MicroRNA-181a-3p as a Diagnostic and Prognostic Biomarker for Acute Myeloid Leukemia

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Abstract. Background: Micro (mi) RNAs play an important role in the pathogenesis and development of acute myeloid leukemia (AML), and their abnormal expression may be sufficient to predict the prognosis and outcomes in AML patients. We evaluated the clinical diagnostic value of miRNA-181a-3p in predicting prognosis and outcomes in patients with AML.

Methods: A total of 119 newly diagnosed adult patients with AML and 60 healthy controls were recruited. Blood specimens were obtained from all AML patients at diagnosis, and 10 blood specimens were obtained on day 28 after induction chemotherapy. The controls also provided blood samples. Relative gene expression was quantified by PCR and determined using the comparative Ct method. Publicly available clinical data and gene expressions for 188 patients with AML were downloaded from TCGA data portal.

Results: Compared with healthy controls, the expression of miRNA-181a-3p was significantly increased in patients with AML. MiR-181a-3p expression could be used to discriminate AML patients from controls, with up-regulated expression correlating with favorable prognosis. Moreover, miRNA-181a-3p expression was significantly decreased in patients who achieved a complete response after induction chemotherapy. The multivariate Cox analysis highlighted the prognostic value of miR-181a-3p for patients with AML. Finally, we found that miR-181a-3p expression was negatively correlated with the expression of the NF-κB essential modulator (NEMO/IKBKG).

Conclusions: MiR-181a-3p may be clinically useful as a disease marker for AML, and enhanced the prediction of patient outcomes to chemotherapy.

Keywords: MicroRNAs; MiR-181a-3p; Biomarkers; Leukemia, Myeloid, Acute; Treatment outcome.

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Introduction. Acute myeloid leukemia (AML) is one of the most common adult leukemias. It is a molecularly heterogeneous disease that is generally associated with poor outcomes. AML patients are classified into distinct risk categories for risk-adjusted chemotherapy, on the basis of cytogenetic and molecular abnormalities. Patients with complex karyotype abnormalities or unfavorable molecular characteristics often have an unfavorable prognosis. However, not all AML patients carry cytogenetic alterations, so new genomic approaches to improve risk stratification are needed.

Micro (mi)RNAs are small, noncoding RNAs that bind their target mRNAs and inhibit the expression of encoded proteins. MiRNAs have critical biological functions, including in hematopoietic cell proliferation, differentiation, and apoptosis, and may also play an essential role in the development and pathogenesis of AML. Several studies have identified that distinctive miRNA profiles are associated with cytogenetic subtypes, mutations, and clinical outcomes of AML. For example, decreased miR-196b expression is associated with the absence of FLT3-ITD and NPM1 mutations, and high miR-196b expression acts as a predictive factor of poor prognosis. Therefore, miRNA expression levels may be suitable to predict prognosis and outcomes in AML patients.

The miR-181 family is thought to be involved in a number of biological processes, including transcription, translation, and signaling transduction. In humans, the miR-181 family has four mature homologs (hsa-miR-181a, hsa-miR-181b, hsa-miR-181c and hsa-miR-181d). MiR-181a-3p belongs to miR-181a mature homologs, acts as a negative post-transcriptional regulator of Nuclear Factor kappa-B (NF-kB) signaling pathway by directly targeting NF-kB essential modulator (NEMO/IKBKG) in Human Umbilical Vein Endothelial Cells (HUVECs). Our previous study showed that abnormal expression of miR-181a-3p was associated with human monocytic leukaemia cell line THP-1 cell. Few studies have focused on the clinical role of miR-181a-3p in AML patients. Therefore, the present study examined the expression of miR-181a-3p in AML patients prior to treatment to evaluate its clinical diagnostic value and predictive role in the prognosis and outcomes of AML patients.

