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ORIGINAL ARTICLE

Lower Serum miR-145 Predicts Poor Prognosis in Patients with Acute Myeloid Leukemia

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SUMMARY

Background: **The current study mainly aims to evaluate the role and clinical significance of miR-145 in the progression of AML.**
Methods: **Serum and bone marrow nucleated cells (BMNc) were collected and the level of miR-145 was detected by RT-PCR. Pearson's correlation assay was carried out to analyze the correlation between serum miR-145 and clinical index. The receiver operating characteristic (ROC) curve was constructed to determine the diagnosis value of serum miR-145.**
Results: **MiR-145 was significantly decreased in serum and BMNc of patients with AML compared with the control group. Pearson's correlation assay showed that serum miR-145 was positively correlated with miR-145 levels in BMNc. Further study showed that the level of serum miR-145 was much lower in AML patients with initial WBC count ≥ 50 x 10^9/L than that of WBC count < 50 x 10^9/L. Moreover, the level of serum miR-145 in prednisone poor responders was significantly lower than that in prednisone good responders. Compared with minimal residual disease (MRD) < 0.01% group, serum miR-145 was much lower in AML patients with MRD ≥ 0.01% group. Pearson's correlation analysis showed that serum miR-145 was positively correlated with MRD. In addition, miR-145 diagnosed AML with an AUC of 0.915 (95% confidence interval: 0.828 to 1.000; p < 0.001).**
Conclusions: **The level of miR-145 in serum and BMNc of AML patients was significantly lower than those of the control group. Serum miR-145 was related to poor prognosis and disease recurrence of AML.**
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KEY WORDS

miR-145, acute myeloid leukemia, serum, bone marrow nucleated cells, prognosis

INTRODUCTION

Acute myeloid leukemia (AML) is the most common acute leukemia in adults [1]. It is a highly heterogeneous hematological malignant disease characterized by an accumulation of immature myeloid blasts in the bone marrow and blood [2,3]. Although a significant improvement has been reported in the 5-year survival rate of AML patients over the past years, the prognosis and relapse of AML is still dismal [4]. It is still difficult to predict the clinical outcome and treatment response of

AML patients [5]. Hence, to identify an effective and sensitive marker might shed light on the diagnosis and prognosis of AML malignancy.

MicroRNA (miRNA, miR) is a newly discovered small non-coding RNA that regulates gene expression after transcription [6]. Over the past years, accumulating evidence has shown that abnormal expression of miRs may be responsible for hematologic malignancies, and some miRNAs can be used as potential biomarkers for AML patients [5-7]. For instance, reduced serum miR-34a has been shown to correlate with poor prognosis of AML patients [5]. Elevated circulating exosomal miR-125b level is shown to predict higher risks of relapse and overall death among AML patients [6]. However, very little is known regarding the role of miRNAs.

miR-145 has been shown to be differentially expressed in many neoplastic diseases, such as lung cancer, colon cancer, etc. [8,9]. Prominently, miR-145 has also been shown to be dysregulated in AML cell lines [10]. However, the expression of miR-145 in patient samples has never been explored. Therefore, this study further elucidates the expression of miR-145 *in vivo*, which may provide a new perspective for the diagnosis and prognosis of AML.

MATERIALS AND METHODS

Patient samples

BMNc and blood samples from 40 patients with AML were taken from patients at the Jinan Infectious Diseases Hospital from June 2017 to March 2018. The study was approved by the Medical Ethics Committee in Jinan Infectious Diseases Hospital and informed consent was obtained. All patients were newly diagnosed, had no history of other malignant tumors, and had not received any anti-tumor drug treatment. The age of AML patients ranged from 19 to 42 years old (average age: 26.35 ± 5.91 years), including 24 males and 16 females. Bone marrow puncture was performed before and after treatment. Blood samples were collected at admission. MRD assessments by flow cytometry were retrieved and low risk groups were categorized with $< 0.01\%$, while high risk group were categorized with $\geq 0.01\%$. Another 20 patients with non-hematopoietic diseases of the same age were selected as the control group, including 11 males and 9 females (aged 21 to 49 years, with an average age of 27.01 ± 6.23 years). There were 9 cases of iron deficiency anemia, 9 cases of allergic purpura, and 2 cases of other diseases. There was no significant difference in gender and age between the two groups ($p > 0.05$).

