MicroRNA-181b-5p insufficiency predicts treatment response failure risk and unfavorable event-free survival as well as overall survival in acute myeloid leukemia patients

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Received November 9, 2021; Accepted February 10, 2022

DOI: 10.3892/ol.2022.13450

Abstract. The present study aimed to explore the correlation of microRNA (miR)-181b-5p expression with treatment response and long-term prognosis in acute myeloid leukemia (AML) patients. miR-181b-5p was detected in the bone marrow of 84 AML patients before therapy. After induction therapy, the patients exhibiting complete remission (CR) were recorded. Next, event-free survival (EFS) and overall survival (OS) were calculated. miR-181b-5p had excellent potential to discriminate AML patients from healthy donors [area under the curve (AUC): 0.922, 95% confidence interval (CI): 0.873-0.971)]. In addition, miR-181b-5p expression was decreased in AML patients with the *FLT3*-ITD mutation (P=0.032) or *WT1* mutation (P=0.017) when compared to AML patients without these genetic mutations. Meanwhile, miR-181b-5p expression was negatively correlated with the National Comprehensive Cancer Network (NCCN) risk classification of AML (P=0.036). Furthermore, miR-181b-5p expression was elevated in CR AML patients compared to non-CR AML patients (P=0.030). Moreover, higher miR-181b-5p expression was associated with favorable accumulating EFS (P=0.001) and OS (P=0.024). In addition, higher miR-181b-5p expression was independently associated with better EFS (hazard ratio: 0.698, P=0.012). In conclusion, miR-181b-5p insufficiency is associated with induction therapy response failure, unfavorable accumulating EFS and OS in AML patients.

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Abbreviations: AML, acute myeloid leukemia; miRNAs, microRNAs; HMGB1, high mobility group protein; Mcl-1, myeloid cell leukemia-1; BMMCs, bone marrow mononuclear cells; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; CR, complete remission; EFS, event-free survival; OS, overall survival; ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval

Key words: miR-181b-5p, acute myeloid leukemia, treatment response, prognosis, induction therapy

Introduction

Acute myeloid leukemia (AML) is a malignancy of the hematopoietic stem cells characterized by the uncontrolled growth of immature myeloid cells in the bone marrow, which interferes with normal hematopoietic function (1,2). Currently, it is the most common acute leukemia among adults, with an occurrence of more than 20,000 cases every year in the US (3). Furthermore, its prognosis is relative unfavorable among all types of leukemia, including the high probability of relapse and low survival rate (2,4-6). Considering that AML is still a malignancy with unsatisfied outcomes dependent on various factors (2), the exploration of novel biomarker for predicting the treatment response of induction therapy and indicating long-term prognosis in AML patients is crucial.

MicroRNAs (miRNAs) participate in various biological processes, including hematopoietic differentiation, proliferation and leukemogenesis (7). Among them, it has been indicated that miR-181b is able to regulate drug sensitivity in AML through targeting high mobility group protein (HMGB1) and myeloid cell leukemia-1 (Mcl-1) (8). Moreover, it also has been illustrated that miR-181b is abnormally expressed in AML compared to the normal populations, as well as it correlates with the treatment response of AML (8-12). Based on the above-mentioned information, we hypothesized that miR-181b-5p expression could be a potential prognostic marker in AML patients who undergo induction therapy. However, such information is obscure.

Therefore, the aim of this study was to explore the correlation of miR-181b-5p with the National Comprehensive Cancer Network (NCCN) risk classification, treatment response and long-term prognosis of AML.

Patients and methods

Subjects. Between January 2016 and December 2019, following approval by the Ethics Committee of Tongji Hospital, School of Medicine, Tongji University (Shanghai, China), 84 *de novo* AML patients and 30 healthy donors were consecutively

recruited in this study. All eligible patients were confirmed as AML rather than acute promyelocytic leukemia, with an age above 18 years, and had no history of other malignancies. All health donors were enrolled after they agreed to donate bone marrow, and the necessary examinations were carried out for them to confirm the eligibility. Pregnant or breast-feeding subjects were excluded from the study. All subjects provided written informed consent.

Clinical data and sample collection. After recording the clinical features of the AML patients, collection of bone marrow sample was performed before they started the induction therapy. Bone marrow samples of 30 healthy donors (age range, 42-65 years; male-to-female ratio, 3:2) were collected during donation. Immediately after sample collection, human bone marrow monocyte separation solution (Beijing Biolabo Technology Co., Ltd.) was used for separation of the bone marrow mononuclear cells (BMMCs), followed by quantitative analysis of miR-181b-5p using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay.