Materials and Methods.
RNA extraction, real-time PCR and miR-181-a-3p Expression Analyses. Total RNA was extracted from blood cells using TRIzol (Invitrogen). Reverse transcription and quantitative real-time PCR (RT-qPCR) for gene expression were performed using the SYBR Green PCR Kit (GenePharma, Shanghai, PR China). The primer sequences were as follows. MiR-181-a-3p forward (5'-3'): AGAATTACACCATCGACCCTTG; MiRNA-181-a-3p reverse (5'-3'): TATGCTTTCTCCTCTCTGTGTC. U6 forward (5'-3'): GGAACGCTTCACGAATTTG. NF-kB forward (5'-3'): CTGAACCCAGGCCATACTGTG; NF-kB reverse (5'-3'): GAGAAGTCCATGTCCGCAAT. NEMO/IKBKG forward (5'-3'): TACTGGGCAGAGTTCTCC; NEMO/IKBKG reverse (5'-3'): AGAATCTGGTTGCTCTGCC. Analysis of relative gene expression was using 2-△△CT method.

TCGA data set. Publicly available clinical, gene expression data for 188 patients with AML were downloaded from TCGA data portal.

Data analysis. We used SPSS (version 24), GraphPad Prism (version 7) software and R language to analyze the data. The unpaired Student’s t test (two-tailed) was performed to compare differences in miRNA expression between different groups. Chi-square tests were used to test the association between the expression of miRNAs and clinicopathological characteristics. Cox proportional hazards models were used to analyze the prognostic utility of miRNA expression for disease-free survival (DFS) and overall survival (OS) in AML patients.

Results.
Patient clinical characteristics and treatment. From September 2014 through December 2016, a total of 119 untreated, newly diagnosed adult AML patients [age range 15-83 years; male, 52.1%] in The First Affiliated Hospital of University of Science and Technology of China were recruited, together with 60 heath controls [age range 16-81 years; male, 57%] with no hematologic disease. The patients represented the major French-American-British (FAB) subtypes: 1M0, 9 M1, 30 M2, 20 M3, 16 M4, and 43 M5. Of the 119 AML patients, 20 had PML-RARA rearrangements, 24 had AML1-ETO rearrangements, 30 had other molecular genetic abnormalities, and 45 had normal karyotypes. 20 M3 patients received ARTA plus anthracycline-based induction chemotherapy with or without ATO, and 99 AML patients received traditional 7+3 induction chemotherapy. A total of 81 patients achieved a complete response (CR), 30 did not, and 8 patients died within 30 days of receiving
Table 1. Clinical characteristics of AML patients at diagnosis.

| Characteristic   | N(%)  |
|-----------------|-------|
| Gender          |       |
| Male            | 62 (52.1) |
| Female          | 57 (47.9) |
| Age             |       |
| < 60 years      | 81 (68.1) |
| ≥60 years       | 38 (31.9) |
| WBC             |       |
| < 100×10^9/L    | 113 (95.0) |
| ≥100×10^9/L     | 6 (5.0) |
| FAB             |       |
| M0              | 1 (0.8) |
| M1              | 9 (7.6) |
| M2              | 30 (25.2) |
| M3              | 20 (16.8) |
| M4              | 16 (13.4) |
| M5              | 43 (36.1) |
| Risk groups     |       |
| Low and intermediate risk | 73 (61.3) |
| High risk       | 46 (38.7) |
| Molecular abnormalities |       |
| PML/RARa        | 20 (16.8) |
| AML1/ETO        | 24 (20.2) |
| FLT3-ITD or -TKD | 12 (10.1) |
| CEBPA           | 5 (4.2) |
| NPM1            | 5 (4.2) |

Abbreviations: WBC = white blood cell; FAB = French-American-British; PML/RARa = PML-RARa rearrangement; AML1/ETO = AML1-ETO rearrangement; FLT3-ITD or -TKD = FLT3-ITD or TKD rearrangement.

The clinical value of miRNA-181a-3p in the diagnosis of AML. To examine whether miRNA-181a-3p was abnormally expressed in patients with AML, we detected miRNA expression in 60 healthy controls and 119 adult patients with newly diagnosed AML. Compared with healthy controls, the expression of miRNA-181a-3p (P<0.001, Figure 1 A) was significantly increased in AML patients, and in the samples of M1, M2, M3, and M4 subtypes (Figure 1 B). Furthermore, we compared the expression of microRNAs in 4 subtypes base on molecular genetic abnormalities. Compared with healthy controls, the expression of miRNA-181a-3p was significantly increased in all four subtypes (Figure 1 C).