RT-PCR

Total RNA was extracted from BMNc and serum samples using RNAVzol LS (Vigorous Biotechnology Beijing Co., Ltd, Beijing, China) according to the manufacturer's protocol. The concentration and the purity of RNA samples was determined by measuring the optical density (OD) 260/280. A poly(A) tail was added using Poly(A) Tailing Kit (Thermo Fisher Scientific). Reverse transcription was carried out using TaqMan™ MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Reverse transcription system: dNTP 2 μL, reverse transcriptase 1 μL, 5 x Buffer 4 μL, RNase inhibitor 1 μL, $DEPCH_2O$ 8 μL, random primer 1 μL, template RNA 3 μL. The reaction conditions were 75°C, 10 minutes; 37°C, 50 minutes; 70°C, 15 minutes; 4°C, forever. To quantify the relative mRNA levels, qPCR was performed using SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA) in an iCycleriQ real-time PCR detection system. The PCR amplifications were performed in a 10 μL reaction system containing 5 μL SYBR Green Supermix, 0.4 μL forward primer, 0.4 μL reverse primer, 2.2 μL double distilled H_2O, and 2 μL template cDNA. Thermocycling conditions were as follows: 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Relative mRNA expression was normalized to U6 using the $2^{-\Delta\Delta Cq}$ method [11].

Statistical analysis

The data were represented as the mean ± standard error of the mean (SEM). The two-tailed unpaired Student's *t*-test was used for comparisons of two groups. The one-way ANOVA multiple comparison test (SPSS 13.0) followed by Tukey's post hoc test were used for comparisons of two or more groups. Pearson's correlation assay was carried out to analyze the correlation between serum miR-145 and clinical index. The receiver operating characteristic (ROC) curve was constructed to determine the diagnosis value of serum miR-145. $p < 0.05$ indicates a statistically significant difference. SPSS software 20.0 and GraphPad Prism 5.0 were used to conduct the statistical analyses in this study.

RESULTS

Decreased miR-145 in serum and BMNc of AML patients

First, we analyzed the level of miR-145 in serum and BMNc of AML patients. Compared with the control group, our data showed that the level of miR-145 in serum and BMNc was much lower in AML patients than in the control group (Figure 1A and 1B).

Serum miR-145 was positively correlated with BMNc miR-145 in AML patients

Furthermore, we analyzed the correlation between serum miR-145 and the level of miR-145 in BMNc. Pearson's correlation assay showed that serum miR-145 was positively correlated with the level of miR-145 in BMNc ($r = 0.791$, $p < 0.001$) (Figure 2).

