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Up-regulation of serum miR-4262 predicts clinical outcome of patients with acute myeloid leukemia

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Abstract. – OBJECTIVE: The aim of this study was to investigate whether miR-4262 was associated with the prognosis of acute myeloid leukemia (AML) patients.

PATIENTS AND METHODS: Serum and bone marrow miR-4262 expression were investigated in 186 AML patients and 115 healthy normal controls by using Real-time PCR. Associations between miR-4262 expressions and various clinicopathological characteristics were analyzed. Overall survival and relapse-free survival were evaluated using the Kaplan-Meier method. Univariate and multivariate analysis were performed using the Cox proportional hazard analysis.

RESULTS: Expression levels of miR-4262 in the bone marrow and serum of AML patients were both significantly higher than those in healthy controls (both $p<0.01$). Moreover, increased miR-4262 expression was significantly associated with FAB classification ($p=0.001$) and cytogenetics ($p=0.001$). Patients with high miR-4262 expression had poorer overall survival time and relapse-free survival time than those with low miR-4262 expression. Finally, multivariate analysis identified high miR-4262 expression as an independent prognostic factor for AML ($p<0.001$).

CONCLUSIONS: Our data revealed that the evaluation of miR-4262 expression in the bone marrow and serum was an ideal tool for predicting the progression of AML and prognosis.

Key Words
miR-4262, Acute myeloid leukemia, Prognosis.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous malignant disease, which arises from the differentiation arrest of myeloid precursor and malignant proliferation in bone marrow and blood[1,2]. Despite recent advances in our understanding of the mechanisms of leukemogenesis, the clinical outcome of AML is still poor in elder patients[3]. The identification of sensitive and specific AML biomarkers, which can recognize the patients who are at the risk of poor outcome and the development of new therapeutic approaches, is essential. miRNAs are small, noncoding RNA molecules that modulate gene expression and regulate many cellular processes[4]. It has become increasingly clear that aberrant expression of miRNAs is an important regulator in various cancers, including AML[5-7]. A variety of miRNAs have been linked to deregulated oncogenic or tumor suppressor pathways[8]. For instance, Xiao et al[9] showed that miR-223 was downregulated in AML patients compared with healthy subjects, and miR-223 inhibits cell proliferation and enhances cell apoptosis in AML cells via targeting FBXW7. Sharifi et al[10] showed that miR-92a may play a positive regulator in progression of AML through modulation of p63 expression. Organista-Nava et al[11] found that miR-24 upregulation was associated with poor prognosis in AML. Thus, exploring these miRNAs, which could be used to predict prognosis of AML patients, was very important. MiR-4262 is a newly identified miRNA and has been shown to be an effective biomarker and active tumor promoter in several tumors including hepatocellular carcinoma[12] and melanoma[13]. However, whether miR-4262 was associated with the progression of AML has not been reported. In the present study, we aimed to analyze the correlation between miR-4262 dysregulation and clinical characteristics and prognosis.

Patients and Methods

Patients and Tissue Samples

Between 2008 and August 2011, a total of 186 blood and narrow bone samples from AML patients were collected in the Department of Hematology, Jining No. 1 People's Hospital.

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AML diagnosis was made in accordance with the revised French-American-British (FAB) classification. All patients were followed up until December 2016 or death. The control group consisted of 80 healthy volunteers. None of these controls had previously been diagnosed with any malignancy or other benign disease. The mean age of the patients and control subjects was 45±5 years and 38±9 years, respectively. This study was approved by the Research Ethics Committee of Jining No. 1 People's Hospital, China. Written informed consent was obtained from all of the patients according to the committee's regulations.

Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

The isolation of miRNA from serum samples was performed with the miRNeasy™ RNA isolation kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. To verify mature miRNA expression levels, qRT-PCR was performed using the standard TaqMan® miRNA assay protocol on an ABI7500 Real-time PCR System. Cycling conditions were 95°C for 10 min followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. The primers were designed and produced by Invitrogen (Carlsbad, CA, USA). Each sample was carried out in triplicate. The qRT-PCR data were normalized using $2^{-\Delta\Delta Ct}$ method relative to U6 small nuclear RNA.

Statistical Analysis

The statistical analyses were performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). The difference in serum and bone narrow miR-4262 expression levels was determined by the Mann-Whitney test. X^2-test was used to analyze the relationship between miR-4262 expression levels and the clinicopathological characteristics. Overall survival and relapse-free survival curves were analyzed with the Kaplan-Meier method and compared using the log-rank test. Multivariate survival analysis was performed using the Cox proportional hazards model. The factors selected from univariate analysis. $p<0.05$ was considered indicative of statistical significance.

