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RESEARCH ARTICLE

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# Serum miR-22 is a novel prognostic marker for acute myeloid leukemia

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## Abstract

**Background:** It has been demonstrated that aberrant expression of serum microRNAs is potential markers for the prognostic prediction of acute myeloid leukemia (AML). However, the clinical significance of serum miR-22 remained uncovered. In this study, we aimed to explore the potential prognostic value of serum miR-22 for AML.

**Methods:** Blood samples were collected from 124 patients with AML and 60 healthy individuals. Serum miR-22 level was detected by quantitative reverse transcription-polymerase chain reaction (qRT-PCR), and its potential clinical value was investigated.

**Results:** Our results showed that serum miR-22 expression was significantly downregulated in AML subjects compared to healthy controls. Serum miR-22 levels were lowest in AML patients with M4/M5 subtypes, and low serum miR-22 expression occurred more frequently in AML patients with higher white blood cell counts or poor cytogenetic risk. Receiver operating characteristic (ROC) analysis revealed that serum miR-22 well differentiated AML cases from healthy controls. In addition, serum miR-22 downregulation was closely associated with worse clinical features and shorter survival. Low serum miR-22 expression was confirmed to be an independent predictor for overall survival and relapse-free survival in AML patients. Moreover, the expression level of serum miR-22 was dramatically increased following treatment. In addition, serum miR-22 levels were significantly higher in AML patients achieving complete remission (CR) than those without CR.

**Conclusion:** Collectively, serum miR-22 might serve as a novel and promising prognostic biomarker for AML.

KEYWORDS

acute myeloid leukemia, complete remission, prognosis, serum miR-22

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## 1 | INTRODUCTION

Acute myeloid leukemia (AML) is an aggressive hematopoietic stem cell malignancy that is characterized by the clonal proliferation of myeloid precursors.[1] Abnormal accumulation of leukemic blasts in the bone marrow, blood, and other tissues results in significant reductions of normal blood cells.[2] AML patients can be divided into 3 risk-based categories according to cytogenetic information: poor, intermediate, and favorable.[3] Though treatment of this disease has made huge progress over the past years, the 5-year overall survival rate of AML patients remains unfavorable, ranging from 11%-55%.[4,5] Therefore, identifying novel and reliable prognostic biomarkers for risk stratification of AML patients is critical for selecting the optimal therapeutic strategies and improving the clinical outcome.[6,7]

MicroRNAs (miRNAs) are small (19-25 nucleotide), noncoding RNAs that control the gene expression at the post-transcriptional level, leading to target mRNAs degradation or translational inhibition.[8,9] Increasing evidence has demonstrated that miRNA dysregulation is associated with the initiation and progression of cancer.[10] In addition, miRNAs are highly stable in the biofluids such as serum, plasma, and saliva.[11] These features of miRNAs enable them to become the promising biomarkers for the diagnosis and prognosis of cancer.

MiR-22, located in chromosome 17p13, has been found to act as a tumor suppressor in AML. For instance, Jiang et al showed miR-22 expression was significantly decreased in AML patients, and miR-22 upregulation markedly inhibited leukemic cell viability in vitro and suppressed leukemia progression in vivo.[12] Similarly, miR-22 was demonstrated to play a tumor suppressive role in AML development.[13] However, the clinical significance of serum miR-22 in AML remains uncertain. The aim of our study was to elucidate the potential prognostic value of serum miR-22 in AML.