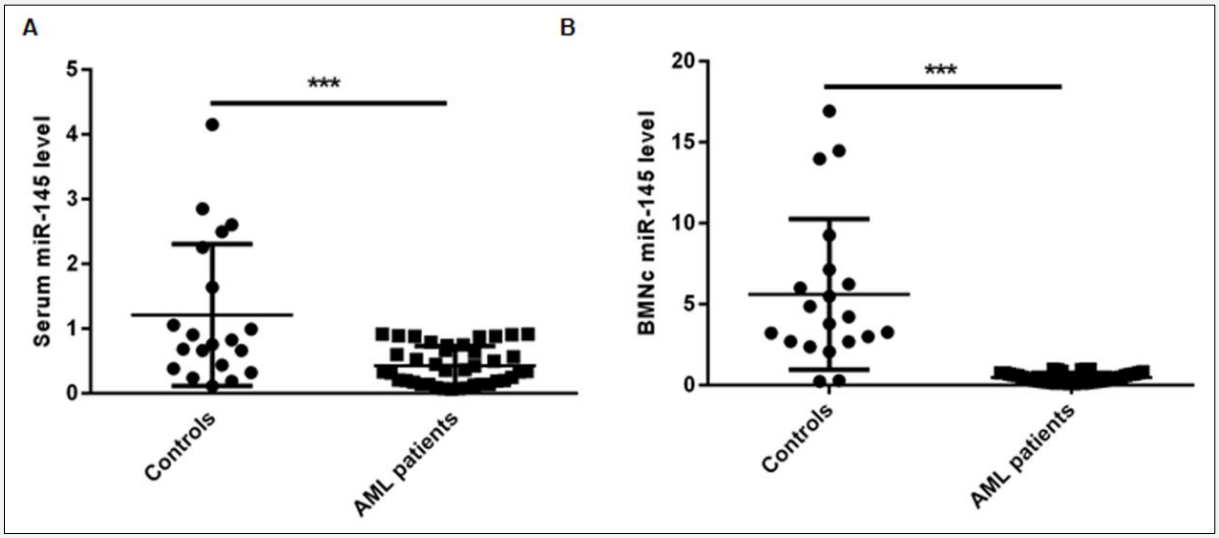

Figure 1. MiR-145 was reduced in serum and BMNc of AML patients compared to the controls.

The results showed that the expression of miR-145 was significantly down-regulated in serum (A) and BMNc (B) of AML patients compared with the control group. $^{***}p < 0.001$ vs. as indicated.

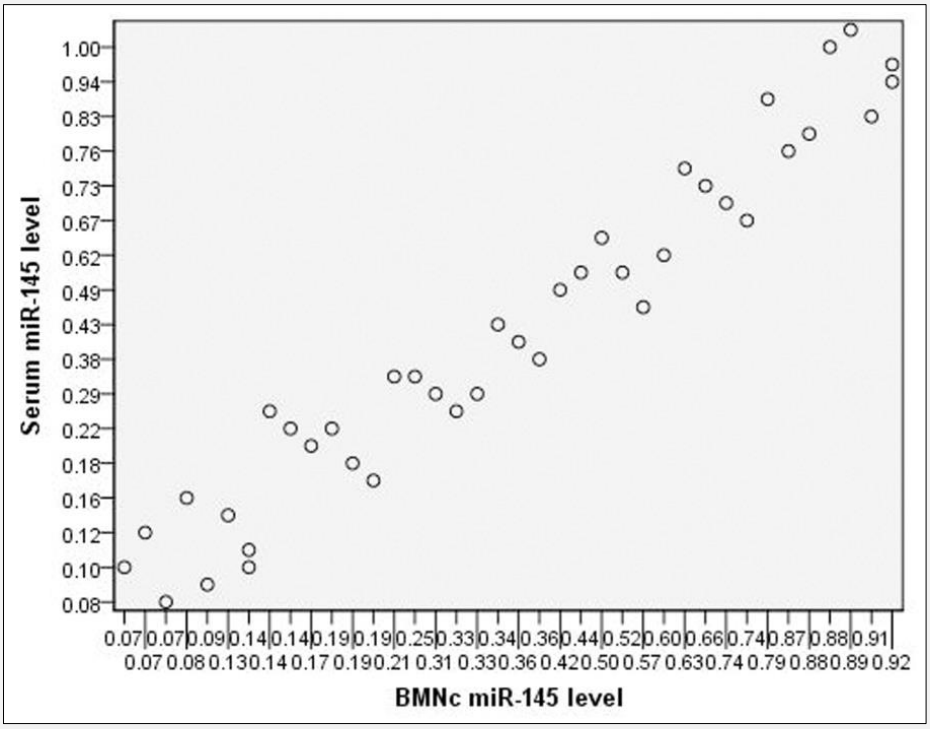

Figure 2. Pearson's correlation assay showed that serum miR-145 was positively correlated with the level of miR-145 in BMNc.

