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Original Article

Serum miR-150 as a novel prognostic biomarker for acute myeloid leukemia

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Abstract: Acute myeloid leukemia (AML) is a hematological malignancy with dismal prognosis. Aberrant expression of microRNAs (miRNAs) is a common feature during the tumorigenesis of AML. miR-150 is downregulated in AML and might act as a tumor suppressor in this malignant disease. However, the prognostic value of serum miR-150 in AML has not been intensively studied. In the present study, we firstly detected the expression pattern of serum miR-150 in AML patients and healthy control subjects using real-time PCR. Then various analyses were performed to evaluate the prognostic significance of serum miR-150 in AML. Serum miR-150 levels were significantly lower in AML patients especially those with M5 subtype or poor risk cytogenetic. Serum miR-150 levels increased significantly in those patients who achieved complete remission. In addition, serum miR-150 levels were associated with FAB classification, percentage of blast in bone marrow and cytogenetics. AML patients with lower serum miR-150 expression had shorter overall survival and event free survival. Multivariate analysis revealed that serum miR-150 was an independent prognostic factor for AML. Collectively, our results demonstrate that serum miR-150 levels are reduced in AML and might have potential prognostic value for this deadly malignancy.

Keywords: miR-150, acute myeloid leukemia, biomarker, prognosis

Introduction

Acute myeloid leukemia (AML) is a genetically heterogeneous disease characterized by uncontrolled proliferation of hematopoietic progenitor cells [1]. The treatment methods for AML includes chemotherapy, targeted therapy, immune regulation therapy and bone marrow transplant [2]. The complete remission rate and long-term prognosis has been significantly improved in the past few decades. However, the prognosis of AML remains poor, with the 5-year survival rate is less than 30%. Therefore it is imperative to explore the underlying molecular mechanisms and novel biomarkers associated with the progression of this malignancy.

MicroRNAs (miRNAs) are a class of evolutionarily conserved, endogenous, small non-coding RNAs which regulate gene expression mainly by promoting mRNA degradation or inhibiting mRNA translation [3]. A growing number of studies have demonstrated miRNAs are not only play an important role in the progression of various tumors including AML, but also significantly associated with clinical outcome [4, 5]. Thus miRNAs have become attractive molecular biomarkers for predicating the prognosis of cancer. For instance, miR-370 levels were significantly reduced in bone marrow and serum samples from patients with AML. In addition, decreased serum miR-370 levels were found to be a risk factor associated with poor prognosis of AML [6]. Serum miR-210 was overexpressed in AML and correlated with unfavorable clinicopathological variables. Moreover, its levels decreased at a large scale in those patients who achieved complete remission [7], indicating serum miR-210 might be used for monitoring the therapeutic responses in the clinical setting.

Aberrant expression of miR-150 has been shown in various types of cancer such as non-small cell lung cancer, pancreatic cancer, breast cancer and AML [8-11]. The expression level of plasma miR-150 was found to be reduced in AML patients compared with that in healthy

Table 1. Correlation between serum miR-150 levels and the clinicopathological parameters of AML

Parameters	Cases	Serum miR-150		*P*
		Low (n=41)	High (n=44)	
Gender				0.152
Male	40	16	24	
Female	45	25	20	
Age				0.247
<60	59	26	33	
≥60	26	15	11	
Blast in bone marrow (%)				0.020
<50	38	13	25	
≥50	47	28	19	
WBC ($\times 10^9$/L)				0.911
<10	43	21	22	
≥10	42	20	22	
PLT ($\times 10^9$/L)				0.221
<50	46	25	21	
≥50	39	16	23	
Extramedullary disease				0.490
No	57	26	31	
Yes	28	15	13	
FAB classification				0.013
M0	6	3	3	
M1	17	3	14	
M2	33	16	17	
M4	20	11	9	
M5	9	8	1	
Cytogenetics				0.012
Favorable	15	4	11	
Intermediate	43	18	25	
Poor	27	19	8	

controls [11]. Also, miR-150 promoted myeloid differentiation of acute leukemia cells, indicating loss of miR-150 might contribute to progression of AML [12]. Therefore, miR-150 probably plays a tumor suppressive role in the development of AML. The aim of current study was to evaluate the prognostic significance of serum miR-150 in AML.