**TABLE 1** The association between serum miR-22 and the clinicopathological parameters of AML

| Characteristics | Number | Serum miR-22 | | *P* |
|---|---|---|---|---|
| | | Low expression | High expression | |
| Age (years) | | | | |
| <60 | 83 | 35 (42.2%) | 48 (57.8%) | .227 |
| ≥60 | 41 | 22 (53.7%) | 19 (46.3%) | |
| Gender | | | | |
| Male | 75 | 33 (44.0%) | 42 (56.0%) | .587 |
| Female | 49 | 24 (48.9%) | 25 (51.1%) | |
| BM Blasts (%) | | | | |
| <50 | 56 | 22 (39.3%) | 34 (60.7%) | .176 |
| ≥50 | 68 | 35 (51.5%) | 33 (48.5%) | |
| WBC counts ($\times 10^9$/L) | | | | |
| <10 | 54 | 19 (35.2%) | 35 (64.8%) | .034 |
| ≥10 | 70 | 38 (54.3%) | 32 (45.7%) | |
| FAB subtype | | | | |
| M0 | 10 | 4 (40.0%) | 6 (60.0%) | .202 |
| M1/M2 | 72 | 29 (40.3%) | 43 (59.7%) | |
| M4/M5 | 42 | 24 (57.1%) | 18 (42.9%) | |
| Platelet counts ($\times 10^9$/L) | | | | |
| <50 | 63 | 24 (38.1%) | 39 (61.9%) | .074 |
| ≥50 | 61 | 33 (54.1%) | 28 (45.9%) | |
| Cytogenetics | | | | |
| Favorable | 49 | 15 (30.6%) | 34 (69.4%) | .008 |
| Intermediate | 58 | 30 (51.7%) | 28 (48.3%) | |
| Poor | 17 | 12 (70.6%) | 5 (29.4%) | |
| Complete Remission | | | | |
| Yes | 55 | 21 (38.2%) | 34 (61.8%) | .120 |
| No | 69 | 36 (52.2%) | 33 (47.8%) | |

## 2 | MATERIALS AND METHODS

### 2.1 | Ethics statement

The current study was approved by the Ethics Committee of Panyu Central Hospital, and written informed consent was obtained from all participants. All specimens were handled and made anonymous according to the ethical and legal standards.

### 2.2 | Patients and sample collection

A total of 124 patients with newly diagnosed AML and 60 healthy individuals as controls were enrolled in this study. The diagnosis and classification of AML patients was made according to French-America-British (FAB) and World Health Organization criteria. The patients' characteristics are summarized and presented in Table 1. The AML patients were stratified into three risk categories based on the cytogenetics (poor, intermediate, and favorable). The detailed information about the cytogenetic information of AML patients was shown in Supplementary Table S1. Complete remission (CR) was defined by <5% blast cells in the bone marrow and normalization of the peripheral blood counts at four weeks after starting induction therapy, as well as no residual evidence of extramedullary disease. Overall survival (OS) was defined as the time from the diagnosis to death due to any cause or the last follow-up. Relapse-free survival (RFS) was defined as the time from the achievement of CR to relapse or the last follow-up. No patient lost to follow-up, and the median follow-up time was 23 months (range 5.4-60 months). All the AML patients received similar chemotherapy treatment. The induction chemotherapy was administered to the AML patients based on their clinical status, and the details of the therapeutic protocols were referred to NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for AML. The expression level of serum miR-22 was detected in AML patients both before and after the treatment.

For the serum sample collection, fasting peripheral vein blood was obtained from 124 AML patients and 60 controls. The samples were centrifugated at 2500 g for 10 minutes, followed by centrifugation at 16 000 g for 10 minutes. The serum samples were stored at −80°C until further processing.

### 2.3 | Total RNA extraction and quantitative RT-PCR

Total RNA was isolated from serum with the miRNeasy Serum/Plasma kit (Qiagen) according to the manufacturer's instructions. The RNA concentration was measured with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Cel-miR-39 was used as the spiked-in control.[14,15] The cDNA was reverse-transcribed from total RNA using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The amplification of cDNAs was performed on the 7500 Real-Time PCR system (Applied Biosystems). The relative serum miR-22 expression levels were normalized to cel-miR-39 and calculated by the $2^{-\Delta\Delta Ct}$ method.