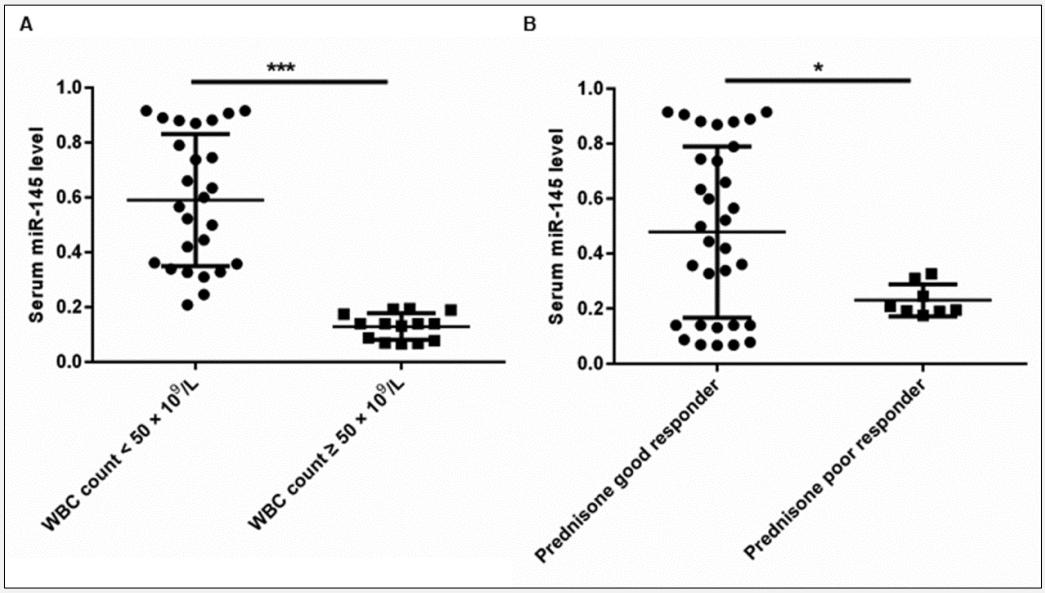

Figure 3. RT-PCR was carried out to analyze the level of serum miR-145 according to clinical indicators in AML patients.

(A) The level of serum miR-145 was significantly lower in AML patients with initial WBC count ≥ 50 x 10^9/L compared to that of patients with WBC count < 50 x 10^9/L. (B) The level of serum miR-145 in pre-sensitized AML patients was significantly lower than that in insensitive AML patients. * - p < 0.05, * - p < 0.001 vs. as indicated.**

