Int J Clin Exp Pathol 2017;10(1):550-555
www.ijcep.com /ISSN:1936-2625/IJCEP0040107

Original Article

Prognostic value of plasma miR-638 in patients with acute myeloid leukemia

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Received September 16, 2016; Accepted November 3, 2016; Epub January 1, 2017; Published January 15, 2017

Abstract: MicroRNAs (miRNAs) play an important role in many human diseases including cancer. The aim of this study was to investigate the plasma expression level of miR-638 and explore its prognostic significance in acute myeloid leukemia (AML). Quantitative reverse transcription-polymerase chain reaction was used to examine plasma miR-638 expression level in the participants. Then the association between plasma miR-638 and clinical features of AML was analyzed. Our results demonstrated that plasma miR-638 was significantly reduced in AML patients compared to the healthy controls, and its level was increased in patients with complete remission. In addition, plasma miR-638 has a high accurate discrimination of the blood samples from AML patients and healthy volunteers. Plasma miR-638 expression level was significantly associated with various clinicopathological parameters including blast percentage and cytogenetics. Moreover, AML patients in the low plasma miR-638 expression group suffered statistically significant worse overall survival. Taken together, plasma miR-638 might be a promising biomarker useful to improve the clinical outcome of AML.

Keywords: Acute myeloid leukemia, plasma miR-638, prognosis, biomarker

Introduction

Acute myeloid leukemia (AML) is characterized by uncontrolled proliferation of immature blood cells [1]. This malignancy is a highly heterogeneous disease which has various cytogenetic abnormalities and genetic alterations [2]. The information of cytogenetic aberration and genetic changes is of great clinical value for the treatment of AML. However, it is difficult to predict the prognosis of AML patients who lack of these information [3]. Thus identifying novel biomarkers that contributing to the assessment of prognosis in AML is very important.

MicroRNAs (miRNAs) are a class of small, highly conserved non-coding RNA molecules that regulate gene expression at the posttranscriptional level [4]. Aberrant expression of miRNAs has been regarded as a new type of "oncomiRs" or "tumor suppressors", which plays critical roles in the progression of a number of cancers including AML [5-8]. miR-22 was significantly downregulated in AML and upregulation of miR-22 suppressed the oncogenic activities of leukaemic cells both *in vitro* and *in vivo*. In addition, multiple oncogenes and oncognic pathways were identified as downstream target of miR-22, suggesting miR-22 acted as a tumor suppressor in AML [9]. Overexpression of let-7c promotes granulocytic differentiation of AML cell lines and primary blasts. Moreover, PBX2 was negatively regulated by let-7c, indicating let-7c was an oncomiR in AML [10]. Considering that miRNAs are very stable in the circulation system, detecting circulating miRNAs expression level is very important for early detection, diagnosis and prognosis of AML [11]. The expression level of serum miR-10a-5p was significantly upregulated in de novo AML patients compared with in healthy controls. In addition, serum miR-10a-5p was increased in the patients with complete remission. Moreover, AML patients with higher serum miR-10a-5p expression level had poorer overall survival compared to those with lower serum miR-10a-5p expression level [3].

Table 1. Association between plasma miR-638 level and the clinicopathological parameters of AML

Parameters	No	Plasma miR-638 level		P
		Low	High	
Gender				
Male	43	22	21	0.6586
Female	41	19	22	
Age				
<50	54	25	29	0.5364
≥50	30	16	14	
BM blast (%)				
<50	39	13	26	0.0082
≥50	45	28	17	
Extramedullary disease				
No	61	27	34	0.1745
Yes	23	14	9	
FAB classification				
M0	3	1	2	0.4258
M1	8	3	5	
M2	44	21	23	
M4	25	12	13	
M5	2	2	0	
M6	2	2	0	
Cytogenetics				
Favorable	25	6	19	0.0109
Intermediate	42	24	18	
Unfavorable	17	11	6	

Deregulation of miR-638 expression has been implicated in the initiation and development of many cancers such as gastric cancer, breast cancer and melanoma [12-14]. Although miR-638 might function as a tumor suppressor in AML, its clinical significance remains poorly known [15]. Therefore, our hypothesis was that circulating miR-638 expression level might be a favorable prognostic biomarker in patients with AML.