RT-qPCR assay. The RT-qPCR procedures were performed as described in a previous study (11), and the following kits were used: TRIzol™ Reagent (Thermo Fisher Scientific, Inc.) for extraction of total RNA; RT-PCR Quick Master Mix (Toyobo) for reverse transcription; SYBR® Premix DimerEraser™ (Takara Bio, Inc.) for qPCR. The expression of miR-181b-5p was normalized to the *U6* gene, and the relative expression of miR-181b-5p was calculated by the $2^{-\Delta\Delta Cq}$ method (13). The primer sequences for miR-181b-5p were as follows (14): Forward, 5'-GCGGATCATTCATTGCTGTCG-3' and reverse, 5'-ATCTGGTGGCTCTCGGAGTAA-3'. For U6, the forward sequence was 5'-CGCTTCGGCAGCACATATACTA-3' and the reverse sequence was 5'-ATGGAACGCTTCACGAATTTGC-3'.

The expression of miR-181b-5p was classified according to 4 quantiles in survival analyses: quantile 1, miR-181b-5p expression in the interval of 0-25% of total AML patients; quantile 2, miR-181b-5p expression in the interval of 26-50% of total AML patients; quantile 3, miR-181b-5p expression in the interval of 51-75% of total AML patients; quantile 4, miR-181b-5p expression in the interval of 76-100% of total AML patients. In particular, miR-181b-5p expression in the interval of 0-25% of total AML patients was defined as miR-181b-5p insufficiency.

Response data and survival data collection. All patients received standard induction therapy with 3 days of an anthracycline (e.g., daunorubicin, at least 60 mg/m^2, idarubicin, 10-12 mg/m^2, or anthracenedione mitoxantrone, 10-12 mg/m^2) and 7 days of cytarabine (100-200 mg/m^2 cont. i.v.). Complete remission (CR) patients after induction therapy were recorded for the study analysis. Follow-up was conducted every 3 months for the first 2 years, and then surveillance continued every 6 months for the following 2-3 years. The final follow-up date for study was December 31, 2020. Event-free survival (EFS) and overall survival (OS) were calculated based on the recorded date of defined events in the AML guideline (15).

Table I. Characteristics of the patients with AML (n=84).

Item	Value
Age, years	58.4±12.8
>60	42 (50.0)
≤60	42 (50.0)
Male sex	51 (60.7)
FAB classification	
M1	5 (6.0)
M2	28 (33.3)
M4	22 (26.2)
M5	29 (34.5)
Cytogenetic abnormities	
Normal karyotype	41 (48.8)
Complex karyotype	9 (10.7)
Inv(16) or t(16;16)	5 (6.0)
Monosomal karyotype	4 (4.8)
+8	4 (4.8)
t(9;11)	4 (4.8)
-7 or 7q-	3 (3.6)
-5 or 5q-	1 (1.2)
inv(3), t(3;3)	1 (1.2)
t(6;9)	1 (1.2)
t(8;21)	1 (1.2)
Others non-defined	14 (16.7)
Genetic mutations	
NPM1 mutation	25 (29.8)
FLT3-ITD mutation	20 (23.8)
WT1 mutation	10 (11.9)
CEBPA mutation	7 (8.3)
WBCs, 1/l	
>10x10^9	57 (67.9)
≤10x10^9	27 (32.1)
BM blasts, %	
>75	40 (47.6)
≤75	44 (52.4)
Risk classification	
High risk	15 (17.9)
Intermediate risk	44 (52.4)
Low risk	25 (29.8)

Values are expressed as the mean ± standard deviation or n (%). AML, acute myeloid leukemia; FAB, French-American-British; *NPM1*, nucleophosmin 1; *FLT3*-ITD, the internal tandem duplication (ITD) representing the most common type of FMS-like tyrosine kinase 3 (FLT3) mutation; *WT1*, Wilms' tumor 1; *CEBPA*, CCAAT/enhancer binding protein α; WBCs, white blood cells; BM, bone marrow.

Statistical analysis. Data analysis and figure plotting were performed using SPSS 20.0 (IBM Corp.) and GraphPad Prism 6.01 (GraphPad Software Inc.). Distribution characteristics of miR-181b-5p in the different subjects were displayed using a Box plot. Comparison of the expression difference of miR-181b-5p among the different subjects was determined

