Technology in Cancer Research & Treatment
Volume 19: 1-8

DOI: 10.1177/1533033820945815
journals.sagepub.com/home/tct
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Long Noncoding RNA Maternally Expressed Gene 3 Is Downregulated, and Its Insufficiency Correlates With Poor-Risk Stratification, Worse Treatment Response, as Well as Unfavorable Survival Data in Patients With Acute Myeloid Leukemia

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Abstract

Objective: Our study aimed to investigate the correlation of long noncoding RNA maternally expressed gene 3 expression with clinical features, treatment response, and survival profiles in patients with acute myeloid leukemia. **Methods:** Bone marrow samples of 122 *de novo* patients with acute myeloid leukemia (prior to treatment) and 30 healthy donors (after enrollment) were collected, and long noncoding RNA maternally expressed gene 3 expression was detected by reverse transcription quantitative polymerase chain reaction. According to median value of long noncoding RNA maternally expressed gene 3 expression in patients with acute myeloid leukemia, they were divided into long noncoding RNA maternally expressed gene 3 high expression and low expression patients (which were further categorized as low—, low–, and low- expression patients). **Results:** Long noncoding RNA maternally expressed gene 3 expression was decreased in patients with acute myeloid leukemia compared to healthy donors. Besides, receiver operating characteristic curve displayed that long noncoding RNA maternally expressed gene 3 distinguished patients with acute myeloid leukemia from healthy donors. In patients with acute myeloid leukemia, long noncoding RNA maternally expressed gene 3 low expression was associated with poor-risk stratification but was not correlated with age, gender, French-American-Britain classification, or white blood cell level. For prognosis, complete remission rate was lowest in long noncoding RNA maternally expressed gene 3 low— expression patients, followed by long noncoding RNA maternally expressed gene 3 low– expression patients, long noncoding RNA maternally expressed gene 3 low- expression patients, and was highest in long noncoding RNA maternally expressed gene 3 high expression patients; Kaplan-Meier curves displayed that lower long noncoding RNA maternally expressed gene 3 expression was associated with reduced event-free survival and overall survival; Cox regression analysis showed that lower long noncoding RNA maternally expressed gene 3 expression independently predicted decreased event-free survival and worse overall survival in patients with acute myeloid leukemia. **Conclusion:** Long noncoding RNA maternally expressed gene 3 may function as a novel marker for effective surveillance and management of acute myeloid leukemia.

Keywords

long noncoding RNA maternally expressed gene 3, acute myeloid leukemia, complete remission, event-free survival, overall survival

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Abbreviations

AML, acute myeloid leukemia; BM, bone marrow; BMMCs, BM mononuclear cells; CEBPA, CCAAT/enhancer-binding protein α; CK, complex karyotype; CI, confidence interval; CML, chronic myeloid leukemia; CMR, complete molecular response; CR, complete remission; EFS, event-free survival; FAB, French-American-Britain; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HR, hazard ratio; HSCT, hematopoietic stem cell transplantation; IQR, interquartile range; lncRNA MEG3, long noncoding RNA maternally expressed gene 3; miRNA, microRNA; MK, monosomal karyotype; MM, multiple myeloma; NK, normal karyotype; NPM1, nucleophosmin 1; OS, overall survival; ROC, receiver operating characteristic; RT-qPCR, reverse transcription quantitative polymerase chain reaction; WBC, white blood cell

Received: February 11, 2020; Revised: May 13, 2020; Accepted: June 26, 2020.

Introduction

Acute myeloid leukemia (AML) is one of the most aggressive hematological malignancies characterized by abnormal cell proliferation and differentiation of immature myeloid cells.[1] It has an incidence of 4 per 100 000 adult-cases per year and contributes to around 20% of all hematologic malignancy-related deaths globally.[2,3] Although potentially curative treatments (including intensive chemotherapy and allogenetic stem cell transplantation) have been applied in patients with AML, only a minority of them would be eligible for these therapies (due to intolerance to therapy and lack of matched donors); for patients with AML who receive transplantation, over 50% of them eventually relapse.[4,5] Therefore, seeking convincing biomarkers for disease progression of AML is needed to help conduct effective surveillance and therapies of patients with AML.[3,6,7]

Long noncoding RNA (lncRNA) has been recognized as the critical actor across diverse biological processes, such as cell proliferation, apoptosis, differentiation, and metabolism.[3,7] Long noncoding RNA maternally expressed gene 3 (MEG3), located on chromosome 14q32.3 DLK1 locus, is gradually realized as a tumor suppressor in hematological malignancies.[8,9] For instance, lncRNA MEG3 inhibits cell proliferation and migration but enhances apoptosis of chronic myeloid leukemia (CML) cells and T-cell lymphoblastic lymphoma cells.[10-12] Meanwhile, lncRNA MEG3 has been reported to be downregulated in patients with multiple myeloma (MM) and patients with CML.[13,14] For AML, a few studies disclose that lncRNA MEG3 represses cell proliferation, induces G0/G1 cell-cycle arrest, and promotes apoptosis in AML cells.[10,13] Based on these indications, we speculated lncRNA MEG3 might play a key role in the AML progression; however, no related investigation revealed the clinical implication of lncRNA MEG3 in patients with AML. We had a preliminary study, by which we observed that lncRNA MEG3 was elevated in the bone marrow (BM) samples of a small number of patients with AML compared to healthy controls. In the present study, we aimed to investigate the correlation of lncRNA MEG3 expression with clinical features, treatment response, and survival profiles in patients with AML, so as to explore its clinical significance in AML management.