Materials and methods

Patients and clinical samples

This study analyzed the plasma specimens that were obtained from healthy volunteers and patients with AML at the Department of Hematology, Qilu Hospital, Shandong University. Plasma was obtained from whole blood by centrifugation at 1500 g for 10 min and the supernatants were subsequently stored in aliquots at -80°C prior to analyses. All participants provided written informed consent to demonstrate their willingness to donate their blood samples for investigation. This study was approved by Research Ethics Committees of Qilu Hospital. The clinical characteristic of the patients with AML is summarized in **Table 1**.

Quantitative reverse transcription-polymerase chain reaction

Total RNA was extracted from plasma samples using Trizol (Invitrogen, Carlsbad, CA, USA). For detection of miRNAs, reverse-transcribed to cDNA using AMV reverse transcriptase (TaKaRa, Dalian, China) and a stem-loop RT primer (Applied Biosystems, Foster City, CA). Real-time PCR was performed using a TaqMan PCR kit on an Applied Biosystems 7500 fast Real-Time PCR System (Applied Biosystems). U6 snRNA was used as an internal control, and the relative quantification of plasma miR-638 was calculated using the comparative Ct method.

Statistical analysis

Kolmogorov-Smirnov test was used to evaluate whether the plasma miR-638 level pf the study population subjected to a normal distribution. Mann-Whitney approach was employed to compared the expression level of plasma miR-638 between AML patients and healthy volunteers as well as pretreatment and post treatment plasma miR-638 level. Chi-square test was performed to analyze the association between plasma miR-638 level and clinicopathological parameters of AML. The receiver operating characteristic (ROC) curve was used to estimate the diagnostic value of the plasma miR-638 in discriminating AML patients from healthy volunteers. To analyze the potential relationship between the plasma miR-638 and overall survival, survival curve were plotted using the Kaplan-Meier method and survival differences were assessed by the log-rank test. The differences were considered to be statistically significant at $P<0.05$.

Results

Plasma miR-638 was downregulated in AML

Our real-time PCR result showed that the expression level of miR-638 was remarkably