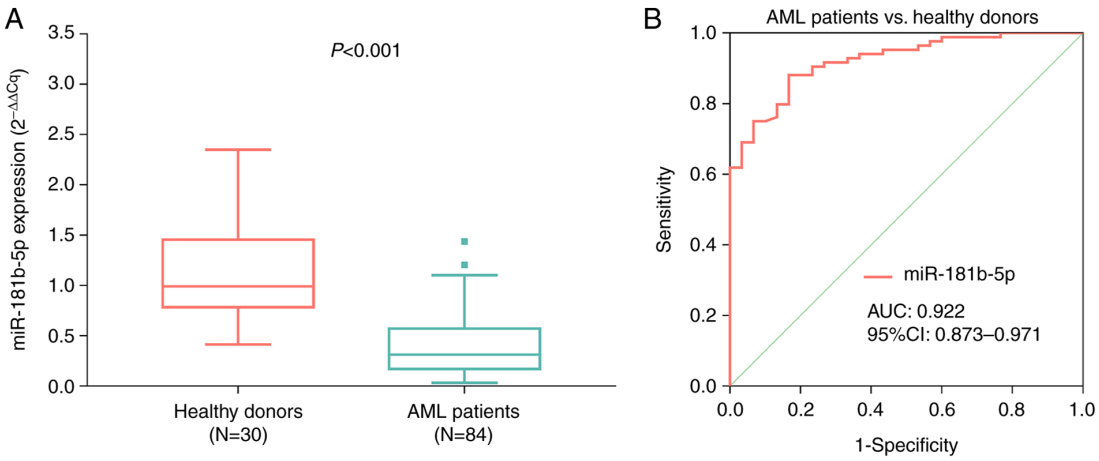


Figure 1. miR-181b-5p in AML patients and healthy donors. (A) Comparison of miR-181b-5p between AML patients and healthy donors. (B) The ability of miR-181b-5p to discriminate AML patients from healthy donors. miR-181b-5p, microRNA-181b-5p; AML, acute myeloid leukemia; AUC, area under the ROS curve; CI, confidence interval.

by Kruskal-Wallis test or Mann-Whitney U test. Correlation analysis between miR-181b-5p expression and NCCN risk classification was determined by Spearman rank correlation test. The receiver-operating characteristic (ROC) curve and area under the curve (AUC) were used for estimating profiles of miR-181b-5p in distinguishing different subjects. Survival data were described using the Kaplan-Meier method. The multiple comparisons of survival data were examined by log-rank test and corrected by Benjamini-Hochberg (B-H) method. Cox proportional hazards regression with forward stepwise method was applied for analysis of the prognostic factors. P-value <0.05 was considered indicative of a statistical significant difference.

Results

Baseline characteristics. For the 84 AML patients, the mean age was 58.4±12.8 years. There were 42 (50.0%) patients >60 years and 42 (50.0%) patients ≤60 years. Moreover, there were 51 (60.7%) males in these AML patients. As for FAB classification, there were 5 (6.0%) patients with M1, 28 (33.3%) patients with M2, 22 (26.2%) patients with M4 and 29 (34.5%) patients with M5. In terms of cytogenetic abnormities, there were 41 (48.8%) patients with a normal karyotype, 9 (10.7%) patients with complex karyotype and 4 (4.8%) patients with a monosomal karyotype. Regarding genetic mutations, there were 25 (29.8%) patients with *NPM1* mutation, 20 (23.8%) patients with *FLT3*-ITD mutation, 10 (11.9%) patients with *WT1* mutation and 7 (8.3%) patients with *CEBPA* mutation. Furthermore, according to risk classification, there were 15 (17.9%) patients with better-risk, 44 (52.4%) patients with intermediate-risk and 25 (29.8%) patients with poor-risk. The detailed characteristics of the AML patients are shown in Table I.

Comparison of miR-181b-5p expression between AML patients and healthy donors. miR-181b-5p expression was reduced in the AML patients [median value, 0.312 (0.170-0.571)] compared to the healthy donors [median value, 0.990 (0.784-1.455)] (P<0.001) (Fig. 1A). Meanwhile, the ROC curve showed that miR-181b-5p had excellent potential in discriminating AML patients from healthy donors with AUC of 0.922 [95% confidence interval (CI): 0.873-0.971]. In addition, miR-181b-5p expression was 0.735 at the best cut-off point (the point with maximum value of the sum of sensitivity and specificity); the sensitivity and specificity were 0.881 and 0.833 at the best cut-off point, respectively (Fig. 1B).

Comparison of miR-181b-5p expression among patients with diverse characteristics. miR-181b-5p expression in patients stratified based on various features is compared in Fig. 2. miR-181b-5p expression was decreased in the patients with *FLT3*-ITD mutation compared to those without *FLT3*-ITD mutation (P=0.032) (Fig. 2F). Moreover, miR-181b-5p expression was attenuated in patients with *WT1* mutation compared to those without *WT1* mutation (P=0.017) (Fig. 2G). In addition, miR-181b-5p expression was highest in patients with better-risk classification, followed by patients with intermediate-risk classification, and lowest in patients with poor-risk classification (P=0.036) (Fig. 2I). However, no difference in miR-181b-5p expression was found among patients with different FAB classification (M1, M2, M4 or M5) (P=0.578) (Fig. 2A). Furthermore, no difference was found in miR-181b-5p expression in patients with or without normal karyotype, complex karyotype, monosomal karyotype, *NMP1* mutation or *CEBPA* mutation (all P>0.05) (Fig. 2B-E and H).