Methods

Patients and Specimens

In this retrospective study, 122 *de novo* patients with AML treated in our hospital between January 2016 and December 2019 were reviewed, and their BM specimens were obtained from pathology department of our hospital. Patients were eligible if they had (1) confirmed diagnosis of *de novo* AML, (2) age within 18 to 80 years, (3) accessible fresh-frozen BM specimens prior to treatment, (4) complete clinical features prior to treatment, (5) intact response data of induction therapy, and (6) detailed follow-up data. While the following patients were excluded if they had (1) secondary or mixed AML, (2) acute promyelocytic leukemia, (3) severe liver or renal dysfunction before therapy, and (4) history of hematopoietic and lymphoid tissue diseases or other malignancies. Furthermore, 30 healthy BM samples were collected from 30 healthy donors undergoing BM donation in our hospital during same period. This study was approved by the ethics committee of our hospital. All patients or their families signed the informed consents.

Specimen Processing and Data Collection

Bone marrow specimens were processed by density-gradient centrifugation to separate the BM mononuclear cells (BMMCs), then the BMMCs were subjected to reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay for the detection of lncRNA MEG3. Besides, clinical features of patients with AML prior to therapy were collected, which included age, gender, French-American-Britain (FAB) classification, cytogenetics, key molecule genetics, risk stratification, and white blood cell (WBC) level. The response data after 2-cycle standard induction therapy were also collected from the assessment records, where the complete remission (CR) was evaluated according to the response criteria recommended by AML guidelines.[15] The survival data (to date of December 31, 2019) were collected from patients' follow-up records, which were used for the calculation of event-free survival (EFS) and overall survival (OS).

Long noncoding RNA maternally expressed gene 3 Detection by RT-qPCR

TRIzol Reagent (Thermo Fisher Scientific) was used to extract total RNA, and subsequently, reverse transcription to complementary DNA was performed using PrimeScript RT reagent Kit (Perfect Real Time; Takara). The process of qPCR was conducted using SYBR Premix DimerEraser (Takara), and the relative expression of lncRNA MEG3 was calculated by $2^{-\Delta\Delta Ct}$ formula as follows: (1) qPCR was performed in triplicate, and the average values of lncRNA MEG3 Ct and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) Ct in every sample were determined, respectively; (2) calculations of $\triangle Ct$ ($Ct_{avg.lncRNA\ MEG3} - Ct_{avg.\ GAPDH}$) were presented in every sample, which was shown as $\triangle Ct_{(sample)}$; (3) the median of $\triangle Ct$ in control was referred as the calibrator, which was shown as $\triangle Ct_{(calibrator)}$; (4) $\triangle\triangle Ct = \triangle Ct_{(sample)} - \triangle Ct_{(calibrator)}$; and (5) the relative expression of lncRNA MEG3 was proceeded via calculating $2^{-\triangle\triangle Ct}$. In addition, GAPDH was used as the internal reference. Sequences of primers were as follows: lncRNA MEG3, forward: TACACCTCACGAGGGCACTA, reverse: CAGGGCTTAATGCCCAATGC; GAPDH, forward: TGACCACAGTCCATGCCATCAC, reverse: GCCTGCTTCACCACCTTCTTGA.

Treatment and Follow-Up

All patients with AML received standard "3 + 7" regimen for induction therapy, which comprised of 3 days of an anthracycline (eg, daunorubicin, at least 60 mg/m^2, idarubicin, 10-12 mg/m^2, or the anthracenedione mitoxantrone, 10-12 mg/m^2) and 7 days of cytarabine (100-200 mg/m^2 continuous intravenous). After 2 cycles of induction therapy, response to induction treatment was evaluated by serial peripheral blood counts and repeat BM examinations. The CR was defined as blast clearance in the BM to <5% of all nucleated cells, morphologically normal hematopoiesis, and return of peripheral blood cell counts to normal levels. The subsequent treatment, such as consolidation therapy, hematopoietic stem cell transplantation (HSCT), or palliative systemic treatment, was administered based on response status after induction therapy. Among 95 CR patients, 14 patients underwent HSCT, and remaining patients received consolidation therapy due to no suitable donor or personal conditions; for non-CR patients, repeated induction therapy or palliative systemic treatment was administered. Besides, clinical visit was conducted every 3 to 6 months or as clinically indicated, and the median follow-up duration was 19.0 months, ranging from 2.0 to 46.0 months.