To assess the clinical diagnostic value of miR-181a-3p in discriminating AML patients from healthy controls, we performed receiver operating characteristic (ROC) curve analyses. The Area Under Curve (AUC) of miR-181a-3p was 0.654, 95% CI, 0.575 to 0.732, P<0.001, Figure 1D). Compared with health controls, miR-181a-3p showed significant difference in the samples of FAB M1, M2, M3 subtypes (Figure 1E) and PML/RARa, AML1/ETO subtypes (Figure 1F) compared with controls, suggesting that it had value in discriminating patients from controls. The AUC of miR-181a-3p in the samples of different subtypes was showed in Table 2.

The expression of miRNA-181a-3p was decreased when patients achieved CR after induction chemotherapy. To examine whether miRNA-181a-3p was decreased in patients who achieved CR after induction chemotherapy, we detected its expression from blood specimens in 10 patients. On day 28 after induction chemotherapy, miRNA-181a-3p expression was significantly decreased in 80% of patients (P<0.001, Figure 2). Moreover, on day 28 after induction chemotherapy, miR-181a-3p was expressed at higher levels compared with healthy controls (P<0.05, Figure 2A).

Association of miR181a-3p expression with AML patient outcome. A total of 119 adult patients with newly diagnosed AML were recruited, 8 patients died within 30 days after chemotherapy, finally 111 patients were included for statistical analysis. A total of 81 patients achieved complete remission (CR), whereas 30 patients failed to. To graphically display the association of miR181a-3p expression with CR achievement, we compared expression levels in patients who achieved CR (n=81) with those who failed to achieve CR (n=30) (Fig 3A). At the time of diagnosis, miRNA-181a-3p expression level was correlated with the response to induction chemotherapy (P<0.05).

Table 2. The AUC of miR-181a-3p in the samples of different subtypes. P-values calculated by Unpaired student's t test. AUC = The Area Under Curve. 95% CI = 95% confidence interval.

| miR-181a-3p | AUC | 95%CI        | P     |
|-------------|-----|--------------|-------|
| M1          | 0.736 | 0.537-0.935 | P<0.05 |
| M2          | 0.805 | 0.701-0.908  | P<0.0001 |
| M3          | 0.759 | 0.612-0.907  | P<0.001 |
| M4          | 0.588 | 0.408-0.767  | P<0.05 |
| M5          | 0.521 | 0.405-0.637  | P<0.05 |
| AML1/ETO    | 0.732 | 0.606-0.859  | P<0.001 |
| PML/RARa    | 0.759 | 0.612-0.907  | P<0.001 |
**Figure 1.** Evaluation of miR-181a-3p expression levels in patients with AML. (A) Expression levels of miR-181a-3p in peripheral blood mononuclear cells derived from AML patients and healthy controls. (B) Expression levels of miR-181a-3p in AML patients of major FAB subtypes (from M1 to M5 subtypes), respectively. (C) Expression levels of miR-181a-3p in subtypes grouped according to molecular genetic abnormalities, respectively. (D) AUC of miR-181a-3p comparison between AML and health controls. (E) AUC of miR-181a-3p comparison between FAB subtypes of AML and health controls. (F) AUC of miR-181a-3p comparison between PML/RARA and AML1/ETO subtypes of AML and health controls. P-values calculated by Unpaired student's t test. * = P<0.05, ** = P<0.01, *** = P<0.001, **** = P<0.0001.

**Figure 2.** Evaluation of miR-181a-3p expression levels in patients with AML at diagnosis and on day 28 after induction chemotherapy. (A) Box plots of miR-181a-3p expression in AML patients at diagnosis and on day 28 after induction chemotherapy, and in healthy controls. P-values calculated by Unpaired student's t test. * = P<0.05, *** = P<0.001. (B) Bar graph of the miR-181a-3p expression level ratio on day 28 after induction chemotherapy compared with disease diagnosis. (C) Line graphs of miR-181a-3p expression levels in AML patients at diagnosis and on day 28 after induction chemotherapy.
According to miR-181a-3p expression, patients with AML were dichotomized into high (above median expression levels) and low (below or at median expression levels) groups. Kaplan–Meier survival curves showed that patients with higher miR-181a-3p expression levels at diagnosis presented with a better OS ($P=0.014$, Fig 3D), but not a better DFS ($P=0.062$, Fig 3C), than those with lower expression levels. In the TCGA analysis, AML patients with higher miR-181a-3p also presented with a better OS ($P=0.008$, Fig 3E).