Comparison of miR-181b-5p expression between CR patients and non-CR patients. miR-181b-5p expression was increased in CR patients [median value: 0.339 (0.209-0.595)] compared to non-CR patients [median value: 0.199 (0.068-0.452)] (P=0.030) (Fig. 3A). Meanwhile, the ROC curve illustrated that miR-181b-5p had certain ability in discriminating CR patients from non-CR patients with AUC of 0.656 (95% CI: 0.511-0.802). In addition, miR-181b-5p expression was 0.142 at the best cut-off point; the sensitivity and specificity were 0.903 and 0.500 at the best cut-off point, respectively (Fig. 3B).

Association of miR-181b-5p expression with accumulating EFS. Higher miR-181b-5p expression was correlated with

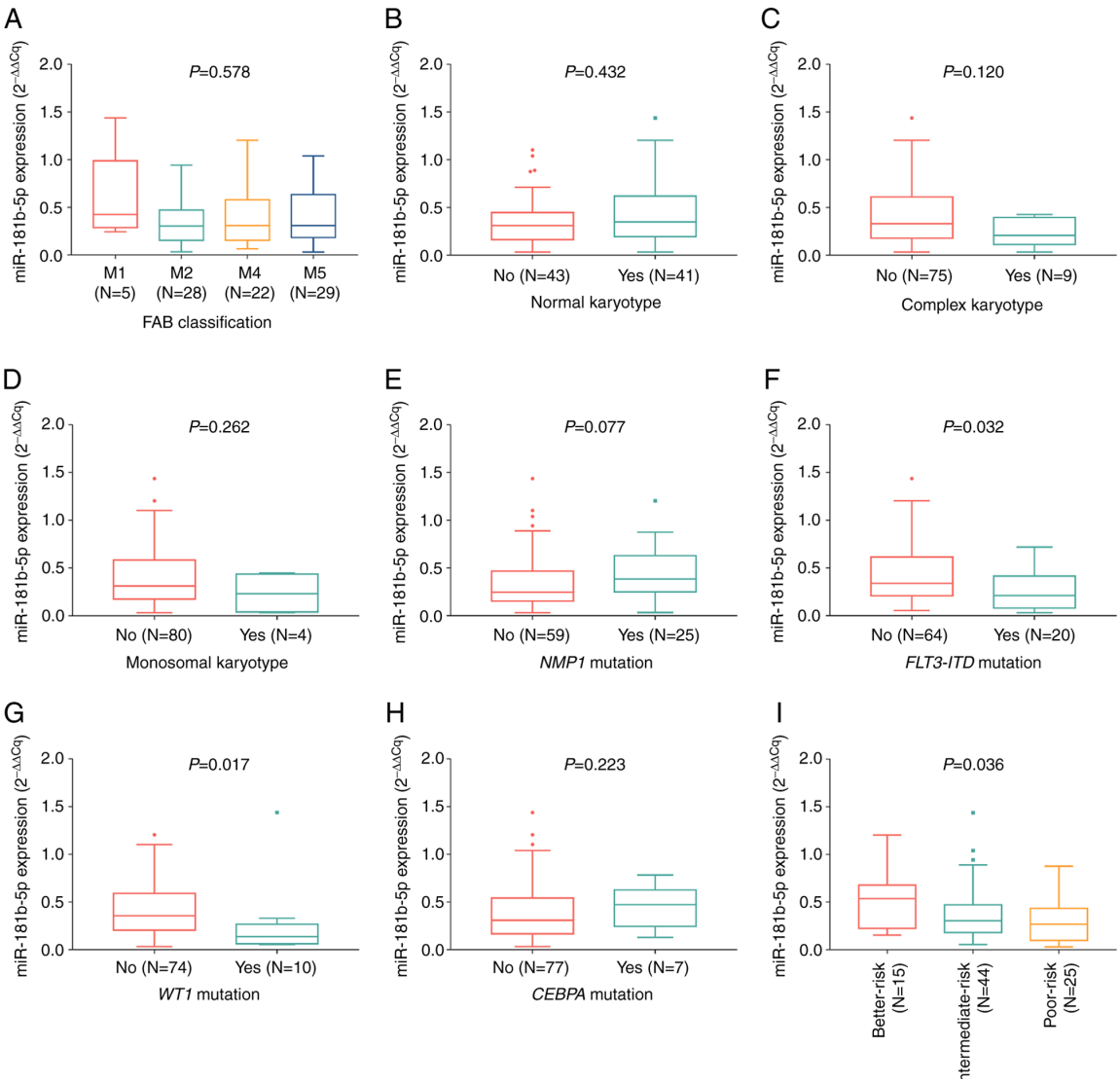


Figure 2. miR-181b-5p in AML patients with distinct clinical features. Association of miR-181b-5p with (A) FAB classification, (B) normal karyotype, (C) complex karyotype, (D) monosomal karyotype, (E) *NMP1* mutation, (F) *FLT3*-ITD mutation, (G) *WT1* mutation, (H) *CEBPA* mutation and (I) National Comprehensive Cancer Network (NCCN) risk classification in AML patients. miR-181b-5p, microRNA-181b-5p; AML, acute myeloid leukemia; FAB, France-American-Britain; *NMP1*, nucleophosmin 1; *FLT3*-ITD, Fms-like tyrosine kinase 3-internal tandem duplication; *WT1*, Wilms' tumor 1; *CEBPA*, CCAAT/enhancer binding protein α.