Statistical Analysis

SPSS version 24.0 was used for statistical analyses, and GraphPad Prism version 7.01 was used for figure plotting. Data were described as mean and SD, median and interquartile range, or number (percentage). For the statistical analysis, patients with AML were divided into lncRNA MEG3 high expression group (in the 50th to 100th percentile) and lncRNA MEG3 low expression group (in the 0 to 50th percentile) by the median value of lncRNA MEG3 relative expression in all patients with AML. In response and survival analysis, lncRNA MEG3 low expression patients were further classified as lncRNA MEG3 low- expression (in the 25th to 50th percentile), lncRNA MEG3 low– expression (in the 10th to 25th percentile), and lncRNA MEG3 low— expression (in the 0 to 10th percentile). Long noncoding RNA maternally expressed gene 3 expression difference between patients with AML and healthy donors was determined by Wilcoxon rank-sum test. Comparisons of clinical features and induction therapy response between or among patients with different lncRNA MEG3 expressions were determined by χ^2 test or Wilcoxon rank-sum test. Receiver operating characteristic (ROC) curve was used to evaluate the value of lncRNA MEG3 in differentiating patients with AML and healthy donors. Event-free survival was evaluated from the date of initial therapy to the date of documentation of failure to induce CR within 2 cycles, relapse or death.[16] Overall survival was evaluated from the date of initial therapy to the date of death. Kaplan-Meier curves were plotted to display the EFS and OS, and the difference of EFS and OS between/among groups was determined by the log-rank test. Factors related to EFS and OS were analyzed by backward stepwise multivariate Cox proportional hazard regression model. A P value <.05 was considered statistically significant.

Results

Characteristics of Patients With AML

In all, 122 patients with AML (including 79 [64.8%] males and 43 [35.2%] females) were enrolled in this study, with the mean age of 54.1 ± 14.3 years and the median age of 56.0 (43.8-65.0) years (Table 1). For disease subtypes, 45 (36.9%), 33 (27.0%), and 44 (36.1%) patients were with FAB classification M2, M4, and M5, respectively; regarding cytogenetics, 65 (53.3%), 14 (11.5%), 11 (9.0%), 4 (3.3%), 4 (3.3%), 4 (3.3%), 2 (1.6%), 1 (0.8%), 1 (0.8%), 1 (0.8%), and 15 (12.3%) patients showed normal karyotype (NK), complex karyotype (CK), inv(16) or t(16;16), −7 or 7q−, t(9;11), t(9;22), +8, 11q23, t(6;9), t(8;21), and others (nondefined), respectively; as to risk stratification, 52 (42.6%), 41 (33.6%), and 29 (23.8%) patients were classified as favorable-risk, intermediate-risk, and poor-risk, respectively. The detailed information of other characteristics is summarized in Table 1.

Comparison of lncRNA MEG3 Expression Between Patients With AML and Healthy Donors

Long noncoding RNA maternally expressed gene 3 expression was decreased in patients with AML compared to healthy donors ($P < .001$; Figure 1A). Besides, ROC curves displayed that lncRNA MEG3 distinguished AML from healthy donors (area under the curve: 0.848, 95% CI: 0.768-0.927; Figure 1B), with the sensitivity of 83.3% and specificity of 78.7% at the best cutoff value.

Table 1. Characteristics of Patients.

Items	Patients with AML, N = 122
Age (years)	
Mean ± SD	54.1 ± 14.3
Median (IQR)	56.0 (43.8-65.0)
Gender, n (%)	
Male	79 (64.8)
Female	43 (35.2)
FAB classification, n (%)	
M2	45 (36.9)
M4	33 (27.0)
M5	44 (36.1)
Cytogenetics, n (%)	
NK	65 (53.3)
CK	14 (11.5)
inv(16) or t(16;16)	11 (9.0)
−7 or 7q−	4 (3.3)
t(9;11)	4 (3.3)
t(9;22)	4 (3.3)
+8	2 (1.6)
11q23	1 (0.8)
t(6;9)	1 (0.8)
t(8;21)	1 (0.8)
Others (nondefined)	15 (12.3)
MK, n (%)	10 (8.2)
Gene mutations, n (%)	
FLT3-ITD mutation	29 (23.8)
Isolated biallelic CEBPA mutation	12 (9.8)
NPM1 mutation	44 (36.1)
Risk stratification, n (%)	
Favorable-risk	52 (42.6)
Intermediate-risk	41 (33.6)
Poor-risk	29 (23.8)
WBC level ($\times 10^9$/L)	
Mean ± SD	21.6 ± 21.4
Median (IQR)	17.7 (9.5-28.5)

Abbreviations: AML, acute myeloid leukemia; CEBPA, CCAAT/enhancer-binding protein α; CK, complex karyotype; FAB, French-American-Britain; FLT3-ITD, internal tandem duplications in the FMS-like tyrosine kinase 3; IQR, interquartile range; MK, monosomal karyotype; NK, normal karyotype; NPM1, nucleophosmin 1; WBC, white blood cell.