Materials and methods

Patients and serum samples

This study was approved by the local ethic committee of the Second Hospital of Shandong University and informed consents were obtained from each participant. Eighty-five newly diagnosed patients with AML and 30 healthy individuals presenting to our department were enrolled in this study. The AML subtypes were classified based on French-American British (FAB) classification system. The criterion for defining complete remission (CR) was as follows: No blast cells in peripheral blood and no more than 5% blasts in the bone marrow; absolute neutrophil counts ≥1,500/μl and platelet counts ≥100,000/μl in peripheral blood; and no extramedullary disease (e.g., CNS, soft tissue diseases). The clinical information of AML patients were listed in **Table 1**. The serum samples were collected from AML patients before receiving any kind of therapy. All blood samples were centrifuged at 1,200×g for 15 min at room temperature. The supernatant was then aliquoted and stored at -80°C for further use.

Real-time quantitative polymerase chain reaction (RT-qPCR)

RNA was isolated from 200 μL of serum with QIAgen miRNeasy Mini Kit (QIAgen, Hilden, Germany) according to manufacturer's recommendations. The quantity and integrity of total RNA were assessed using a NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Caenorhabditis elegans miRNA-39 (cel-miR-39) was added for normalization. Complementary DNA was synthesized using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Amplification of cDNA was performed with SYBR Green Real-Time PCR Master Mixes (Applied Biosystems). RT-qPCR was conducted using the 7900 HT Fast Real-Time PCR System (Applied Biosystems). The serum miR-150 level was calculated by the 2(-Delta Delta C(T)) method relative to cel-miR-39 expression.

Statistical analysis

The expression level of serum miR-150 was compared among different groups using Mann-Whitney U test or Krusal-Wallis test. Association between clinical variables and was evaluated with the Chi-squared test. Kaplan-Meier curves and log-rank tests were used to examine the correlation of serum miR-150 levels with overall survival and event free survival. A multivariate Cox regression model was used to examine the

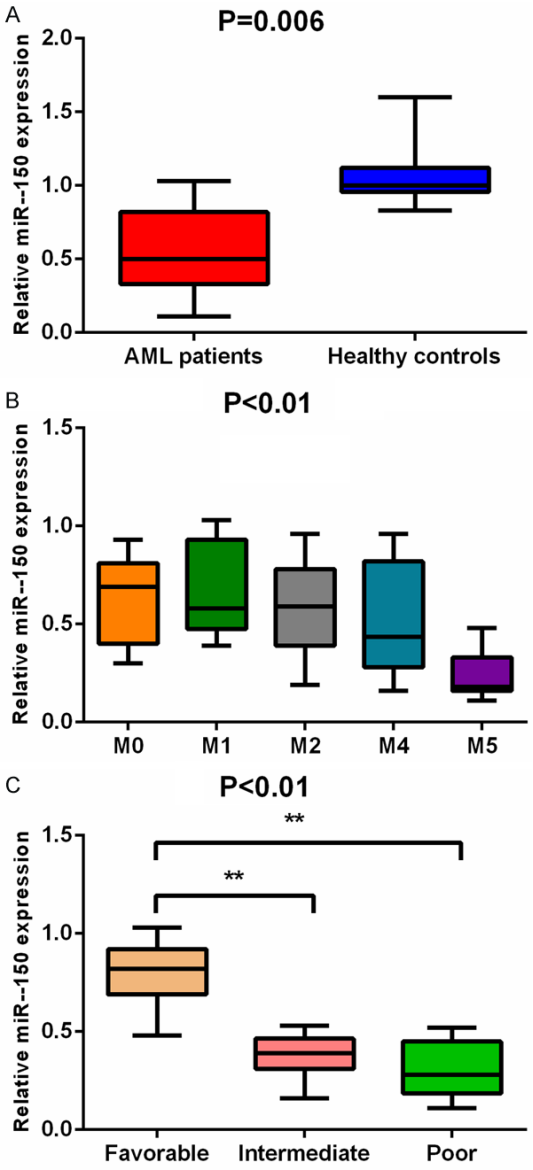

Figure 1. Serum miR-150 was reduced in patients in AML.

independent contribution of each variable to overall survival. Differences with P<0.05 were considered statistically significant. All *P* values were 2-sided. All the data were analyzed using the GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA) and SPSS 20.0. software (SPSS, Inc., Chicago, IL, USA).