The Mantel–Cox test and Gehan–Breslow–Wilcoxon test were performed to determine the relationship between miR-181a-3p expression and OS or DFS (Fig 3B). The Gehan–Breslow–Wilcoxon test highlighted the prognostic value of increased miR-181a-3p expression at diagnosis both for disease relapse (HR: 1.597; 95% CI: 0.980-2.559; $P=0.03$) and death (HR: 2.062; 95% CI: 1.160-3.456; $P=0.02$). Thus, a higher miR-181a-3p expression at diagnosis was significantly associated with patient outcome.

Finally, multivariate Cox analysis was performed to determine the relationship between independent prognostic value and OS or DFS. The multivariate Cox analysis was adjusted for patients’ age, gender, WBC count and disease risk stratification. Multivariate analysis (Table 3) highlighted the of miR-181a-3p levels on AML diagnosis not for disease relapse ($P=0.05$) but for death (HR: 1.923; 95% CI: 1.035-3.574; $P<0.05$).

![Figure 3. Association of miR-181a-3p expression in AML patients at diagnosis with clinical outcomes.](image)

| Disease-Free Survival (DFS) |
|-----------------------------|
| Covariants: tested vs control (HR=1) HR 95%CI $P$ |
| miR-181a-3p levels (diagnosis): low vs high 1.848 1.070-3.192 $P<0.05$ |
| Age: ≥ 60 years vs <60 years 1.947 1.197-3.169 $P<0.001$ |
| High risk group vs low/intermediate risk 2.694 1.549-4.687 $P<0.0001$ |
| Gender: male vs female $P=0.204$ |
| WBC count: >100000 cells/μl vs <100000 cells/μl $P=0.588$ |

**Table 3.** Multivariate Cox regression analysis of independent risk factors influencing OS and DFS. P-values calculated by Unpaired student’s t test. WBC = white blood cell. 95% CI = 95% confidence interval.

**Multivariate Cox regression analysis**

### Overall Survival (OS)

| Covariants : tested vs control (HR=1) | HR | 95%CI | $P$ |
|--------------------------------------|----|------|-----|
| miR-181a-3p levels (diagnosis): low vs high | 1.923 | 1.035-3.574 | $P<0.05$ |
| Age: ≥ 60 years vs <60 years | 1.848 | 1.070-3.192 | $P<0.05$ |
| High risk group vs low/intermediate risk | 2.694 | 1.549-4.687 | $P<0.0001$ |
| Gender: male vs female | $P=0.204$ |
| WBC count: >100000 cells/μl vs <100000 cells/μl | $P=0.588$ |

### Disease-Free Survival (DFS)

| Covariants: tested vs control (HR=1) | HR | 95%CI | $P$ |
|--------------------------------------|----|------|-----|
| miR-181a-3p levels (diagnosis): low vs high | 1.947 | 1.197-3.169 | $P<0.001$ |
| Age: ≥ 60 years vs <60 years | 1.947 | 1.197-3.169 | $P<0.001$ |
| High risk group vs low/intermediate risk | 2.694 | 1.549-4.687 | $P<0.0001$ |
| Gender: male vs female | $P=0.970$ |
| WBC count: >100000 cells/μl vs <100000 cells/μl | $P=0.709$ |
Assessment of the relationship between miR-181a-3p ectopic expression and IKBKG and NF-κB family. Since miR-181a-3p blocks the NF-κB signaling pathway by targeting NEMO/IKBKG in Human Umbilical Vein Endothelial Cells (HUVECs),8 whether IKBKG and NF-κB expressions are affected by miR-181a-3p in AML cells needed to be determined. NF-κB family contains NF-κB1, NF-κB2, RelA, RelB and Rel. Firstly, we investigated the relation of miR-181a-3p ectopic expression with NEMO/IKBKG and NF-κB family in a set of primary TCGA AML patients. NEMO/IKBKG expression was positively correlated with expression of NF-κB family (NF-κB1, NF-κB2, RelA, RelB) (Person correlation= 0.377, \(P<0.01\)), while ROC analyses verified the ability of miR-181a-3p to distinguish AML from control blood samples.4,14