Correlation of lncRNA MEG3 Expression With Clinical Features in Patients With AML

In patients with AML, lncRNA MEG3 low expression was associated with poor-risk stratification ($P = .034$). No association of lncRNA MEG3 expression with age ($P = .714$), gender ($P = .185$), FAB classification ($P = .359$), cytogenetics ($P = .469$), monosomal karyotype (MK; $P = 1.000$), FLT3-ITD mutation ($P = .288$), isolated biallelic CCAAT/enhancer-binding protein α (CEBPA) mutation ($P = .068$), nucleophosmin 1 (NPM1) mutation ($P = .258$), or WBC level ($P = .839$) was observed in patients with AML (Table 2). Notably, we chose the value of WBC $\geq 10.0 \times 10^9$/L according to previous studies.[17,18]

Correlation of lncRNA MEG3 Expression With CR Achievement in Patients With AML

Totally, of the 122 patients, 95 (77.9%) achieved CR, but 27 (22.2%) patients did not (Figure 2A). Besides, CR rate was decreased in lncRNA MEG3 low expression patients compared to lncRNA MEG3 high expression patients ($P = .016$; Figure 2B). In detail, 42 (68.9%) of 61 lncRNA MEG3 low expression patients achieved CR, but 19 (31.1%) lncRNA MEG3 low expression patients did not achieve CR; 53 (86.9%) of 61 lncRNA MEG3 high expression patients achieved CR, but 8 (13.1%) lncRNA MEG3 high expression patients did not achieve CR (Figure 2B). Moreover, in order to further investigate whether lncRNA MEG3 expression was related to the CR achievement, we divided lncRNA MEG3 low expression patients into lncRNA MEG3 low— expression patients, lncRNA MEG3 low– expression patients, and lncRNA MEG3 low- expression patients, and the further analysis displayed that CR rate was lowest in lncRNA MEG3 low— expression patients (58.3%), followed by lncRNA MEG3 low– expression patients (66.7%), lncRNA MEG3 low- expression patients (74.2%), and highest in lncRNA MEG3 high expression patients (86.9%; $P = .008$; Figure 2C).

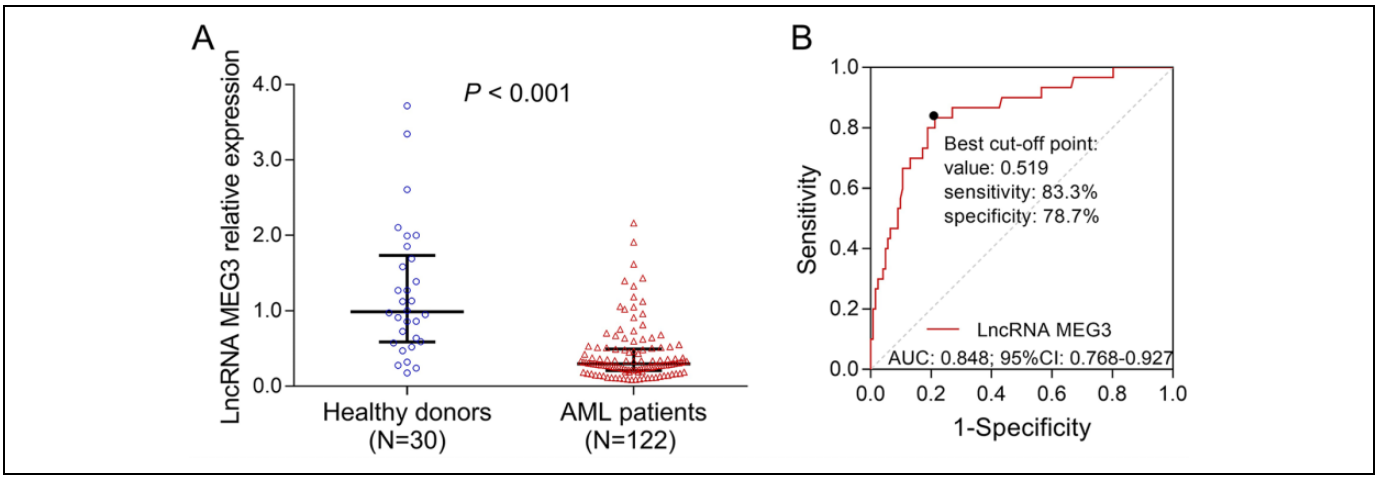

Figure 1. LncRNA MEG3 expression in patients with AML and healthy donors. LncRNA MEG3 expression in patients with AML and healthy donors (A). ROC curve for lncRNA MEG3 to distinguish patients with AML from healthy controls (B). AML indicates acute myeloid leukemia; AUC, area under the curve; LncRNA MEG3, long noncoding RNA maternally expressed gene 3; ROC, receiver operating characteristic.