### 2.4 | Statistical analysis

Student's t test or one-way ANOVA was conducted to compare the difference in serum miR-22 concentrations between two or three groups, respectively. The chi-square test was used to analyze possible associations between serum miR-22 expression and clinical variables. Receiver operating characteristic (ROC) curve was performed, and area under the ROC curve (AUC) was used to assess the discriminative power of serum miR-22. The Kaplan-Meier method was performed to construct the survival curves of AML patients, and the difference in survival curves was compared by log-rank test. Multivariate Cox proportional hazards regression analysis was used to identify the independent prognostic factors. All analyses were performed with the GraphPad Prism 5 for Windows (GraphPad software, San Diego, CA, USA) and SPSS 16.0 software (SPSS Inc). *P* value <.05 was considered statistically significant.

## 3 | RESULTS

### 3.1 | Serum miR-22 was downregulated in AML patients

The serum miR-22 expression levels were measured in 124 AML subjects and 60 normal controls by qRT-PCR. As shown in Figure 1A, serum miR-22 levels were significantly lower in AML patients than in the healthy controls ($P < .0001$). In addition, high serum miR-22 expression occurred more frequently in AML patients with M1/M2 subtypes compared with those with M4/M5 subtypes ($P = .016$). Moreover, AML patients with white blood cells (WBC) $<10 \times 10^9$/L had