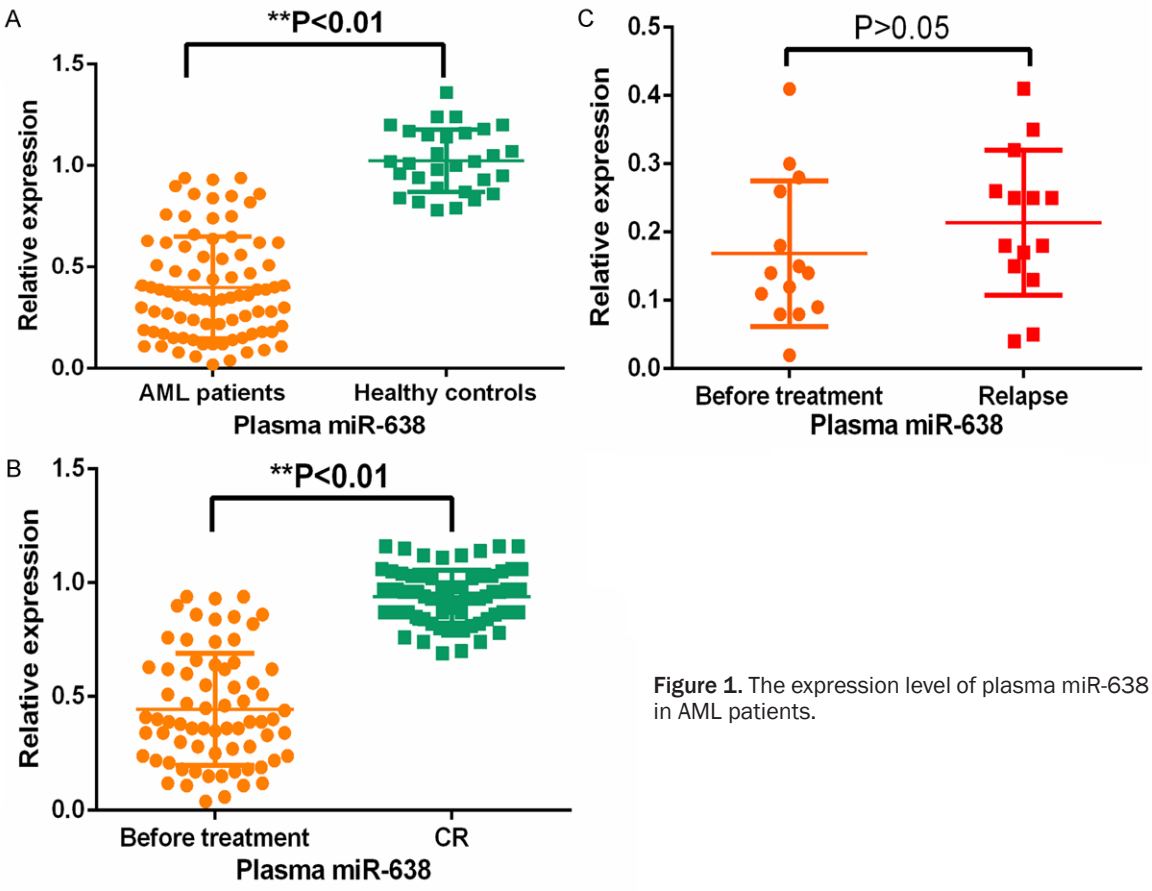

Figure 1. The expression level of plasma miR-638 in AML patients.

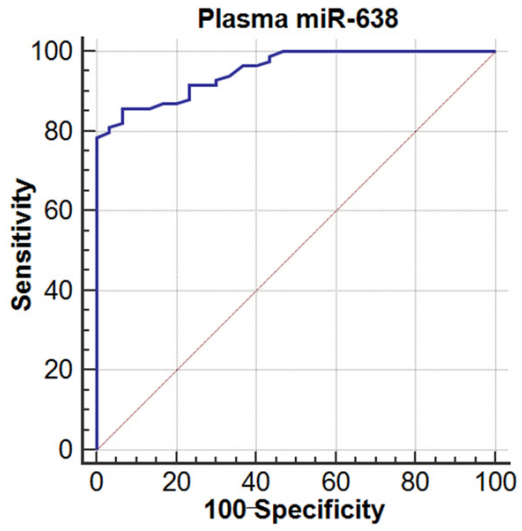

Figure 2. The diagnostic value of plasma miR-638 in AML patients.

decreased in the plasma samples from patients with AML compared with that in healthy volunteers (P<0.01) (**Figure 1A**).

Plasma miR-638 was upregulated in AML patients with complete remission

The expression level of plasma miR-638 was enhanced to normal levels in the patients who achieved complete remission (P<0.01) (**Figure 1B**). However, its expression level changed little in the patients experiencing a relapse compared to the pretreatment plasma miR-638 expression level (P>0.05) (**Figure 1C**).

The diagnostic accuracy of plasma miR-638 in AML

The ROC curve analysis was used to evaluate the diagnostic accuracy of plasma miR-638. The results showed that plasma miR-638 could discriminate AML from healthy controls with an AUC (the areas under the ROC curve) of 0.925 (95% CI: 0.856-0.971; P<0.001) (**Figure 2**).