Table 2. Association of lncRNA MEG3 With Clinical Features of Patients With AML.

Features	lncRNA MEG3		*P* value
	Low	High	
Age (years), n (%)			.714
<60	36 (59.0)	34 (55.7)	
≥60	25 (41.0)	27 (44.3)	
Gender, n (%)			.185
Male	43 (70.5)	36 (59.0)	
Female	18 (29.5)	25 (41.0)	
FAB classification, n (%)			.359
M2	24 (39.3)	21 (34.4)	
M4	13 (21.4)	20 (32.8)	
M5	24 (39.3)	20 (32.8)	
Risk stratification, n (%)			.034
Favorable-risk	9 (14.8)	20 (32.8)	
Intermediate-risk	28 (45.9)	24 (39.3)	
Poor-risk	24 (39.3)	17 (27.9)	
Cytogenetics, n (%)			.469
NK	33 (54.1)	32 (52.5)	
CK	9 (14.8)	5 (8.2)	
inv(16) or t(16;16)	2 (3.3)	9 (14.8)	
−7 or 7q−	2 (3.3)	2 (3.3)	
t(9;11)	3 (4.9)	1 (1.6)	
t(9;22)	2 (3.3)	2 (3.3)	
+8	1 (1.6)	1 (1.6)	
11q23	0 (0.0)	1 (1.6)	
t(6;9)	0 (0.0)	1 (1.6)	
t(8;21)	1 (1.6)	0 (0.0)	
Others (nondefined)	8 (13.1)	7 (11.5)	
MK, n (%)			1.000
No	56 (91.8)	56 (91.8)	
Yes	5 (8.2)	5 (8.2)	
FLT3-ITD mutation, n (%)			.288
No	44 (72.1)	49 (80.3)	
Yes	17 (27.9)	12 (19.7)	
Isolated biallelic CEBPA mutation, n (%)			.068
No	52 (85.2)	58 (95.1)	
Yes	9 (14.8)	3 (4.9)	
NPM1 mutation, n (%)			.258
No	42 (68.9)	36 (59.0)	
Yes	19 (31.1)	25 (41.0)	
WBC level, n (%)			.839
$<10 \times 10^9$/L	17 (27.9)	16 (26.2)	
$\geq 10 \times 10^9$/L	44 (72.1)	45 (73.8)	

Abbreviations: AML, acute myeloid leukemia; CEBPA, CCAAT/enhancer-binding protein α; CK, complex karyotype; FAB, French-American-Britain; FLT3-ITD, internal tandem duplications in the FMS-like tyrosine kinase 3; MK, monosomal karyotype; NK, normal karyotype; NPM1, nucleophosmin 1; WBC, white blood cell.

Correlation of lncRNA MEG3 Expression With EFS and OS in Patients With AML

Event-free survival was decreased in lncRNA MEG3 low expression patients compared to lncRNA MEG3 high expression patients ($P = .012$; Figure 3A). Besides, further analysis showed that EFS was shortest in lncRNA MEG3 low— expression patients, followed by lncRNA MEG3 low– expression patients and lncRNA MEG3 low- expression patients, and was longest in lncRNA MEG3 high expression patients ($P = .011$; Figure 3B**)**. As to OS, it was reduced in lncRNA MEG3 low expression patients compared to lncRNA MEG3 high expression patients ($P = .008$; Figure 4A). Furthermore, OS was shortest in lncRNA MEG3 low— expression patients, followed by lncRNA MEG3 low– expression patients, lncRNA MEG3 low- expression patients, and was longest in lncRNA MEG3 high expression patients ($P = .002$; Figure 4B).

Analysis of Factors Affecting EFS and OS in Patients With AML

Backward stepwise multivariate Cox regression analysis was used to assess the factors affecting EFS, which displayed that lower lncRNA MEG3 expression ($P = .021$; hazard ratio [HR]: 1.282 [95% CI, 1.038-1.582]) was an independent factor predicting decreased EFS, meanwhile poor-risk stratification ($P < .001$; HR: 2.494 [95% CI: 1.760-3.536]) and increased WBC ($\geq 10.0 \times 10^9$/L; $P < .001$; HR: 2.892 [95% CI: 1.641-5.096]) independently predicted worse EFS in patients with AML as well (Table 3). For the factors affecting OS, lower lncRNA MEG3 expression ($P = .024$; HR: 1.385 [95% CI: 1.045-1.836]) was an independent predictive factor for unfavorable OS, and poor-risk stratification ($P < .001$; HR: 2.746 [95% CI: 1.646-4.583]) also independently predicted worse OS in patients with AML.