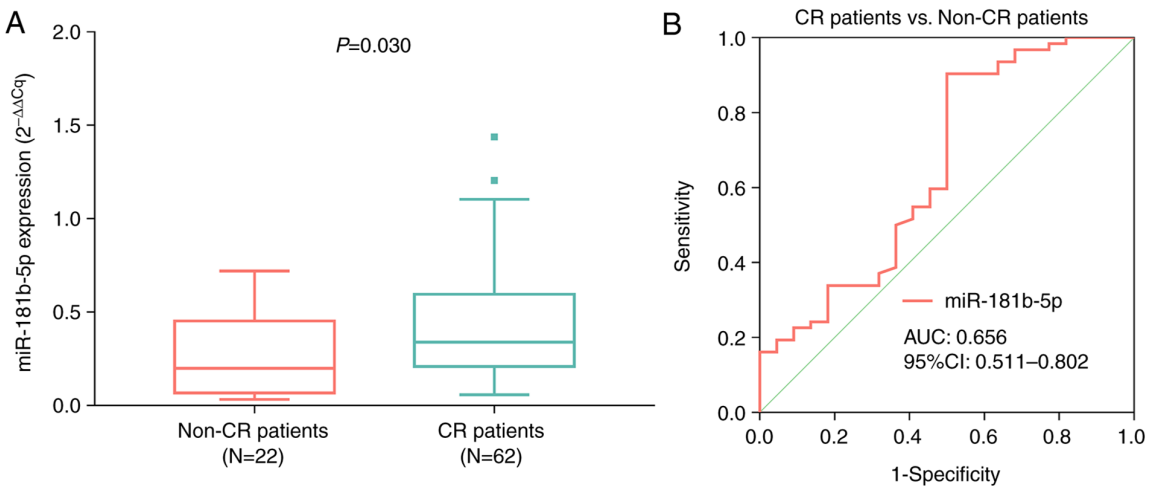
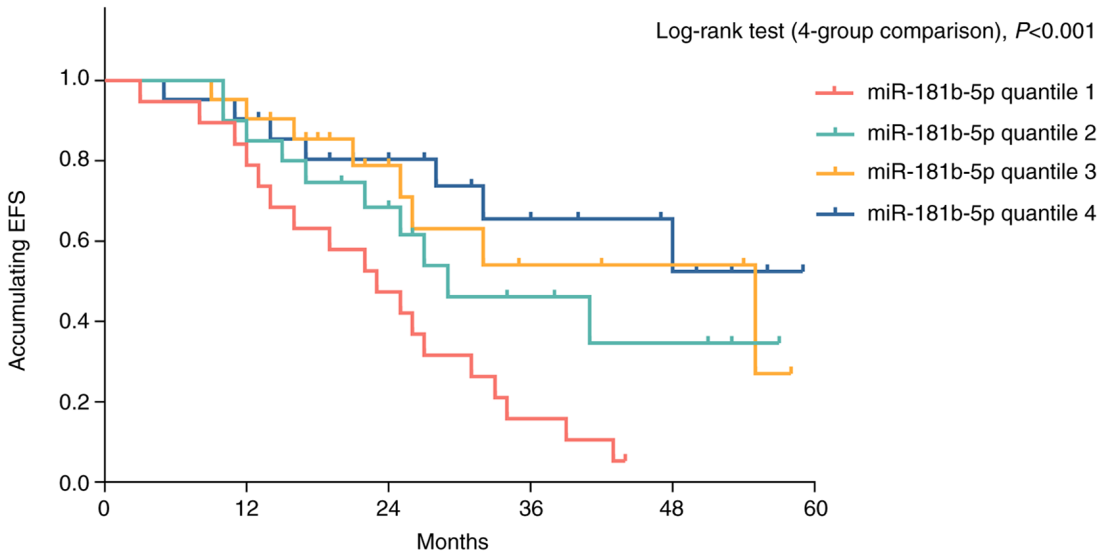


