European Review for Medical and Pharmacological Sciences 2015; 19: 4841-4850

Overexpressed miR-155 is associated with initial presentation and poor outcome in Chinese pediatric acute myeloid leukemia

L.-H. XU[1,2], Y. GUO[2], J.-N. CEN[3], W.-Y. YAN[4], H.-L. HE[1], Y.-N. NIU[5], Y.-X. LIN[4], C.-S. CHEN[6], S.-Y. HU[1]

[1]Department of Hematology and Oncology, Children's Hospital of Soochow University, Suzhou, Jiangsu, China
[2]The First People's Hospital of Lianyungang, Lianyungang, Jiangsu, China
[3]The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China
[4]Center for Systems Biology, Soochow University, Suzhou, Jiangsu, China
[5]School of Laboratory Medicine, Xinxiang Medical University, Xinxiang, Henan, China
[6]Department of Internal Medicine, Division of Hematology and Medical Oncology and Biospecimen Laboratory, Loma Linda University, Loma Linda, CA, USA

Lihua Xu and *Yang Guo* contributed equally to this article

Abstract. – OBJECTIVE: Acute myeloid leukemia (AML) is the second leading leukemia in children. There is growing evidence that microRNAs (miRNAs) are crucial regulators involved in leukemogenesis. This study aimed to investigate the role of miR-155 in Chinese pediatric AML by evaluating its diagnostic and prognostic significance.

PATIENTS AND METHODS: The expression of miR-155 and miR-25 in bone marrow specimens from 83 AML and 29 non-malignancies children were analyzed by TaqMan probe-based real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR).

RESULTS: The expression level of miR-155 was significantly higher in AML patients than in controls. Besides, a lowest miR-155 level was found in favorable prognosis group and *t* (15; 17)/M3 subgroup compared to the rest, while a higher level in C-Kit/FLT3-ITD mutation and relatively lower level existed in "Negative" mutation group. Moreover, miR-155 level was positively associated with the white blood cell (WBC) count, serum lactate dehydrogenase (LDH) and C-reaction protein (CRP) value in peripheral blood (PB), as well as miR-25/miR-196b expression levels. Survival analysis showed a statistically negative association with overall survival (OS) in the expression of miR-155 in chemotherapy group.

CONCLUSIONS: These finding suggested that miR-155 expression cannot only be promising biomarker for the early detection of pediatric AML but also predict poor outcome. MiR-155 would be a novel biomarker for diagnosis, prognosis and therapy in pediatric AML.

Key Words:
MiR-155, MiR-25, Pediatric acute myeloid leukemia, Diagnosis, Prognosis.

Introduction

Childhood acute myeloid leukemia (AML) is a highly heterogeneous hematopoietic system malignancy different from adult AML in cytogenetic and molecular genetics[1,2]. The five-year survival rate of childhood AML has been improved from 40% to 60%-75% over three decades[3-5], owing to risk-adapting therapy[6,7]. However, even with intensive chemotherapy for high risk pediatric AML, the outcome of relapsed and refractory AML remains dismal. Identification of novel markers for diagnosis, prognosis and treatment stratification is likely the keys for further refine the treatment strategies and achieve better outcome[8].

MicroRNAs (miRNAs) are a family of short, single strand and non-coding RNAs with highly conserved sequences about 22 nucleotides in length. They inhibit translation or degrade mRNA in post-transcriptional level by binding to 3'-UTR of special target genes, acting as oncogenes or tumor suppressor genes[9]. The relationship between aberrant miRNAs and adult AML has been well-identified[10,11], while the miRNA data is less correlated with childhood AML. The potential of miRNAs as novel biomarkers in pediatric AML is still on the brink of realization.

Corresponding Author: Shaoyan Hu, MD; e-mail: hsy139@126.com

In this exploratory study, we used miRNAs and mRNAs microarray expression data (Accession number: GSE35320 and GSE43176) reported from the National Center for Biotechnology Information Gene Expression Omnibus database (NCBI GEO DataSets), combined with the improved "POMA" (Pipeline of Outlier microRNA Analysis, from Center for Systems Biology of Soochow University in China), we predicted a series of candidate miRNA biomarkers involved with the leukemogenesis of pediatric AML[12,13]. Among them, miR-155 and miR-25, the top two which have the highest value of NOD (number of degree) and TFP (transcription factor percentage) (Wilcoxon test, $p < 0.05$; NOD = 28 and 32, TFP = 0.289 and 0.242, respectively), were considered as two potential diagnostic biomarkers of pediatric AML. In previous studies, miR-155 has been demonstrated be altered in some adult AML[14]. There has been much investigation as to the influence on other pediatric carcinoma such as brain tumors[15]. As for the children with AML, previously our laboratory found that high miR-196b expression existed in M4/5 subtype of initial AML and was related to poor outcome. Besides that, high expression of miR-196b can also be found in pediatric leukemia with aberrant activation of HOXA genes[16]. This study mainly aims to further explore the underlying biological value of miR-155 and miR-25 in pediatric AML.