Discussion

Some emerging studies focus on the influence of lncRNA MEG3 in pathology of hematological malignancies.[10,12,19-21] For example, lncRNA MEG3 represses cell migration and invasion via suppressing the phosphoinositide 3-kinase/mechanistic target of rapamycin pathway and inhibits drug resistance through decreasing the P-glycoprotein in T-cell lymphoblastic lymphoma cells.[12] Besides, lncRNA MEG3 represses cell proliferation via sponging miR-21 in CML cells.[14] Furthermore, lncRNA MEG3 enhances apoptosis in imatinib-resistant human leukemic cells.[19] Also, lncRNA MEG3 is downregulated and inhibits cell proliferation, induces G0/G1 cell-cycle arrest, and promotes apoptosis in AML cell lines.[10,21] These studies reveal that lncRNA MEG3 may have anticancer effect in hematological malignancies, including AML.

A few clinical practices also uncover the downregulation of lncRNA MEG3 in patients with hematological malignancies, including T-cell lymphoblastic lymphoma, MM, and CML.[13,14,22] For AML, little evidence discloses the clinical significance of lncRNA MEG3 expression in patients with AML. Hence, our study enrolled 122 *de novo* patients with AML and 30 healthy donors, and we observed that lncRNA MEG3 was downregulated in patients with AML compared to healthy controls; meanwhile, it showed a well performance in distinguishing patients with AML from healthy controls. These results might be due to the fact that lncRNA MEG3 might

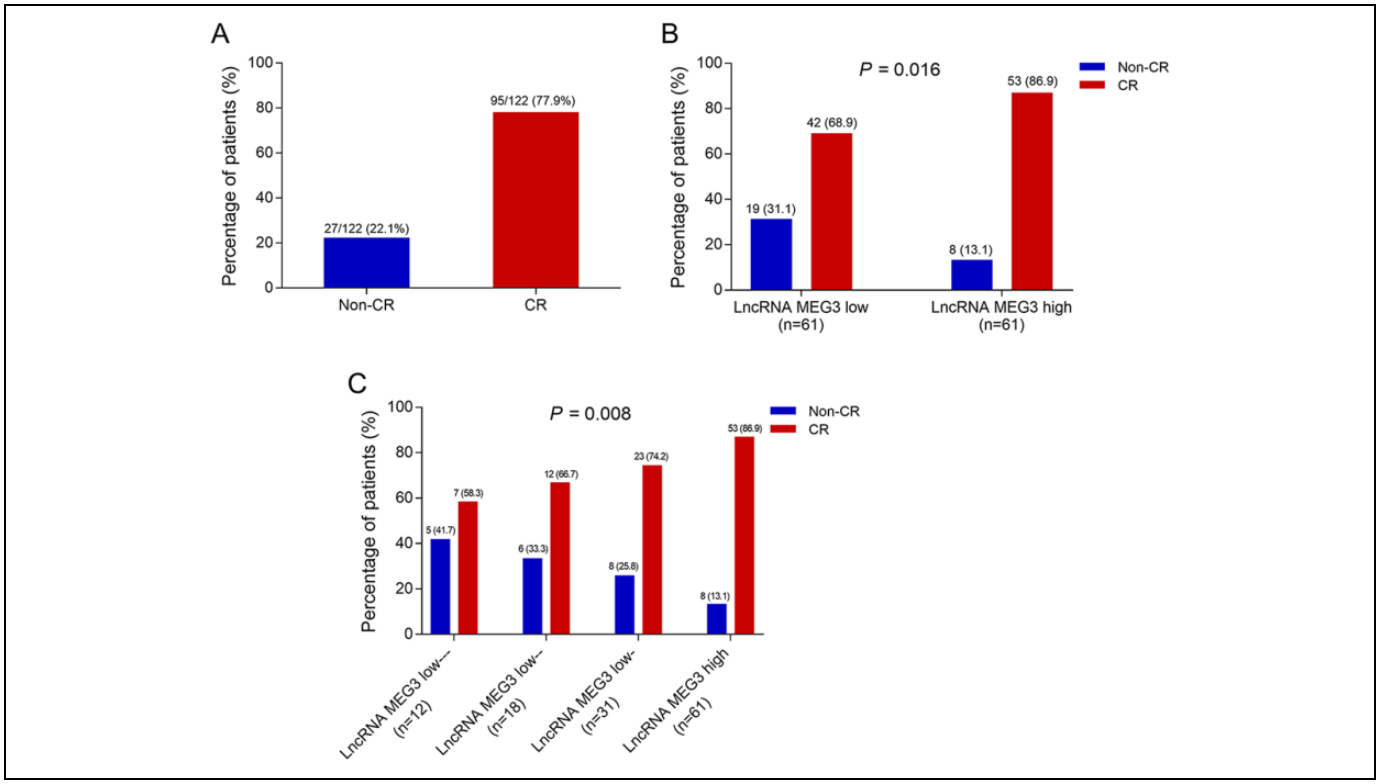

Figure 2. Complete remission (CR) achievements in patients with acute myeloid leukemia. Percentages of CR patients and non-CR patients (A). Percentages of CR patients in lncRNA MEG3 low expression patients and lncRNA MEG3 high expression patients (B). CR rate in lncRNA MEG3 low— expression patients, lncRNA MEG3 low– expression patients, lncRNA MEG3 low- expression patients, and lncRNA MEG3 high expression patients (C). lncRNA MEG3 indicates long noncoding RNA maternally expressed gene 3.