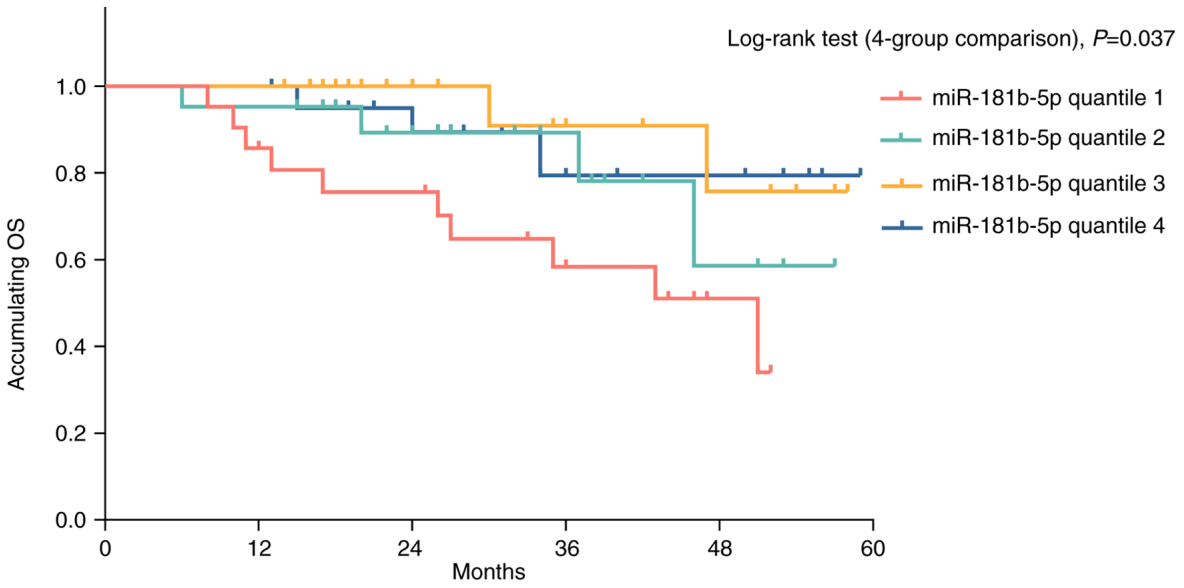
Figure 3. Correlation between miR-181b-5p and CR. (A) Comparison of miR-181b-5p between CR patients and non-CR AML patients. (B) The ability of miR-181b-5p to discriminate CR patients from non-CR patients. miR-181b-5p, microRNA-181b-5p; CR, complete remission; AML, acute myeloid leukemia; AUC, area under the ROS curve; CI, confidence interval.



2-group multiple comparisons (B-H adjusted *P* values)

	Mean EFS (95%CI)	Median EFS (95%CI)	miR-181b-5p quantile 2	miR-181b-5p quantile 3	miR-181b-5p quantile 4
miR-181b-5p quantile 1	21.1 (15.6–26.6)	22.0 (11.5–32.5)	0.076	0.009	<0.001
miR-181b-5p quantile 2	33.5 (24.4–42.5)	29.0 (22.3–35.7)	–	0.475	0.251
miR-181b-5p quantile 3	40.7 (31.6–49.7)	55.0 (23.0–87.0)	–	–	0.588
miR-181b-5p quantile 4	44.0 (35.1–52.9)	–	–	–	–

Figure 4. Correlation between miR-181b-5p and accumulating EFS. miR-181b-5p, microRNA-181b-5p; EFS, event-free survival; CI, confidence interval.



2-group multiple comparisons (B-H adjusted *P* values)

	Mean OS (95%CI)	Median OS (95%CI)	miR-181b-5p quantile 2	miR-181b-5p quantile 3	miR-181b-5p quantile 4
miR-181b-5p quantile 1	37.5 (30.1–44.9)	51.0 (30.3–71.7)	0.280	0.096	0.126
miR-181b-5p quantile 2	48.0 (40.6–55.4)	–	–	0.456	0.738
miR-181b-5p quantile 3	53.8 (48.4–59.1)	–	–	–	0.680
miR-181b-5p quantile 4	52.4 (45.5–52.2)	–	–	–	–

Figure 5. Correlation between miR-181b-5p and accumulating OS. miR-181b-5p, microRNA-181b-5p; OS, overall survival; CI, confidence interval.

Table II. Cox proportional hazards regression analysis for event-free survival.

A, Univariate Cox regression analysis

Item	P-value	HR (95% CI)
Higher miR-181b-5p expression	<0.001	0.589 (0.445-0.780)
Age >60 years	0.778	0.920 (0.515-1.642)
Male sex	0.034	1.977 (1.051-3.718)
FAB classification		
M1	Reference	
M2	0.577	1.521 (0.349-6.631)
M4	0.639	0.694 (0.151-3.197)
M5	0.929	0.935 (0.215-4.066)
Cytogenetic abnormities		
Normal karyotype	0.258	0.709 (0.390-1.287)
Complex karyotype	0.181	1.739 (0.773-3.913)
Monosomal karyotype	0.268	1.947 (0.599-6.328)
Genetic mutations		
NPM1 mutation	0.683	0.868 (0.439-1.715)
FLT3-ITD mutation	0.005	2.414 (1.312-4.443)
WT1 mutation	0.066	2.066 (0.953-4.476)
CEBPA mutation	0.417	0.614 (0.189-1.994)
WBCs >10x10^9/l	0.013	2.450 (1.209-4.968)
BM blasts >75%	0.337	1.329 (0.743-2.377)
Poor risk classification	<0.001	2.432 (1.538-3.846)

