2016.1190969.

28. Appelbaum FR, Gundacker H, Head DR, Slovak ML, Willman CL, Godwin JE, et al. Age and acute myeloid leukemia. Blood. 2006;107(9):3481–5. doi: 10.1182/ blood-2005-09-3724.

29. Abelson S, Collord G, Ng SWK, Weissbrod O, Cohen NM, Niemeyer E, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. Nature. 2018;559(7714):400-4. doi:10.1038/s41586-018-0317-6.
30. Cun J, Yang Q. Bioinformatics-based interaction analysis of miR-92a-3p and key genes in tamoxifen-resistant breast cancer cells. Biomed Pharmacother. 2018;107:117-28. doi:10.1016/j.biopha.2018.07.158.

31. Song H, Zhang Y, Liu N, Zhao S, Kong Y, Yuan LD, et al. miR-92a-3p Exerts Various Effects in Glioma and Glioma Stem-Like Cells Specifically Targeting CDH1/β-Catenin and Notch-1/Akt Signaling Pathways. Int J Mol Sci 2016;17(11). doi:10.3390/ijms17111799.

32. Zhang G, Zhou H, Xiao H, Liu ZL, Tian HP, Zhou T. MicroRNA-92a functions as an oncogene in colorectal cancer by targeting PTEN. Dig Dis Sci. 2014;59(1):98-107. doi:10.1007/s10620-013-2858-8.

33. Zhang H, Cao H, Xu D, Zhu K. MicroRNA-92a promotes metastasis of nasopharyngeal carcinoma by targeting the PTEN/AKT pathway. Onco Targets Ther. 2016;9:3579-88. doi:10.2147/OTT.S105470.

Figures
Figure 1

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

Figure 2

ROC curve analysis of circ_0002232 for distinguishing AML patients from controls: A: Whole AML; B: non-APL AML.

Figure 3

Pearson correlation analysis: A: correlation between patients’ age and circ_0002232 expression in AML; B: correlation between platelet count and circ_0002232 expression in AML.
Kaplan-Meier analysis showed the differences in overall survival between circ_0002232low and circ_0002232high group: A: overall survival among whole AML; B: overall survival among non-APL AML; C: overall survival among AML (age<40y).

A: Relative expression level of PTEN in controls and whole AML. B: Pearson correlation analysis between the expression of PTEN and circ_0002232 in AML.
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: Pearson correlation analysis between the expression of circ_0002232 and miR-92a-3p in AML. G: Pearson correlation analysis between the expression of miR-92a-3p and PTEN in AML.