The association between plasma miR-638 expression level and clinical parameters of AML

The median value of plasma miR-638 was used as a cutoff point to divide the AML into two

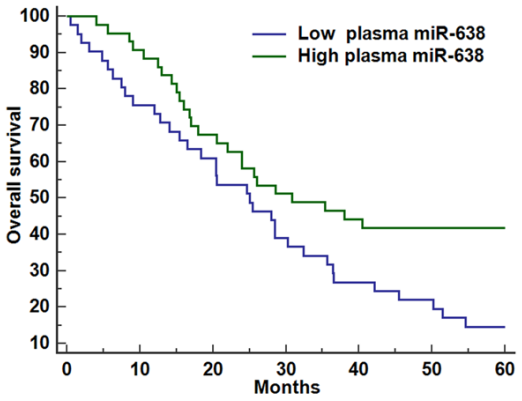

Figure 3. The association between plasma miR-638 and 5 year overall survival in AML.

groups. Forty-one patients were included in low plasma miR-638 expression group while 43 in high plasma miR-638 expression group. Our Chi-square analysis showed that low plasma miR-638 expression level was significantly associated with higher blast percentage (P=0.0082) and unfavorable cytogenetics (P= 0.0109) (**Table 1**).

The association between plasma miR-638 expression level and overall survival of AML

For the survival analysis, the AML patients in the low miR-638 expression group had a significantly shorter five year overall survival than the patients in the high miR-638 expression group (P=0.0183) (**Figure 3**).

Discussion

Circulating miRNAs derived from the tumors can be stably detected in various biofluids such as saliva, urine, serum and plasma, which contributes to early detection and diagnosis of various types of cancers [16]. In this study, our results showed that the expression level of plasma miR-638 was significantly reduced in AML patients compared to the healthy controls, and its level returned to normal range in those who achieved complete remission. In addition, plasma miR-638 discriminated AML patients from healthy volunteers with high accuracy. Plasma miR-638 expression level was significantly associated with blast percentage and cytogenetics. Moreover, AML patients with lower plasma miR-638 suffered worse overall survival. Taken together, our data indicates that miR-638 plays a tumor suppressive role in the progression of AML and downregulation of miR-638 might promote the tumorigenesis. Similarly, the expression level of miR-638 was downregulated in primary AML blast while dramatically upregulated in the cancer cells undergoing myeloid differentiation. In addition, overexpression of miR-638 suppressed proliferation and promoted differentiation of leukemic cell lines, and opposite findings were observed when miR-638 was inhibited. Moreover, cyclin-dependent kinase 2 was identified as a downstream target of miR-638, indicating that dyregulation of miR-638 was important for leukemogenesis [15].

miR-638 has also reported to function as a tumor suppressor in other types of cancers. The expression level of miR-638 was downregulated in colorectal carcinoma (CRC) tissues. In addition, suppression of miR-638 promoted cell invasion and a mesenchymal-like transition of CRC cells, and SOX2 was a downstream target of miR-638 [17]. Similarly, Zhang et al showed that miR-638 expression level was reduced in gastric cancer tissues compared with that in the adjacent normal tissues. Upregulation of miR-638 inhibited the proliferative capacity of gastric cancer cells by decreasing the expression of cyclin D1, and *vice verse.* Moreover, specificity protein 2 was identified as its regulatory target [18]. Cisplatin could significantly enhance the apoptosis of non-small cell lung cancer (NSCLC) cells by increasing the expression of miR-638 level. In addition, serum miR-638 was upregulated in the mice that subjected to cisplatin treatment. Lower serum miR-638 level was also associated with poorer prognosis of NSCLC, indicating miR-638 acted as a tumor suppressor in NSCLC [19].

However, the role of miR-638 is very complicated in tumor microenvironment. Some studies also reported that miR-638 promoted the initiation and development of cancer. Ectopic expression of miR-638 enhanced the tumorigenic properties of melanoma cells both *in vitro* and *in vivo*. Reduced expression of miR-638 induced expression of p53 as well as promoted apoptosis and autophagy [14]. Interestingly, miR-638 was also showed to promote the carcinogenesis of CRC [20], which was contradictory to the other studies [17, 21]. One possible reason was that the role of miR-638 might be also cell type dependent and might play different roles in the development of cancer.

In conclusion, our data demonstrate that down-regulation of plasma miR-638 is associated with poor prognosis of AML; indicating miR-638 might be a promising novel biomarker that will be beneficial to the treatment of this malignancy.

Acknowledgements

This project was supported by the fund from National Natural Science Foundation of China (No. 30960446).

Disclosure of conflict of interest

None.

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