B, Forward stepwise multivariate Cox regression analysis

Item	P-value	HR (95% CI)
Higher miR-181b-5p expression	0.012	0.698 (0.528-0.924)
Male sex	0.012	2.353 (1.211-4.571)
Poor risk classification	<0.001	2.476 (1.503-4.079)

HR, hazard ratio; CI, confidence interval; FAB, French-American-British; *NPM1*, nucleophosmin 1; *FLT3*-ITD, the internal tandem duplication representing the most common type of FMS-like tyrosine kinase 3 mutation; *WT1*, Wilms' tumor 1; *CEBPA*, CCAAT/enhancer binding protein α; WBCs, white blood cells; BM, bone marrow.

Table III. Cox proportional hazards regression analysis for overall survival.

A, Univariate Cox regression analysis

Item	P-value	HR (95% CI)
Higher miR-181b-5p expression	0.017	0.583 (0.374-0.908)
Age >60 years	0.311	0.624 (0.251-1.555)
Male sex	0.017	4.490 (1.301-15.490)
FAB classification		
M1	Reference	
M2	0.630	1.678 (0.205-13.748)
M4	0.921	1.112 (0.136-9.107)
M5	0.509	0.477 (0.053-4.298)
Cytogenetic abnormities		
Normal karyotype	0.979	0.988 (0.398-2.452)
Complex karyotype	0.452	1.607 (0.467-5.531)
Monosomal karyotype	0.102	3.477 (0.782-15.459)
Genetic mutations		
NPM1 mutation	0.832	0.895 (0.322-2.491)
FLT3-ITD mutation	0.081	2.301 (0.902-5.871)
WT1 mutation	0.185	2.125 (0.698-6.468)
CEBPA mutation	0.442	0.452 (0.059-3.431)
WBCs >10x10^9/l	0.694	1.215 (0.461-3.204)
BM blasts >75%	0.436	1.441 (0.575-3.611)
Poor risk classification	<0.001	4.947 (2.050-11.941)

B, Forward stepwise multivariate Cox regression analysis

Item	P-value	HR (95% CI)
Male sex	0.004	6.877 (1.881-25.141)
Poor risk classification	<0.001	7.401 (2.836-19.311)

HR, hazard ratio; CI, confidence interval; FAB, French-American-British; *NPM1*, nucleophosmin 1; *FLT3*-ITD, the internal tandem duplication representing the most common type of FMS-like tyrosine kinase 3 mutation; *WT1*, Wilms' tumor 1; *CEBPA*, CCAAT/enhancer binding protein α; WBCs, white blood cells; BM, bone marrow. P-values showing significance differences are indicated in bold print.

increased accumulating EFS (P<0.001). Meanwhile, adjusted multiple comparisons showed that accumulating EFS was attenuated in patients with AML with miR-181b-5p quantile 1 compared to those with miR-181b-5p quantile 3 (P=0.009) and miR-181b-5p quantile 4 (P<0.001). However, no difference in accumulating EFS was found in patients with AML with miR-181b-5p quantile 1 vs. miR-181b-5p quantile 2 (P=0.076), miR-181b-5p quantile 2 vs. miR-181b-5p quantile 3 (P=0.475), miR-181b-5p quantile 2 vs. miR-181b-5p quantile 4 (P=0.251) or miR-181b-5p quantile 3 vs. miR-181b-5p quantile 4 (P=0.588) (Fig. 4). In addition, forward stepwise multivariate Cox regression analysis showed that higher miR-181b-5p expression (HR: 0.698, P=0.012) was independently associated with better EFS (Table II). These above-mentioned data imply that miR-181b-5p insufficiency is correlated with worse EFS.

Association of miR-181b-5p expression with accumulating OS. Higher miR-181b-5p expression was found to be associated with enhanced accumulating OS (P=0.037). Furthermore, adjusted multiple comparisons showed that no difference in accumulating OS was found in patients with AML with miR-181b-5p quantile 1 vs. miR-181b-5p quantile 2 (P=0.280), miR-181b-5p quantile 1 vs. miR-181b-5p quantile 3 (P=0.096), miR-181b-5p quantile 1 vs. miR-181b-5p quantile 4 (P=0.126), miR-181b-5p quantile 2 vs. miR-181b-5p quantile 3 (P=0.456), miR-181b-5p quantile 2 vs. miR-181b-5p quantile 4 (P=0.738), or miR-181b-5p quantile 3 vs. miR-181b-5p quantile 4

(P=0.680) (Fig. 5). In addition, forward stepwise multivariate Cox regression analysis illustrated that higher miR-181b-5p expression was not independently associated with accumulating OS (Table III).