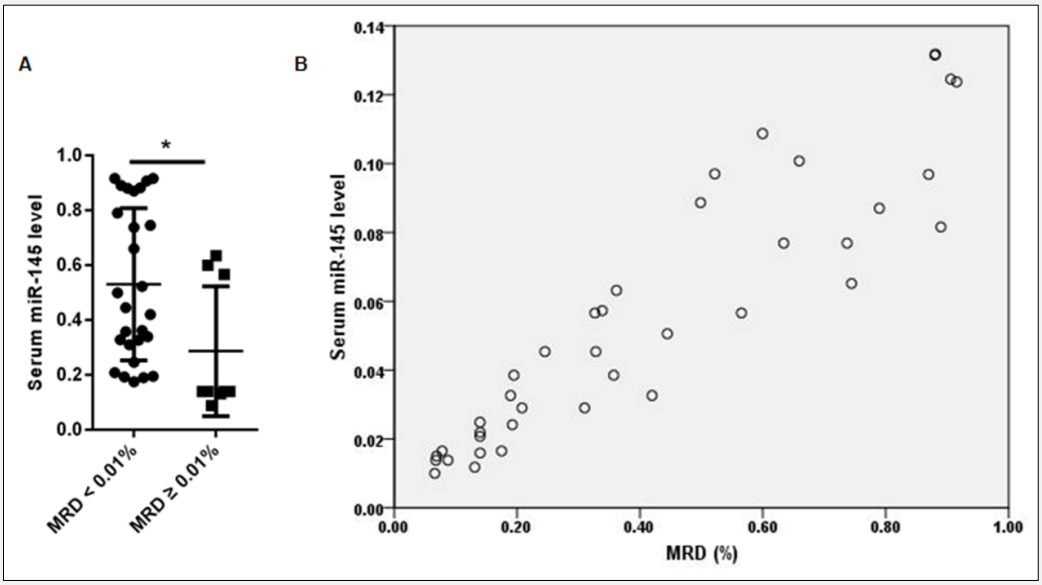

Figure 4. Serum miR-145 negatively correlated with MRD.

(A) Compared with MRD < 0.01% group, serum miR-145 was much lower in AML patients with MRD ≥ 0.01% group. (B) Pearson's correlation analysis showed a positive correlation between serum miR-145 and MRD. * - p < 0.05 vs. as indicated.

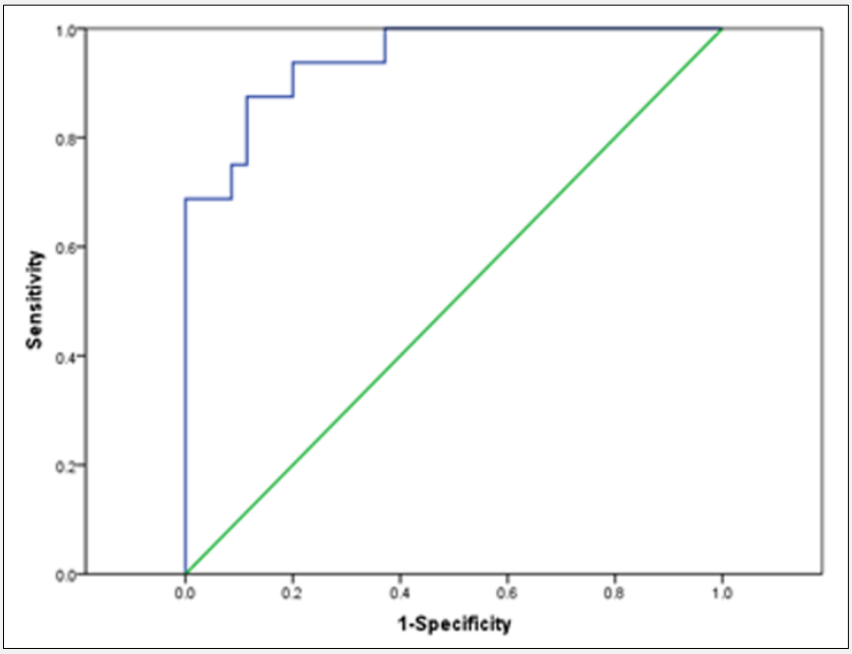

Figure 5. ROC analysis showed that serum miR-145 could screen AML patients from controls.

Relationship between serum miR-145 and clinical indicators in AML patients

Furthermore, we analyzed the level of serum miR-145 in AML patients based on clinical indicators. Our data showed that the level of serum miR-145 was significantly lower in AML patients with initial WBC count $\geq 50 \times 10^9$/L (n = 14) than in patients with WBC count $< 50 \times 10^9$/L (n = 26) (Figure 3A). In addition, the level of serum miR-145 in prednisone poor responders (n = 8) was significantly lower than in prednisone good responders (n = 32) (Figure 3B). However, the expression of serum miR-145 was not related to gender, age, and chromosomal abnormalities (data not shown).