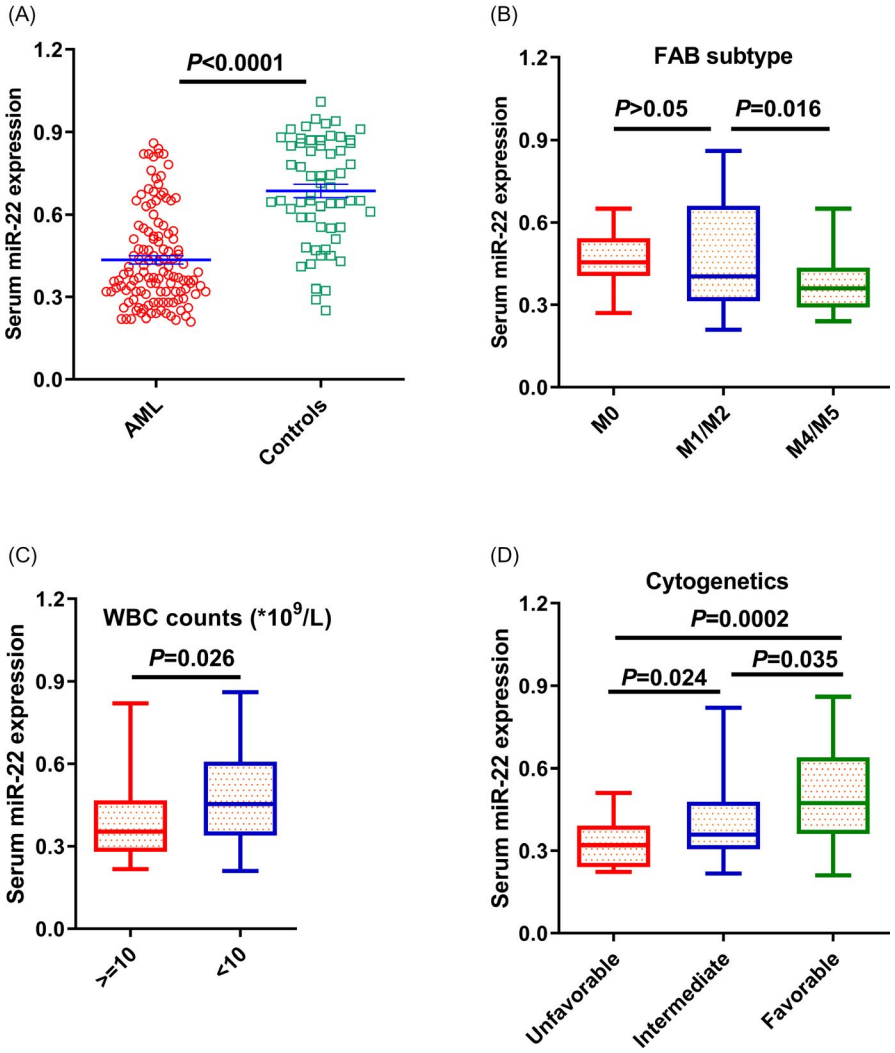


**FIGURE 1** Reduced serum miR-22 in AML patients. A, Serum miR-22 levels were significantly reduced in AML patients compared to controls. B, Serum miR-22 levels were significantly downregulated in M4/M5 subtypes compared to M1/M2 subtypes. C, Serum miR-22 levels were significantly downregulated in AML patients with higher WBC counts. D, Serum miR-22 levels were gradually downregulated in AML patients with favorable risk cytogenetic group, intermediate risk cytogenetic group, and unfavorable risk cytogenetic group

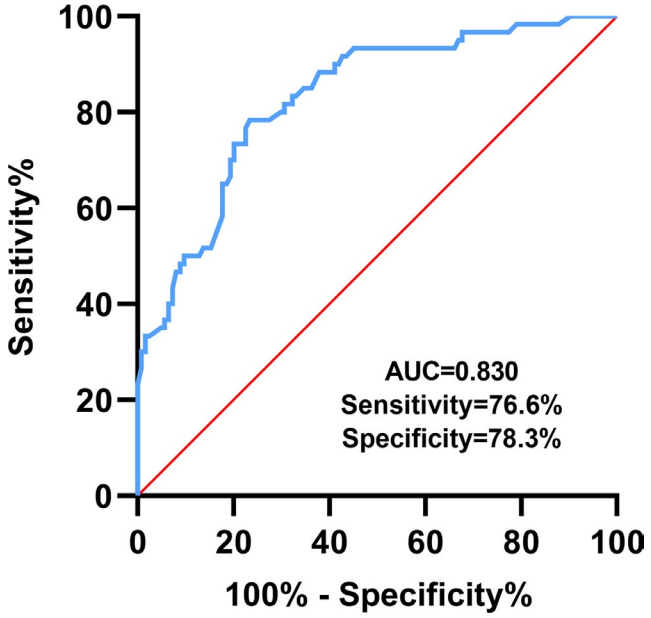


**FIGURE 2** Serum miR-22 exhibited good performance for discriminating AML from normal controls

**TABLE 2** Multivariate analysis of the independent prognostic factors for AML

| Characteristics | Risk Ratio | 95% CI | *P* |
|---|---|---|---|
| OS (all AML, N = 124) | | | |
| WBC counts (× $10^9$/L) | 2.46 | 1.40-3.68 | .020 |
| Cytogenetics | 2.82 | 1.51-4.32 | .013 |
| Serum miR-22 | 2.73 | 1.44-4.17 | .015 |
| RFS (all AML, N = 124) | | | |
| WBC counts (× $10^9$/L) | 2.61 | 1.47-4.05 | .018 |
| Cytogenetics | 3.02 | 1.65-4.58 | .011 |
| Serum miR-22 | 3.23 | 1.84-4.97 | .007 |

higher serum miR-22 expression than those with WBC ≥ 10×$10^9$/L (*P* = .026, Figure 1C). Furthermore, serum miR-22 levels were significantly reduced in poor cytogenetic risk group compared to intermediate cytogenetic risk subgroup (*P* = .024) and favorable risk cytogenetic group (*P* = .0002), and significant lower serum miR-22 expression was observed in intermediate cytogenetic risk group than in favorable cytogenetic risk group (*P* = .035, Figure 1D). ROC analysis showed that serum miR-22 well differentiated AML subjects from normal controls with AUC value of 0.830; the specificity and the sensitivity were 78.3% and 76.6%, respectively (Figure 2).