Results

Serum miR-150 levels were downregulated in AML patients

We first compared the expression levels of miR-150 in the serum samples from 85 AML

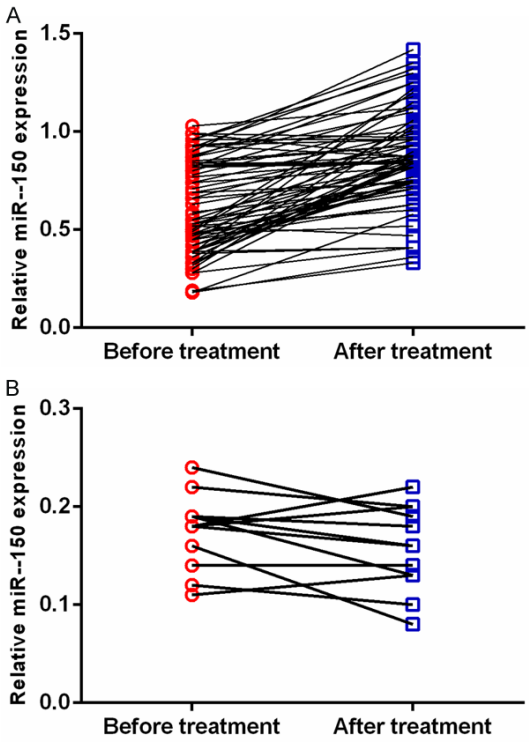

Figure 2. Serum miR-150 was upregulated in AML patients who achieved CR.

patients and 30 healthy controls subjects by real-time PCR. Our results showed that serum miR-150 levels were significantly downregulated in AML patients compared with that in the controls (P=0.006) (**Figure 1A**). In addition, the AML patients with the M5 subtype had lower serum miR-150 levels than those with other subtypes including M0, M1, M2 and M4 (P< 0.01). Although no significant difference in the serum miR-150 was found among patients with M0, M1, M2 and M4, serum miR-150 was progressively downregulated from M0 to M4 subtypes (**Figure 1B**). AML patients in the poor or intermediate risk cytogenetic group had remarkably reduced serum miR-150 levels than the patients in the favorable risk cytogenetic group (P<0.01) (**Figure 1C**).

Serum miR-150 levels were upregulated in patients who achieved CR

We then compared the expression level of serum miR-150 in the AML patients before and after treatment. Our analysis showed that serum miR-150 levels were significantly increased in the AML patients who achieved CR (P<0.01) (**Figure 2A**). However, no significant changes in the serum miR-150 levels were found in AML patients who did not achieved CR (**Figure 2B**).

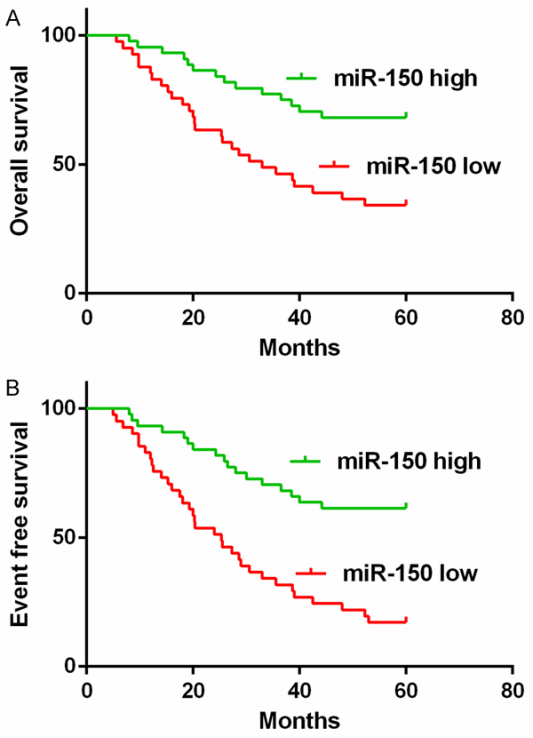

Figure 3. The prognostic significance of serum miR-150 in patients with AML.

The association between serum miR-150 and clinicopathological parameters of AML

The correlation between serum miR-150 levels and clinicopathological variables was further analyzed. Our results showed that serum miR-150 was significantly associated with FAB classification (P=0.013), percentage of blast in bone marrow (P=0.020) and cytogenetics (P=0.012). However, no significance difference was found between serum miR-150 and gender, age, WBC number, PLT number and extramedullary disease (P>0.05) (**Table 1**).