Since miR-181a-3p blocks the NF-κB signaling pathway, we investigated the relation of miR-181a-3p expression with NEMO/IKBKG and NF-κB family (NF-κB1, NF-κB2, RelA, RelB) (Person correlation= -0.209, \(P<0.01\)). Then we investigated the relation of miR-181a-3p expression with NEMO/IKBKG and NF-κB in 59 AML patients, and found that miR-181a-3p expression was negatively correlated with the expression of NF-κB (Person correlation= -0.2795, \(P=0.0321\) and NEMO/IKBKG (Person correlation= -0.313, \(P<0.01\)). We found that miR-181a-3p down-regulation of miR-181a-3p expression on day 28 of induction chemotherapy, while higher miR-181a-3p expression at diagnosis was associated with favorable prognosis. Finally, we found that miR-181a-3p expression was negatively correlated with the expression of NEMO/IKBKG. Our findings should be confirmed using a larger sample size.

Precursor miR-181a can be processed into two mature strands: miR-181a-3p and miR-181a-5p. MiR-181a belongs to the miR-181 family, its role in tumors is still controversial, and it may function as a tumor promoter or suppressor depending on tumor type.16 In hematologic malignancies, miR-181a functions as a tumor suppressor in cellular division and differentiation. AML patients with higher miR-181 expression at diagnosis have a better prognosis than those with lower miR-181 expression, miR-181 may be a diagnostic biomarker and predictor of prognosis in AML patients.17-19

Precursor miR-181a can be processed into two mature strands: miR-181a-3p and miR-181a-5p. MiR-181a-3p is highly expressed in RPMI8226 cell-derived extracellular vesicles (R-EVs) and regulates cell proliferation.20 Mir-181a-3p blocks the NF-κB signaling pathway by targeting NEMO/IKBKG in Human Umbilical Vein Endothelial Cells (HUVECs), and miR-181a-3p mimics treatment prevents myeloid cell recruitment and decreased the expression of TNF-α in apoE−/− mice.8 NF-κB is an important transcription factor, which plays a crucial cancer-promoting role in Acute myeloid leukemia (AML).21-22 It has been known that chromosomal translocations or gene mutations leading to the increase in NF-κB activity. NEMO/IKBKG acts as a crucial antiapoptotic transcription factor, which is crucial for the activation of NF-κB.23 NF-κB family contains RelA, RelB, NF-κB1, NF-κB2 and Rel. We found that miR-181a-3p expression was negatively correlated with the expression of NEMO/IKBKG. Our findings should be confirmed using a larger sample size.

Table 4. The linear correlation analysis in AML samples. P-values calculated by Pearson's Correlation. 95% CI = 95% confidence interval.

| DATA from TCGA | vs NEMO/IKBKG | vs miR181a-3p |
|----------------|---------------|---------------|
| Person correlation | p-value | Person correlation | p-value |
| NF-κB1 | 0.377 | 2.723e-06 | -0.209 | 1.12e-02 |
| NF-κB2 | 0.598 | 1.653e-15 | -0.555 | 3.66e-13 |
| RelA | 0.557 | 2.844e-13 | -0.19 | 2.184e-02 |
| RelB | 0.524 | 1.13e-11 | -0.309 | 1.478e-04 |
| Rel | -0.013 | 8.799e-01 | -0.121 | 1.45e-01 |
| NEMO/IKBKG | -0.313 | 1.168e-04 | |

59 patients with primary AML

| vs NEMO/IKBKG | vs miR181a-3p |
|---------------|---------------|
| Person correlation | p-value | Person correlation | p-value |
| NF-κB | 0.2601 | 0.0466 | -0.2795 | 0.0321 |
| NEMO/IKBKG | -0.2613 | 0.0456 | |

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expression was negatively correlated with the expression of NF-κB family (NF-κB1, NF-κB2, RelA, RelB) and NEMO/IKBKG in TCGA samples. In our study, miR-181a-3p expression was negatively correlated with the expression of NF-κB and NEMO/IKBKG in 59 AML patients. Maybe mir-181a-3p affects AML cell proliferation and apoptosis by targeting NEMO/IKBKG. We should make more efforts to test this hypothesis.