## 3.2 | Correlation between serum miR-22 expression and clinical characteristics

All AML subjects were classified into high serum miR-22 expression group (n = 67) and low serum miR-22 expression group (n = 57) using the mean value of serum miR-22 level. As shown in Table 1,

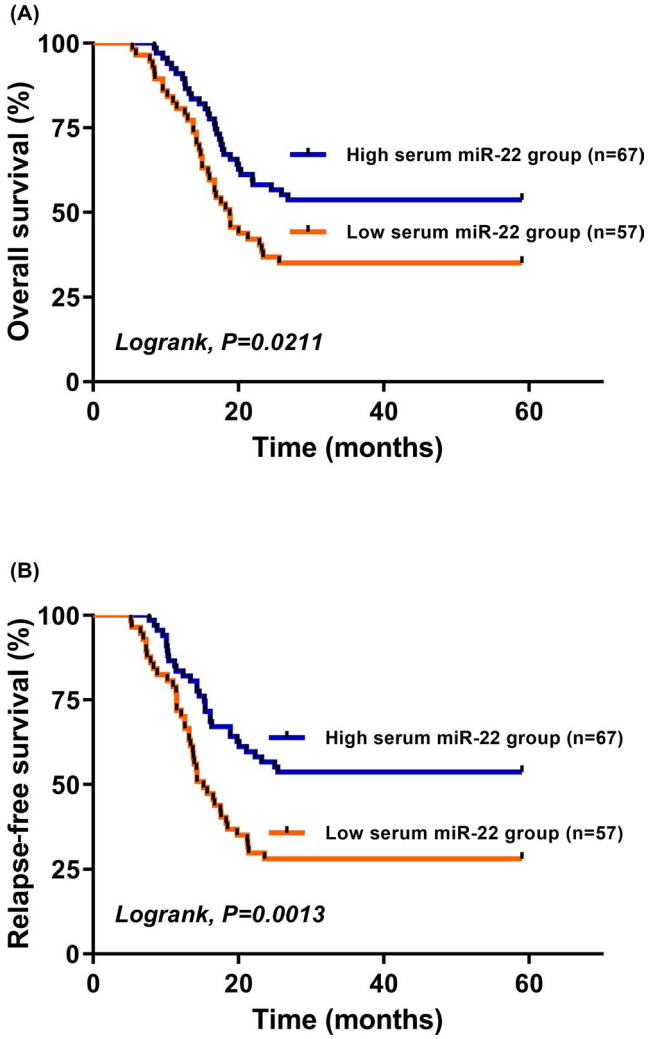


**FIGURE 3** The associations between serum miR-22 level and survival of AML. A, The AML patients in the low serum miR-22 group had worse OS than those in the high serum miR-22 group. (B) The AML patients in the low serum miR-22 group had worse RFS than those in the high serum miR-22 group

significant associations were found between low serum miR-22 expression and WBC counts (*P* = .034) and cytogenetics (*P* = .008). However, no significant correlation was found between serum miR-22 and age (*P* = .227), gender (*P* = .587), bone marrow blasts (*P* = .176), FAB subtype (*P* = .202), platelet counts (*P* = .074) and CR (*P* = .120) (Table 2).

## 3.3 | Low serum miR-22 expression was associated with worse survival in AML

Kaplan-Meier method and log-rank test were used to analyze the association between serum miR-22 expression and survival. Both the OS (*P* = .0211, Figure 3A) and RFS (*P* = .0013, Figure 3B) rates were markedly lower in AML cases in low serum miR-22 expression group (n = 57) than in high serum miR-22 expression group (n = 67).