Serum miR-145 negatively correlated with minimal residual disease (MRD)

MRD plays an important role in the evaluation of prognosis among AML patients, and it is also considered as the root cause of leukemia recurrence [12]. Hence, we evaluated the level of miR-145 in AML patients according to the level of MRD. Compared with MRD < 0.01% group (n = 27), serum miR-145 was much lower in AML patients with MRD ≥ 0.01% group (n = 13) (Figure 4A). Pearson's correlation analysis showed a positive correlation between serum miR-145 and MRD ($r = 0.715$, $p < 0.01$) (Figure 4B), indicating lower serum miR-145 might indicate the recurrence of AML.

Serum miR-145 could screen AML patients from controls

In addition, ROC analysis was carried out to evaluate the diagnostic value of serum miR-145 in the diagnosis of AML, and our data showed that the AUC of miR-145 diagnosed AML was 0.915 (95% confidence interval: 0.828 - 1.000; $p < 0.0001$). When the cutoff value was 0.21, miR-145 has a sensitivity of 80.8% and a specificity of 100% for diagnosis of AML (Figure 5).

DISCUSSION

AML is a highly heterogeneous disease characterized by an accumulation of abnormal primordial cells and immature cells in bone marrow [13]. miRNAs are shown to affect the cellular proliferation and differentiation in AML patients [14,15]. Here, for the first time, we showed novel data that miR-145 was significantly lower in the serum and BMNc of patients with AML than those of the control group. Further study showed a positive correlation between serum miR-145 and BMNc miR-145 levels, indicating serum miR-145 and BMNc miR-145 may originate from the same source. Since serum is a non-invasive sample, it is better as a biomarker for early screening of AML.

It is reported that accurate typing and prognosis analysis before treatment are important for developing personal-

ized treatment plans and ensuring long-term survival [16]. Age, immunophenotyping, chromosomal abnormalities, genetic abnormalities, early treatment response, etc. are all important factors affecting the prognosis of AML patients [17,18]. Here, we showed that the relative expression of miR-145 in the serum of AML patients is related to the WBC count and prednisone sensitivity. This indicates that serum miR-145 expression levels may be associated with poor patient prognosis.

In AML patients who achieve a complete remission (CR), MRD has emerged as an important prognostic factor for relapse and survival in AML [19]. It is reported that eradication of MRD may enhance the survival rate among AML patients [20]. In the present study, we showed that serum miR-145 has a positive correlation with MRD. The expression of serum miR-145 was significantly lower in AML patients with MRD $\geq$ 0.01% group than that of MRD < 0.01% group, suggesting that low expression of miR-145 predicts poor prognosis. Hence, miR-145 can be used as an indicator of prognosis in adult AML patients. Furthermore, the AUC demonstrated the high diagnostic value of miR-145 for AML, indicating serum miR-145 has a high diagnostic efficacy for the diagnosis of AML.

Although the data are promising, several limitations remain in the present study. First, as the sample size is still small, large cohorts are required for further validation. Second, it is uncertain whether this downregulation of serum miR-145 is specific for hematological malignancies. Therefore, it is necessary to compare serum miR-145 in different types of cancer to validate the specificity for AML diagnosis.

CONCLUSION

In summary, low serum miR-145 expression is associated with aggressive clinical features and poor prognosis in AML patients. Hence, serum miR-145 may be a promising marker for the diagnosis and prognosis of AML.

Declaration of Interest:

We declare no conflicts of interest.