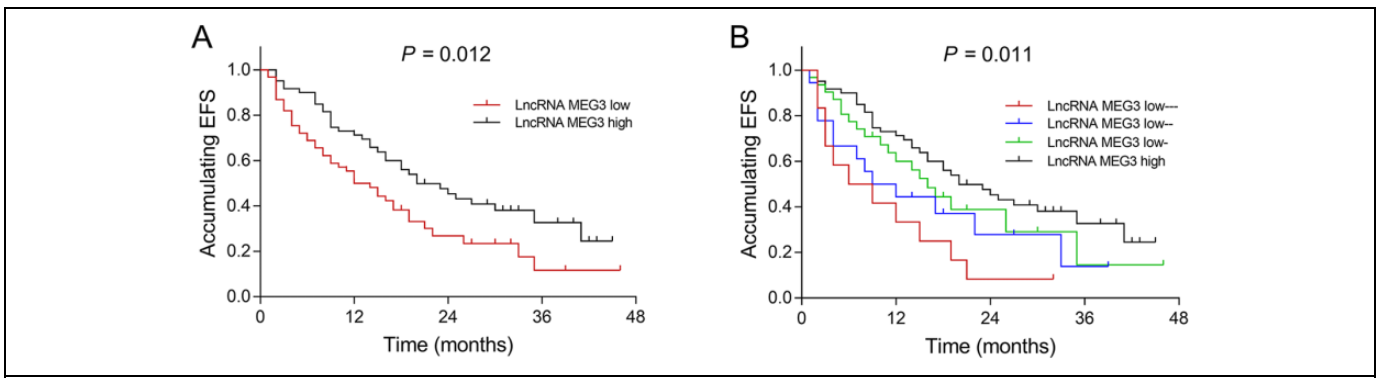

Figure 3. Event-free survival (EFS) in patients with acute myeloid leukemia. EFS in lncRNA MEG3 low expression patients and lncRNA MEG3 high expression patients (A). EFS in lncRNA MEG3 low— expression patients, lncRNA MEG3 low– expression patients, lncRNA MEG3 low- expression patients, and lncRNA MEG3 high expression patients (B). lncRNA MEG3 indicates long noncoding RNA maternally expressed gene 3.

inhibit cell proliferation via sponging targeted microRNAs (miRNAs, such as sponging miR-21 in CML as we discussed above), while its downregulated expression failed to inhibit AML cell proliferation and further promoted the initiation of AML.[11] Notably, the patients' samples were almost completely "leukemic" while the healthy mononuclear fraction contained mature or differentiating cells; therefore, the composition of the evaluated samples between patients with AML and healthy controls was different, which might result in the difference of lncRNA MEG3 expression. Whereas the common detection of lncRNA expression (or other genes) in healthy controls (in comparison of patients with leukemia) was detecting lncRNA from BMMCs of healthy donors, which was observed in many previous studies.[23-25] Besides, these findings reflected the actual lncRNA expressions in patients with AML and healthy controls and suggested the clinical significance of lncRNA MEG3 in AML, no matter whether the difference of lncRNA MEG3 expression was affected by the different composition of

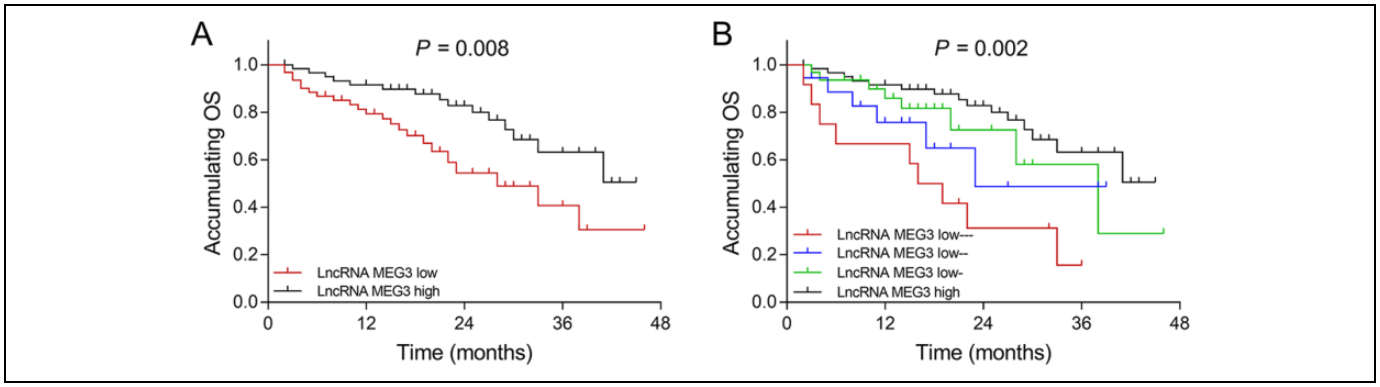

Figure 4. Overall survival (OS) in patients with acute myeloid leukemia. OS in lncRNA MEG3 low expression patients and lncRNA MEG3 high expression patients (A). OS in lncRNA MEG3 low— expression patients, lncRNA MEG3 low– expression patients, lncRNA MEG3 low- expression patients, and lncRNA MEG3 high expression patients (B). lncRNA MEG3 indicates long noncoding RNA maternally expressed gene 3.