In summary, we reported a clinical role for miR-181a-3p in AML patients for the first time. MiRNA-181a-3p expression was shown to have value in discriminating AML patients from healthy controls, and to correlate with the response to induction chemotherapy. Patients with higher miR-181a-3p expression levels at diagnosis demonstrated an improved OS. Therefore, the miR-181a-3p expression may be an independent prognostic biomarker for AML patient outcomes.

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**References:**

1. Coombs CC, Tallman MS, Levine RL: Molecular therapy for acute myeloid leukemia. Nature reviews Clinical oncology 2016, 13(5):305-318. https://doi.org/10.1038/nrclinonc.2015.210 PMid:26602072 PMCid:PMC5525060
2. Marcucci G, Mrozek K, Radmacher MD, Garzon R, Bloomfield CD: The prognostic and functional role of microRNAs in acute myeloid leukemia. Blood 2011, 117(4):1121-1129. doi:10.1182/blood-2010-09-191312. https://doi.org/10.1182/blood-2010-09-191312 PMid:21045193 PMCid:PMC3056468
3. Wallace JA, O’Connell RM: MicroRNAs and acute myeloid leukemia: therapeutic implications and emerging concepts. Blood 2017, 130(11):1301-1309. https://doi.org/10.1182/blood-2016-10-697698 PMid:28731524 PMCid:PMC5600138
4. Diaz-Bejar M, Bruzzi S, Nomura J, Tejero R, Diaz T, Pratcorona M, Tormo M, Ribera JM, Escudero L, Duarte R et al: MicroRNA expression at diagnosis adds relevant prognostic information to molecular categorization in patients with intermediate-risk cytogenetic acute myeloid leukemia. Leukemia 2014, 28(4):804-812. https://doi.org/10.1038/leu.2013.281 PMid:24072101
5. Chuang MK, Chiu YC, Chou WC, Hou HA, Chuang EY, Tien HF: A 3-microRNA scoring system for prognostication in de novo acute myeloid leukemia patients. Leukemia 2015, 29(5):1051-1059. https://doi.org/10.1038/leu.2014.333 PMid:25429253
6. Yang Z, Wan X, Gu Z, Zhang H, Yang X, He L, Mao R, Zhong Y, Zhao H: Evolution of the mir-181 microRNA family. Computers in biology and medicine 2014, 52:82-87. https://doi.org/10.1016/j.compbiomed.2014.06.004 PMid:25016292
7. Su R, Lin HS, Zhang XH, Yin XL, Ning HM, Liu B, Zhai PF, Gong JN, Shen C, Song L et al: MiR-181 family: regulators of myeloid differentiation and acute myeloid leukemia as well as potential therapeutic targets. Oncogene 2015, 34(25):3226-3239. https://doi.org/10.1038/onc.2014.274 PMid:25174404
8. Su Y, Yuan J, Zhang F, Lei Q, Zhang T, Li K, Guo J, Hong Y, Bu G, Lv X et al: MicroRNA-181a-5p and microRNA-181a-3p cooperatively restrict vascular inflammation and atherosclerosis. Cell death & disease 2019, 10(5):365. https://doi.org/10.1038/s41419-019-1599-9 PMid:31064980 PMCid:PMC6504957
9. Sun XX, Zhang SS, Dai CY, Peng J, Pan Q, Xu LF, Ma XL: Lusk-S-PV-Regulated MicroRNA-125a-3p Promotes THP-1 Macrophages Differentiation and Apoptosis by Down-Regulating NF1 and Bcl-2. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 2017, 44(3):1093-1105. https://doi.org/10.1159/000485415 PMid:29179212
10. Bullinger L, Dohner K, Dohner H: Genomics of Acute Myeloid Leukemia Diagnosis and Pathways. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2017, 35(9):934-946. https://doi.org/10.1200/JCO.2016.71.2208 PMid:28297624