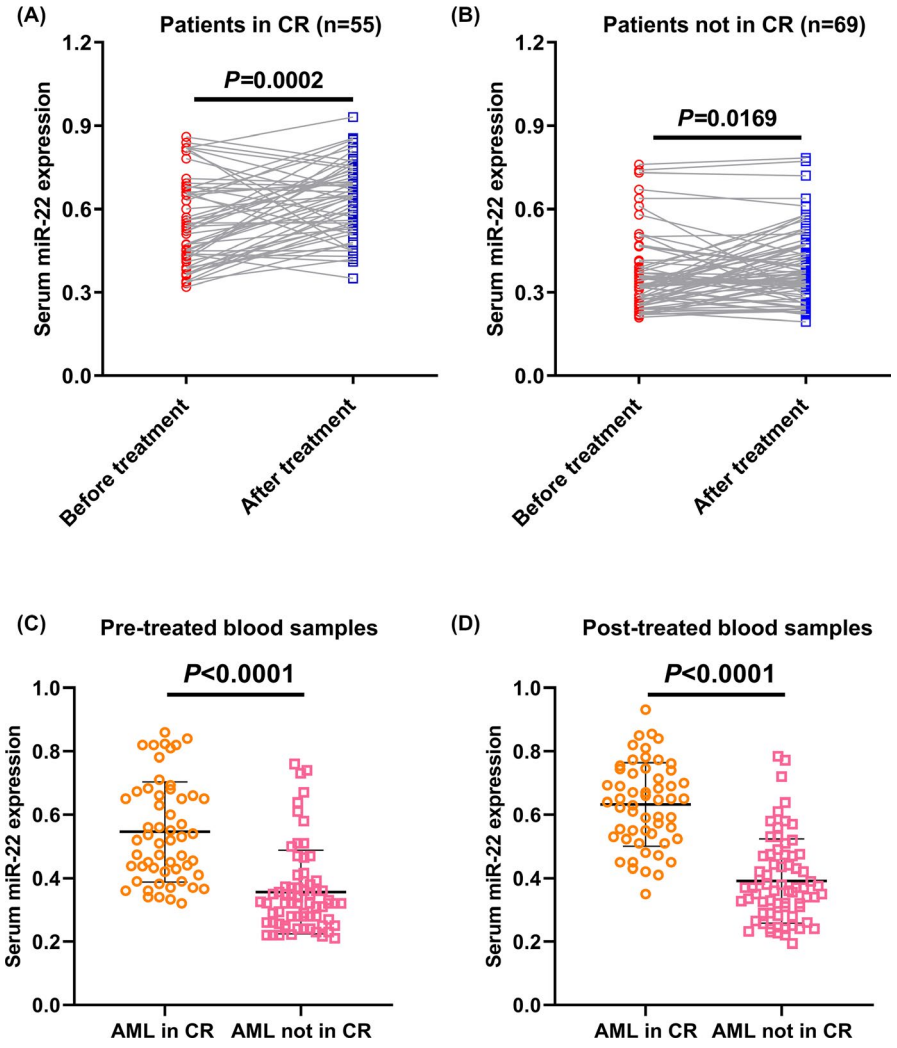


**FIGURE 4** Serum miR-22 was sensitive for monitoring therapeutic responses. A, For the AML cases achieving CR, serum miR-22 levels were markedly elevated in the post-treated blood samples compared to the pre-treated blood samples. B, For the AML cases not achieving CR, serum miR-22 levels were increased in the post-treated blood samples compared to the pre-treated blood samples. C-D, For both pre-treated and post-treated blood samples, serum miR-22 levels were significantly higher in AML achieving CR than in those not achieving CR

### 3.4 | Serum miR-22 was an independent prognostic factor for both OS and RFS

Multivariable analysis showed that WBC counts (RR = 2.46; 95% CI = 1.40-3.68, *P* = .020), cytogenetics (RR = 2.82; 95% CI = 1.51-4.32, *P* = .013), and serum miR-22 expression (RR = 2.73; 95% CI = 1.44-4.17, *P* = .015) were significant prognostic factors for poorer OS. Similarly, WBC counts (RR = 2.61; 95% CI = 1.47-4.05, *P* = .018), cytogenetics (RR = 3.02; 95% CI = 1.65-4.58, *P* = .011), and serum miR-22 expression (RR = 3.23; 95% CI = 1.84-4.97, *P* = .007) were also demonstrated to be independent prognostic markers for RFS in AML patients.