Table 3. Backward Stepwise Multivariate Cox Regression Analysis of EFS and OS.

	Cox proportional hazard regression model			
			95% CI	
Items	*P* value	HR	Lower	Higher
EFS				
Lower lncRNA MEG3[a]	.021	1.282	1.038	1.582
Poor-risk stratification	<.001	2.494	1.760	3.536
WBC ($\geq$10.0 $\times$ 10^9/L)	<.001	2.892	1.641	5.096
OS				
Lower lncRNA MEG3[a]	.024	1.385	1.045	1.836
Poor-risk stratification	<.001	2.746	1.646	4.583

Abbreviations: EFS, event-free survival; HR, hazard ratio; OS, overall survival; WBC, white blood cell.
[a]LncRNA MEG3 was categorized as lncRNA MEG3 high = 0, lncRNA MEG3 low- = 1, lncRNA MEG3 low– = 2, and lncRNA MEG3 low— = 3.

samples. Furthermore, we also discovered that lncRNA MEG3 low expression was associated with poor-risk stratification in patients with AML, and this might be caused by the fact that lncRNA MEG3 insufficiency might influence risk stratification via affecting cytogenetics or molecular abnormalities (such as CK, inv(16), or t(16;16) and isolated biallelic CEBPA mutation), although no statistical significance was observed in our data; thus, lncRNA MEG3 low expression was correlated with poor-risk stratification in patients with AML, while the relevant mechanism was still not clear, which needed further investigation.

Dysregulated lncRNA MEG3 may not only reflect the abnormities in clinical features but also correlate with prognosis in patients with hematological malignancies.[13,19] For instance, lncRNA MEG3 expression is elevated in patients with CML who achieve complete molecular response (CMR) compared to the patients with CML who do not achieve CMR[19]; patients with MM with lncRNA MEG3 high expression exhibit longer EFS compared to patients with MM with lncRNA MEG3 low expression.[13] In our study, we found that lower lncRNA MEG3 expression was correlated with the less CR achievement, decreased EFS, and reduced OS in patients with AML. These results could be explained as follows: (1) lncRNA MEG3 could repress cell proliferation, migration, and invasion but enhance apoptosis through regulating specific signaling pathways (such as deactivating JAK/STAT signaling pathway) or sponging targeted miRNAs (such as sponging miR-21); thus, it contributed to improve the treatment efficacy and its insufficient expression was correlated with less CR achievement[11,14]; (2) lncRNA MEG3 could facilitate the treatment response (as we discussed above), which led to the increased possibility of longer survival in patients with AML; thus, lower lncRNA MEG3 expression was correlated with worse EFS and OS; (3) lncRNA MEG3 might decrease the drug resistance in AML cells and eventually improved the treatment outcomes; thus, lncRNA MEG3 insufficiency might fail to decrease drug resistance and eventually led to poor survival profiles; and (4) lncRNA MEG3 insufficiency resulted in poor-risk stratification (as abovementioned), which led to aggravated disease progression and worse survival profiles in patients with AML.[12]

There were still some limitations in our study: (1) The recruited patients with AML in our study mainly came from the North China, which might limit the generalizability of our findings; (2) only the lncRNA MEG3 expression prior to treatment was detected, while the change of lncRNA MEG3 expression from treatment initiation to the date of treatment response assessment, relapse, or death was not detected in this present study, which was needed to be further investigated; (3) patients with relapsed AML were not enrolled in this study, so a validation of our findings in a further study enrolling patients with relapsed AML was necessary; (4) the difference of lncRNA MEG3 expression might be influenced by the different composition of the evaluated samples (the "leukemic" samples of patients with AML and BMMC samples of healthy controls), while the common detection of lncRNA expression in healthy controls was detecting lncRNA from BMMCs of healthy donors referring to previous studies, and further detections

were needed to validate our findings; and (5) sample sizes of patients with AML and healthy controls were both relatively small, and further study with larger sample size was needed.

In conclusion, lncRNA MEG3 is downregulated, and its insufficiency correlates with poor-risk stratification, worse treatment response, as well as unfavorable survival data in patients with AML.

Authors' Note

This study was approved by the Ethics Committee of The Second Affiliated Hospital of Shaanxi University of Chinese Medicine (approval no. SZFYIEC-PJ-2019 No.29). All patients or their families signed the informed consents.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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