11. Ferrando AA, Lopez-Otin C: Clonal evolution in leukemia. Nature medicine 2017, 23(10):1135-1145. https://doi.org/10.1038/nm.4410 PMid:28985206
12. Short NJ, Ryting ME, Cortes JE: Acute myeloid leukaemia. Lancet (London, England) 2018, 392(10147):593-606. https://doi.org/10.1016/S0140-6736(18)31041-9
13. Medinger M, Passweg JR: Acute myeloid leukaemia genomics. British journal of haematology 2017, 179(4):530-542. https://doi.org/10.1111/bjh.14823 PMid:28653397
14. de Leeuw DC, Verhagen HJ, Denkers F, Kavelaars FG, Valk PJ, Schuurhuis GJ, Ossenkoppele GJ, Smit L: MicroRNA-515b is highly expressed in hematopoietic stem cells and a biomarker for relapse and poor prognosis in acute myeloid leukemia. Leukemia 2016, 30(3):742-746. https://doi.org/10.1038/leu.2015.160 PMid:26108690
15. Marcucci G, Radmacher MD, Mrozek K, Bloomfield CD: MicroRNA expression in acute myeloid leukemia. Current hematologic malignancy reports 2009, 4(2):83-88. https://doi.org/10.1007/s11899-009-0012-7 PMid:20425419
16. Roth E, Cao Q: MiR-181 suppresses metastasis via MMP-14. Aging 2015, 7(10):740-741. https://doi.org/10.7150/thno.27550 PMid:26527690
17. Schwind S, Maharry K, Radmacher MD, Mrozek K, Holland KB, Margeson D, Whitman SP, Hickey C, Becker H, Metzeler KH et al: Prognostic significance of expression of a single microRNA, miR-181a, in cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010, 28(36):5257-5264. https://doi.org/10.1200/JCO.2010.29.2953 PMid:21079133 PMCid:PMC3018359
18. Buttryn A, Rybka J, Baczyńska D, Poreba R, Mazur G, Kulickowski K: Expression of microRNA-181 determines response to treatment with azacitidine and predicts survival in elderly patients with acute myeloid leukemia. Oncotarget 2016, 7(42):68729-68739. https://doi.org/10.18632/oncotarget.90024 PMid:27698792 PMCid:PMC5038519
19. Weng H, Lal K, Yang FF, Chen J: The pathological role and prognostic impact of miR-181 in acute myeloid leukemia. Cancer genetics 2015, 208(5):225-229. https://doi.org/10.1016/j.cancergen.2014.12.006 PMid:25686674 PMCid:PMC4466607
20. Zhang L, Lei Q, Wang H, Xu C, Liu T, Kong F, Yang C, Yan G, Sun L, Zhao A et al: Tumor-derived extracellular vesicles inhibit osteogenesis and exacerbate myeloma bone disease. Theranostics 2019, 9(1):196-209. https://doi.org/10.7150/thno.27550 PMid:30662562 PMCid:PMC6332790
21. Kagoya Y, Yoshimi A, Kataoka K, Nakagawa M, Kumanoo K, Arai S, Kobayashi H, Saito T, Iwakura Y, Kurokawa M: Positive feedback between NF-kappaB and TNF-alpha promotes leukemia-initiating cell capacity. The Journal of clinical investigation 2014, 124(2):528-542. https://doi.org/10.1172/JCI61801 PMid:24382549 PMCid:PMC3904603
22. Bosman MC, Schuringa JJ, Vellenga E: Constitutive NF-kappaB activation in AML: Causes and treatment strategies. Critical reviews in oncology/hematology 2016, 98:35-44. https://doi.org/10.1016/j.critrevonc.2015.10.001 PMid:26490297

23. Brahler S, Ising C, Barrera Aranda B, Hohne M, Schermer B, Benzing T, Brinkkoetter PT: The NF-kappaB essential modulator (NEMO) controls podocyte cytoskeletal dynamics independently of NF-kappaB. American journal of physiology Renal physiology 2015, 309(7):F617-626. https://doi.org/10.1152/ajprenal.00059.2015 PMid:26268269