Patients and Methods

Patient Samples

Bone marrow (BM) specimens were collected from 83 *de novo* AML and 29 non-malignancies children before any interventional measure during the period between January 2012 and October 2014 in the Affiliated Children's Hospital of Soochow University. The study was approved by the hospital Ethical Committee, with informed consent obtained from their legal guardians. The enrolled individuals require compliance with age ≤ 216 months. AML patients were classified and treated according to the protocol for Chinese AML children by Subspecialty Group of Hematology Diseases, the Society of Pediatrics, and Chinese Medical Association[17]. 29 non-malignancies pediatric marrow specimen were used as controls, including the diagnosis of infectious disease (6/29), healthy donor (3/29), and idiopathic thrombocytopenic purpura (20/29).

RNA Extraction, cDNA Synthesis, and Real-time Quantitative Reverse Transcriptase-polymerase Chain Reaction (qRT-PCR)

MiR-155 and miR-25 expression levels were detected by Taqman probe based qRT-PCR method, and U6 was used as reference gene. All primers and probes emanated from Applied Biosystems (ABI) (Refer to: miR-155: Number 000479-PN4427975; miR-25: Number 002442-PN4427975; U6: Number 001973-PN4427975).

Mononuclear cells (MNCs) were isolated through Ficoll-Hypaque gradient centrifugation (Invitrogen, Shanghai, China) and stored at –80°C. Total RNA was extracted from MNCs using Trizol reagent (Invitrogen, Shanghai, China) according to the manufacture's protocol. RNA concentration was determined by measurement of the optical density at 260 nm on MULTISKAN GO (Thermo Scientific, Shanghai, China). The synthesis of cDNA using Taqman® MicroRNA Reverse Transcription Kit were carried out by PCR System 9700 GeneAmp. RT reaction system (15 μl) and parameters were set as: 16°C for 30 min, then 42°C for 30 min, 85°C for 5 min, and 4°C forever, for one cycle.

The expression of mature miRNAs was determined by Taqman probe based qRT-PCR on ABI 7500 Real-Time PCR System. Reaction parameters were set as: 50°C 2 min, 95°C 10 min, 95°C 15 sec, 60°C 60 sec, 20 μl of reaction system, and 40 cycles, according to the protocol of ABI. Triplicates were performed for all qRT-PCR reactions. Ct threshold was manually set at 0.08. Ct values of U6SnRNA are required to ≤ 25. The quantitative PCR results of all samples were normalized to U6snRNA. Using the comparative Ct method[18], gene expressions of 112 samples were calculated relative to the median level of 29 controls and expressed in $2^{-\Delta\Delta Ct}$.

Statistical Analysis

Data analysis were performed using the two software, Statistical Package for Social Sciences program version 18.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.01 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). Mann-Whitney and Kruskal-Wallis test were used for comparison among groups. Spearman's nonparametric correlation analysis was executed between miRNAs expressions and clinical features. The impact of miRNAs level on the survival time was assessed

by Kaplan-Meier survival analysis. All used test were two-sided, and p-value < 0.05 was considered as statistically significant.

Results

Patient Characteristics

Table I displays the clinical features of 83 AML children. AML samples available were detected for cytogenetic and molecular abnormalities, including *t* (15; 17), inv (16), *t* (8; 21), MLL (11q23)-rearrangement, and mutational screening for C-Kit, CEBPA, FLT3. 83 patients were divided into various subgroups (Table II), based on the World Health Organization (WHO) classification 2008 and NCCN AML Guidelines 2015[19,20]. Remission (BM blast < 5%) and non-remission group (BM blast ≥ 5%) could be stratified on BM blast percentage in the 26th day of initial chemotherapy. Survival follow-up was executed for single chemotherapy group.