References:

1. Chen K, Chen Y, Chen Z, et al. miR-134 increases the antitumor effects of cytarabine by targeting Mnks in acute myeloid leukemia cells. Onco Targets Ther 2018;11:3141-7 (PMID: 29872325).
2. Lowenberg B. Acute myeloid leukemia: the challenge of capturing disease variety. Hematology Am Soc Hematol Educ Program 20081-11 (PMID: 19074046).
3. Tenen DG. Disruption of differentiation in human cancer: AML shows the way. Nat Rev Cancer 2003;3:89-101 (PMID: 12563 308).
4. Hong Z, Zhang R, Qi H. Diagnostic and prognostic relevance of serum miR-195 in pediatric acute myeloid leukemia. Cancer Biomark 2018;21:269-75 (PMID: 29226854).
5. Huang Y, Zou Y, Lin L, Ma X, Chen H. Identification of serum miR-34a as a potential biomarker in acute myeloid leukemia. Cancer Biomark 2018;22:799-805 (PMID: 29945348).
6. Jiang L, Deng T, Wang D, Xiao Y. Elevated Serum Exosomal miR-125b Level as a Potential Marker for Poor Prognosis in Intermediate-Risk Acute Myeloid Leukemia. Acta Haematol 2018; 140:183-92 (PMID: 30304715).
7. Guo Y. Clinical significance of serum MicroRNA-203 in patients with acute myeloid leukemia. Bioengineered 2019;10:345-52 (PMID: 31411110).
8. Zhang J, Guo H, Zhang H, et al. Putative tumor suppressor miR-145 inhibits colon cancer cell growth by targeting oncogene Friend leukemia virus integration 1 gene. Cancer 2011;117:86-95 (PMID: 20737575).
9. Lei C, Du F, Sun L, et al. miR-143 and miR-145 inhibit gastric cancer cell migration and metastasis by suppressing MYO6. Cell Death Dis 2017;8:e3101 (PMID: 29022908).
10. Havelange V, Stauffer N, Heaphy CC, et al. Functional implications of microRNAs in acute myeloid leukemia by integrating microRNA and messenger RNA expression profiling. Cancer 2011; 117:4696-706 (PMID: 21455993).
11. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) Method. Methods 2001;25:402-8 (PMID: 11846609).
12. Gomez-Arteaga A, Guzman ML. Minimal Residual Disease in Acute Myeloid Leukemia. Adv Exp Med Biol 2018;1100:111-25 (PMID: 30411263).
13. Li Q, Song W, Wang J. TUG1 confers Adriamycin resistance in acute myeloid leukemia by epigenetically suppressing miR-34a expression via EZH2. Biomed Pharmacother 2019;109:1793-801 (PMID: 30551433).
14. Ma QL, Wang JH, Yang M, Wang HP, Jin J. MiR-362-5p as a novel prognostic predictor of cytogenetically normal acute myeloid leukemia. J Transl Med 2018;16:68 (PMID: 29540187).
15. Peng D, Wang H, Li L, et al. miR-34c-5p promotes eradication of acute myeloid leukemia stem cells by inducing senescence through selective RAB27B targeting to inhibit exosome shedding. Leukemia 2018;32:1180-8 (PMID: 29479064).
16. Wang Z, Luo H, Fang Z, et al. MiR-204 acts as a potential therapeutic target in acute myeloid leukemia by increasing BIRC6-mediated apoptosis. BMB Rep 2018;51:444-9 (PMID: 29764561).
17. Yang C, Shao T, Zhang H, et al. MiR-425 expression profiling in acute myeloid leukemia might guide the treatment choice between allogeneic transplantation and chemotherapy. J Transl Med 2018;16:267 (PMID: 30285885).
18. Ying X, Zhang W, Fang M, Zhang W, Wang C, Han L. miR-345-5p regulates proliferation, cell cycle, and apoptosis of acute myeloid leukemia cells by targeting AKT2. J Cell Biochem 2018 (PMID: 30278103).
19. Jongen-Lavrencic M, Grob T, Hanekamp D, et al. Molecular Minimal Residual Disease in Acute Myeloid Leukemia. N Engl J Med 2018;378:1189-99 (PMID: 29601269).
20. Lowenberg B, Valk PJM. Molecular Minimal Residual Disease in Acute Myeloid Leukemia. N Engl J Med 2018;378:2443 (PMID: 29924951).