Results

MiR-4262 was up-regulated in bone marrow and serum of AML patients

To determine the expression levels of miR-4262 in AML patients and normal controls, Real-time PCR was performed. The results showed that miR-4262 expression was significantly increased in the bone narrow of AML patients compared with the normal controls ($p<0.01$ Figure 1A). Similarly, the serum miR-4262 level was significantly higher in AML patients when compared with normal controls ($p<0.01$ Figure 1B). Those data revealed that miR-4262 may play a positive regulator in AML.

miR-4262 was correlated with the clinicopathological features of AML

To investigate the association between miR-4262 expression and clinicopathological parameters, the 186 blood samples were divided into two subgroups (low and high expression group) based on their miR-4262 expression. Table I showed increased miR-4262 expression was significantly associated with FAB classification ($p=0.001$) and cytogenetic ($p=0.001$). However, there were no significant associations between miR-4262 expression and other clinical features including age, gender, WBC, complete remission (all $p>0.05$).

Relationship between miR-4262 expression and survival outcomes in AML patients

We further examined whether miR-4262 expression correlated with outcome in AML patients. Results of the Kaplan-Meier survival analysis showed that patients with high levels of serum miR-4262 had a poorer overall survival rate than those with low levels of miR-4262 (Figure 2, $p<0.001$). Moreover, the high serum miR-4262 expression group also exhibited a significantly lower 5-year relapse-free survival than the low serum miR-4262 expression group (Figure 3, $p<0.001$).

Prognostic factors determined by univariate and multivariate Cox regression analysis

Finally, univariate and multivariate analyses were utilized to evaluate whether miR-4262 expression level was independent prognostic parameters of AML patient outcomes. Our results showed that miR-4262 expression level, cytogenetic and FAB classification were independent prognostic indicators for overall survival ($p<0.05$, Table II). These findings suggest that serum miR-4262 expression is a biomarker of poor prognoses in AML patients.

Table I. Correlation of serum miR-4262 expression with clinicopathological features of AML.

Variable	No. of cases	miR-4262 expression		*p*
		Low (n %)	High (n %)	
Gender				0.351
Male	103	50	53	
Female	83	46	37	
Age				0.519
<55	116	62	54	
≥55	70	34	36	
WBC				0.715
<10	54	29	25	
≥10	132	67	65	
Blast in BM				0.493
<50%	75	41	34	
≥50%	111	55	56	
FAB classification				**0.001**
M1-M6	146	85	61	
M7	40	11	29	
Complete remission				0.809
Y	112	57	55	
N	74	39	35	
Cytogenetics				**0.001**
Favorable	55	46	9	
Intermediate	71	32	39	
Unfavorable	60	18	42	

Table II. Univariate and multivariate Cox regression analyses for overall survival.

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*		*p*
Cytogenetics				
Favorable/intermediate vs. unfavorable	2.147 (1.337-4.883)	0.007	1.896 (1.156-4.135)	0.011
FAB classification	2.783 (1.547-5.592) M1-M6 vs. M7	0.003	2.341 (1.138-4.882)	0.004
MiR-4262 expression	3.114 (1.611-6.873) Low vs. High	0.001	2.791 (1.355-5.421)	0.001

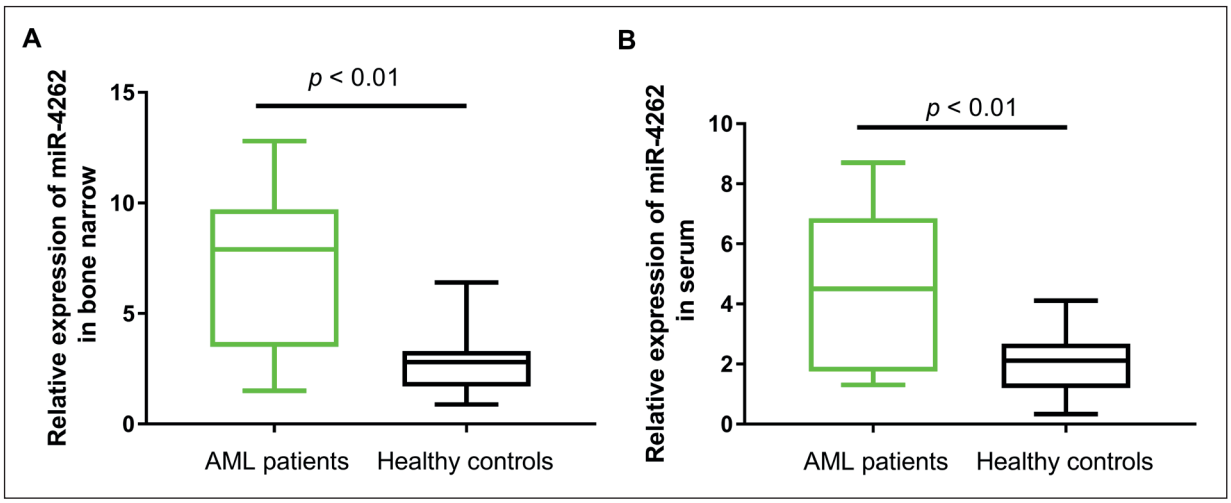

Figure 1. Over-expression of miR-4262 in AML patients as determined by RT-PCR.