The prognostic significance of serum miR-150 in AML

We evaluated the prognostic value of serum miR-150 in patients with AML. Our survival analysis showed that AML patients in the low serum miR-150 group had significantly shorter five year overall survival (P=0.009) (**Figure 3A**) and event free survival (P=0.004) (**Figure 3B**) than the patients in the high serum miR-150 group.

Our multivariate analysis demonstrated that serum miR-150 was an independent prognostic factor (P=0.012, HR=1.752, 95%=1.242-3.309) for overall survival in patients with AML (**Table 2**).

Discussion

In this study, we first compared miR-150 levels in the serum samples from patients with AML and healthy control subjects. Then the prognostic significance of miR-150 was further analyzed. Our data showed that the expression level of serum miR-150 was drastically decreased in AML patients especially those with M5 subtype or poor risk cytogenetic. Serum miR-150 levels increased significantly in those patients who achieved CR. Serum miR-150 levels were associated with FAB classification, percentage of blast in bone marrow and cytogenetics. AML patients in the low serum miR-150 group suffered a significantly lower five year overall and event free survival than those in the high serum miR-150 group. More importantly, reduced serum miR-150 was demonstrated to be an independent risk factor for AML. These data further corroborate that miR-150 functions as a tumor suppressor in AML and its downregulation promotes the progression of this malignant disease.

Previous study also reported that miR-150 levels were decreased in other hematological disorders such as acute lymphoblastic leukemia, chronic myeloid leukemia and malignant lymphoma [13, 14]. Thus whether serum miR-150 levels can discriminate AML from other types of hematopoietic malignancies needs further investigation. In addition, future studies with larger sample size are needed to confirm the clinical significance of serum miR-150 in AML.

Consistent to the findings of our study, miR-150 was downregulated in leukemia stem cells (LSCs) and clinical samples. Ectopic expression of miR-150 suppressed the biological behaviors of LSCs both *in vitro* and *in vivo* by influencing Nanog signaling pathway [15]. Similarly, the expression levels of serum miR-150 were decreased in AML. Overexpression of miR-150 promoted myeloid differentiation of cancer cells and suppressed their proliferation [16]. In addition to AML, miR-150 also acted as a tumor suppressor in other types of cancer. For instance, miR-150 was downregulated in hepatocellular carcinoma (HCC) samples compared with the adjacent normal tissues. In addition,

Table 2. Multivariable analysis with overall survival in the patients with AML

Variable	Overall survival	
	HR (95% CI)	*P*
Gender (Male vs female)	1.032 (0.803-1.869)	0.427
Age (years) (≥60 vs <60)	1.431 (0.985-2.583)	0.055
Blast in bone marrow (%) (<50 vs ≥50)	1.381 (0.971-2.490)	0.082
WBC ($\times 10^9$/L) (≥10 vs <10)	1.153 (0.882-2.072)	0.238
PLT ($\times 10^9$/L) (<50 vs ≥50)	1.239 (0.918-2.344)	0.108
Extramedullary disease (Yes vs no)	1.192 (0.907-2.280)	0.165
FAB classification (M5 vs others)	1.610 (1.126-2.817)	0.031
Cytogenetics (Poor/Intermediate vs others)	1.840 (1.304-3.866)	0.007
Serum miR-150 (Low vs high)	1.752 (1.242-3.309)	0.012

low levels of miR-150 were correlated with worse clinical outcome of HCC. miR-150 overexpression suppressed the proliferation, migration and invasion of cancer cells *in vitro* and tumor growth and metastasis *in vivo* [17]. miR-150 levels were decreased in advanced cutaneous T-cell lymphoma and its downregulation was significantly correlated with tumor invasion and metastasis [18].

However, miR-150 was also found to act as an oncomiR in some types of cancer. For example, the expression level of miR-150 was overexpressed in lung cancer tissues. In addition, overexpression of miR-150 promoted the oncogenic activities of cancer cells by SRC kinase signaling inhibitor 1 [19]. miR-150 expression was upregulated in prostate cancer stem cells. It could increase the tumor volume and the expression of cancer stem cell related biomarkers, indicating that miR-150 might promote the carcinogenesis of prostate cancer [20].

In conclusion, serum miR-150 was significantly reduced in patients with AML, and decreased serum miR-150 was correlated with poor prognosis. It may serve as a useful prognostic biomarker and a promising novel therapeutic target for the treatment of AML.

Disclosure of conflict of interest

None.

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