### 3.5 | The changes in serum miR-22 expression in AML patients following treatment

On the 28th day of the first chemotherapy cycle, blood samples were obtained from all the AML patients and serum miR-22 levels were detected for monitoring treatment response. Of 124 AML patients, 55 patients achieved a CR while 69 cases failed to achieve a CR. We found serum miR-22 levels were remarkably increased both in the subgroup of patients achieving CR (*P* = .0002, Figure 4A) and the subgroup of patients failing to achieve a CR (*P* = .0169, Figure 4B). Also, AML patients achieving CR exhibited significant higher serum miR-22 expression than those without CR achievement for both pre-treated and post-treated blood samples (*P* < .0001, Figure 4C-4D). The data demonstrated serum miR-22 might be used to monitor the treatment response of AML patients.

## 4 | DISCUSSION

In the present study, we found that serum miR-22 was significantly lower in AML patients compared to healthy individuals. ROC analysis showed that serum miR-22 exhibited good performance for discriminating AML patients from healthy controls. The possible reason accounting for the reduced serum miR-22 level in AML patients might be that the cancer cells synthesized and secreted much less miR-22 into the peripheral blood. The AML patients with low serum miR-22 level might receive more aggressive therapeutic strategies. In addition, we found that low serum miR-22 expression was significantly

correlated with aggressive clinicopathological variables and shorter survival. The multivariate analysis demonstrated that low serum miR-22 expression was an independent risk factor for both OS and RFS, suggesting that the serum miR-22 level might be closely correlated with AML progression and serve as a promising prognostic indicator for AML. Interestingly, serum miR-22 levels were significantly increased in AML patients receiving treatments, especially those achieving CR. These findings suggest that serum miR-22 level is sensitive for monitoring the therapeutic responses. Collectively, low serum miR-22 might act as a promising biomarker for monitoring the initiation and development of AML. Therefore, the results of our study were consistent with previous studies that miR-22 played a tumor suppressive role in AML.[12,13]

Except for AML, the tumor suppressive role of miR-22 has also been reported in many other types of cancers. For instance, Wan et al found that miR-22 expression was markedly reduced in epithelial ovarian cancer tissues compared to normal tissues, and downregulation of miR-22 was closely associated with worse clinical parameters.[16] In breast cancer (BC), miR-22 expression was significantly downregulated in BC tissues and cells, and ectopic expression of miR-22 significantly inhibited the tumorigenesis and enhanced radiosensitivity of BC cells.[17,18] Similarly, miR-22 expression was decreased in gastric cancer (GC) tissues, and its downregulation was associated with aggressive phenotype of GC and reduced survival. Restoration of miR-22 remarkably suppressed cancer cell growth, migration, and invasion in vitro.[19,20] In hepatocellular carcinoma (HCC), reduced miR-22 expression occurred more frequently in HCC tissues and cell lines. In vitro and in vivo analysis showed that miR-22 overexpression significantly inhibited carcinogenesis of HCC by regulating CD147.[21,22] In bladder cancer, overexpression of miR-22 markedly reduced MAPK1 and Snail expression, and attenuated cancer cell proliferation, migration, and invasion.[23]

Interestingly, miR-22 might also act as an oncomiR in cancer progression. Serum miR-22 was overexpressed in patients with papillary thyroid cancer, and its upregulation was strongly associated with metastasis.[24] The controversial findings reflect that the role of miR-22 in tumorigenesis might be cancer-type specific, and the hidden molecular mechanisms about the role of miR-22 in tumorigenesis need to be further explored.

One possible limitation of our study was the relatively small sample size. Larger independent cohorts are warranted to validate the clinical significance of serum miR-22 in AML. In addition, serum miR-22 might also be deregulated in other malignancies or other human diseases. Therefore, serum miR-22 should be combined with other known biomarkers and the clinical information to accurately predicts the prognosis of AML. Moreover, the molecular mechanisms for the role of miR-22 in AML progression need deeper investigation.

In conclusion, our study has demonstrated that the serum miR-22 expression is markedly downregulated in AML. In addition, downregulation of serum miR-22 is significantly associated with aggressive clinical variables and poor prognosis of AML. Therefore, serum miR-22 might serve as a promising prognostic biomarker for AML.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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