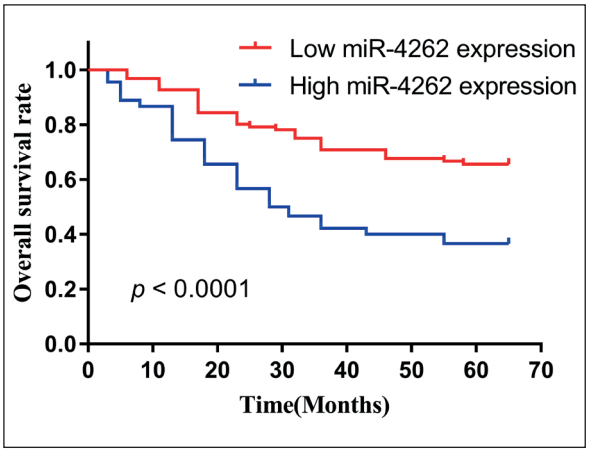

Figure 2. Kaplan-Meier curves of the overall survival of 186 AML patients. Patients with high levels of miR-4262 expression were correlated with a poor overall survival in AML patients (p<0.001).

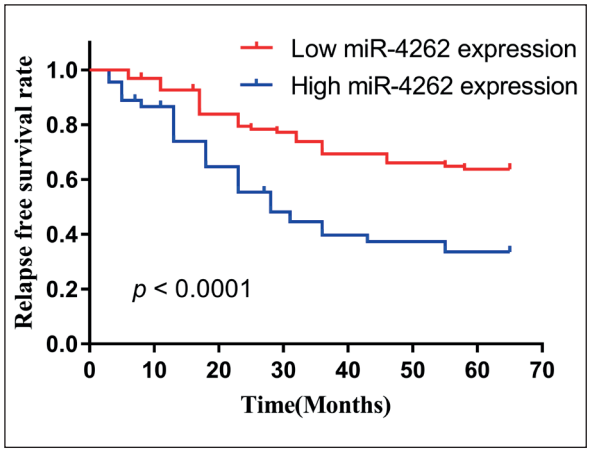

Figure 3. Kaplan-Meier curves of the overall survival of 186 AML patients. Patients with high levels of miR-4262 expression were correlated with a poor relapse-free survival in AML patients (p<0.001).

Discussion

Increasing evidence has shown that miRNAs are involved in cancer development[14,15]. Recently, many miRNAs from cancer tissues have been observed to be dysregulated in various cancers[16,17]. However, MiRNAs are not only expressed in cancer tissues but also in sera. More importantly, circulating miRNAs are more stable in serum and plasma compared with those in tissues[18]. A previous study[19] showed that miRNAs in blood may originate from the damaged cells or circulating cells, suggesting that circulating miRNAs may be used as predictors for diagnosis and prognosis of tumor patients. Indeed, several miRNAs such as miR-215[20] and miR-34c[21] have been reported to be associated with poor clinical outcome in AML. In the current study, we are looking forward to the discovery of suitable circulating miRNAs for prognosis of AML.

As a newly found miRNA, the effect of miR-4262 has remained largely elusive. Previous studies showed that miR-4262 functions either as oncogenes or tumor suppressor genes depending on various cancer types. For instance, Lu et al[12] found that miR-4262 expression was up-regulated in HCC, and it enhances the proliferation of hepatocellular carcinoma cells by activating the NF-kB. Zhang et al[13] showed that miR-4262 promoted the proliferation of human cutaneous malignant melanoma cells by targeting KLF6. However, findings by Song et al[22] indicated the levels of miR-4262 were significantly decreased in osteosarcoma tissues. Moreover, they proved that miR-4262 overexpression inhibited tumor progression via modulation of osteopontin in osteosarcoma. The above findings revealed that miR-4262 played an important role in the carcinogenesis process. However, to our best knowledge, the role of plasma miR-4262 in AML during the carcinogenesis process of AML has never been studied.

In the present study, we first found the expression of miR-4262 was significantly upregulated in the bone narrow and serum of AML patients and positively associated with FAB classification and cytogenetic. Meanwhile, Kaplan-Meier survival analyses and log-rank tests further showed that patients with high miR-4262 expression had poorer overall survival time and relapse-free survival time than those with low miR-4262 expression, suggesting its potential prognostic value for AML. Furthermore, according to multivariate analysis, increased miR-4262 expression was an independent poor prognostic factor for pancreatic patients.

Conclusions

We provided compelling clinical evidence that miR-4262 can be served as an independent prognostic marker for survival in AML. Additional investigation is required to confirm the findings before the clinical application of miR-4262.

Conflict of interest

The authors declare no conflicts of interest.

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