Discussion

In the present study, it was found that: i) miR-181b-5p had excellent potential in discriminating AML patients from non-AML populations; ii) miR-181b-5p insufficiency was correlated with *FLT3*-ITD mutation, *WT1* mutation and poor NCCN risk classification; iii) miR-181b-5p insufficiency was associated with treatment response failure and unfavorable long-term prognosis of AML. Previous research indicated that miR-181a-5p is a prognostic marker for AML (10). To date, only one study has proposed a correlation of miR-181b with treatment response (11), while the correlation of miR-181b-5p with survival in AML remains obscured. To the best of our knowledge, this is the first study to explore the clinical role of miR-181b-5p as a biomarker of both treatment response and survival in AML.

Regarding miR-181b expression in AML patients, it has been demonstrated that miR-181b is abnormally expressed in AML patients compared to healthy populations (11,16). In addition, it also has been illustrated that miR-181b expression is decreased in relapsed/refractory AML patients (17). In this study, we discovered that miR-181b-5p expression was attenuated in AML patients. A possible reason might be that miR-181b-5p could regulate several leukemogenic signaling pathways, including Wnt, protein kinase B and Notch 1 pathways, which are correlated with the pathogenesis of AML (18-23). Therefore, its expression was attenuated in the AML patients.

In terms of the correlation between miR-181b expression and AML clinical features, it has been found that miR-181b expression is correlated with genetic mutations in AML, such as DNA methyltransferase 3 α (*DNMT3a*), tet methylcytosine dioxygenase 2 (*TET2*) and isocitrate dehydrogenase 1/2 (*IDH1/2*) (17). In the present study, it was demonstrated that miR-181b-5p expression was correlated with the *FLT3*-ITD and *WT1* mutation, respectively. This finding was partially consistent with a previous study (17). In addition, insufficient expression of miR-181b-5p was correlated with poor NCCN risk classification of AML. A possible reason might be that *FLT3*-ITD and *WTI* mutations are two important factors involved in NCCN risk classification of AML (24-27). Furthermore, miR-181b-5p expression is associated with the *FLT3*-ITD mutation or *WT1* mutation (as mentioned above). Therefore, miR-181b-5p insufficiency is correlated with poor NCCN risk classification of AML.

As for the association between miR-181b expression and prognosis of AML, it was demonstrated that reduced miR-181b expression is correlated with a lower complete remission (CR) rate in AML patients (17). Another study also illustrated that miR-181b expression is attenuated in AML patients with unfavorable overall survival (OS) (9). In the present study, we discovered that insufficient expression of miR-181b-5p was correlated with lower CR, unfavorable event-free survival (EFS) and OS. Possible explanations may be that: i) decreased expression of miR-181b-5p reduces drug sensitivity via promotion of HMGB1 and Mcl-1 expression (8). Furthermore, miR-181b-5p insufficiency was correlated with poor NCCN risk classification of AML (mentioned above), which could result in the treatment response failure of AML (28). Therefore, miR-181b-5p insufficiency is associated with poor treatment response. ii) Reduced expression of miR-181b-5p might have the capability of inhibiting apoptosis, as well as accelerating the proliferation of AML cells, which may indirectly lead to a worse long-term prognosis of AML patients (9,17,29). Thus, insufficient expression of miR-181b-5p is correlated with the unfavorable survival profile of AML.

In this study, there were several limitations: i) the sample size was not large enough and might have led to reduced strong statistical power in the analyses; ii) the follow-up period was not long enough, thus the association between miR-181b-5p expression and long-term EFS or OS of AML could be investigated in the future; iii) more comprehensive and in-depth understanding of the underlying mechanisms of miR-181b-5p in AML need to be investigated in the future, which may facilitate the development of miR-181b-5p-based treatments; iv) the correlation of miR-181b-5p with other genetic mutations in AML could be explored in the future, such as *DNMT3a*, *TET2* and *IDH1/2*; and v) the correlation of miR-181b-5p with extramedullary diseases could be explored in further study.

In conclusion, miR-181b-5p insufficiency was found to be associated with high disease risk, poor induction therapy response and unfavorable survival of AML, indicating that miR-181b-5p may serve as a potential biomarker in AML and consequently improve the management of AML.

Acknowledgements

Not applicable.

Funding

Not applicable.

Availability of data and materials

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

Authors' contributions

AL and WZ were responsible for the conception of the present study. HL acquired the clinical data. YDi and YDo analyzed and interpreted the data. XL was responsible for statistical analysis. XW and BX made substantial contributions to analysis and interpretation of data, drafted the work and revised it critically for important intellectual content. All authors have read and approved the final manuscript. AL, WZ and HL confirm the authenticity of all the raw data. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Tongji Hospital, School of Medicine, Tongji University (Shanghai,

China) with approval number 279 on 15th April 2019. All subjects provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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