Significantly Differential Expression of miR-155 Existed in AML Patients' Entire Group and Cytogenetic/Molecular Subgroups, and Linked to Clinical Outcome

Predominant overexpression of miR-155 was found in newly diagnosed AML children (3.95-fold expression compared to controls, n = 83, p < 0.0001) (Table II, Figure 1A). We also found miR-155 level was significantly lower in favorable prognostic group when compared to the rest (0.47-fold expression, p = 0.0002) (Table II). There was no significant difference for miR-155 levels between remission and non-remission group after initial induction therapy (M = 3.86 *vs.* 4.05, p = 0.511) (Table II). Further analysis indicated that a lowest level of miR-155 existed in *t* (15; 17) (0.12-fold expression, M = 0.48, p = 0.0004) and M3 (0.11-fold expression, M = 0.48, p < 0.0001), with the miR-155 level higher in C-Kit (3.06-fold expression, M = 10.99, p = 0.004) and lower in "Negative" mutation group (0.69-fold expression, M = 0.69, p = 0.016) (Table II). Moreover, using Spearman's nonparametric correlation analysis, it was demonstrated that miR-155 level was positively correlated with the WBC count, serum lactate dehydrogenase (LDH) and C-reaction protein (CRP) value of initial peripheral blood (PB) (r = 0.30, 0.26, 0.25; p = 0.006, 0.020, 0.022, respectively) (Figure 1B-D), not with WBC, age, as well as sex (p > 0.05, Table II).

Table I. FAB (French-America-British) subtypes and clinical/cytogenetic/molecular characteristics of 83 Chinese pediatric AML patients.

Characteristic	Pediatric AML full cohort (n = 83) value (%)
Age at diagnosis, M	
Median	121
Range	4.6-216
Sex, No. (%)	
Male	43 (51.81)
Female	40 (48.19)
WBC counts, ×10^9/L	
Median	24.7
Range	0.64-459.35
HB counts, g/L	
Median	80
Range	39-142
PLT counts, ×10^9/L	
Median	30
Range	10-262
Peripheral blood blast percentage (%)	
Median	43
Range	0-96
Bone marrow blast percentage (%)	
Median	69
Range	21.5-98
LDH, U/L	
Median	529.05
Range	111-3910.8
CRP, mg/L	
Median	13.74
Range	0.01-201.94
FAB subtypes, No. (%)	
M1	4 (4.82)
M2	26 (31.33)
M3	12 (14.46)
M4	18 (21.69)
M5	18 (21.69)
M6	1 (1.20)
M7	1 (1.20)
Not determined	3 (3.61)
Cytogenetic abnormalities, No. (%)	
11q23 (MLL)	8 (9.64)
t (8:21)	22 (26.51)
t (15:17)	11 (13.25)
inv(16)	9 (10.84)
CN[a]	18 (21.69)
Other[b]	11 (13.25)
Not determined	4 (4.82)
Molecular abnormalities, No. (%)	
C-kit	9 (10.84)
CEBPA	10 (12.05)
FLT3-ITD	5 (6.02)
FLT3-TKD	2 (2.41)
Negative[c]	35 (42.17)
Not determined	22 (26.51)

[a]CN: cytogenetically normal, without cytogenetic aberrations; [b]Other: Other karyotype, with miscellaneous cytogenetic aberrations; [c]Negative: No positive results were detected.

Table II. MiR-155 relative expression among clinical/cytogenetic/molecular subgroups of AML.

	miR-155			
	n	Median expression (rang)[a]	Fold expression vs. rest[b]	*p*-value[c]
Cytogenetic abnormalities				
11q23 (MLL)	8	4.73 (2.33-11.75)	1.20	0.500
inv (16)	9	3.50 (0.49-11.48)	0.88	0.811
t (8;21)	22	5.59 (0.41-13.37)	1.45	0.100
t (15;17)	11	0.48 (0.03-7.40)	0.12	0.0004
CN[d]	18	3.48 (0.89-27.53)	0.88	0.620
Other[e]	11	3.95 (0.02-26.26)	1	0.994
Molecular abnormalities				
C-kit	9	10.99 (3.66-13.37)	3.06	0.004
CEBPA	10	4.33 (1.14-27.52)	1.12	0.778
FLT3-ITD	5	7.40 (2.87-20.28)	1.9	0.145
FLT3-TKD	2	1.01 (0.89-1.12)	0.26	***
Negative[f]	35	3.48 (0.03-11.75)	0.69	0.016
FAB Subtypes				
M1	4	5.84 (4.73-20.28)	1.49	0.155
M2	26	5.92 (0.41-27.52)	1.67	0.021
M3	12	0.48 (0.02-7.40)	0.11	< 0.0001
M4-5 (mononuclear cell group)	36	3.94 (0.49-26.26)	0.98	0.660
NCCN 2015 Prognosis group				
Favorable prognosis	40	2.94 (0.03-11.72)	0.47	0.0002
Intermediate prognosis	29	6.53 (0.02-27.52)	1.87	0.004
Poor prognosis	14	5.21 (0.89-26.26)	1.33	0.217
One Regimen Response (day 26)				
non-Remission	15	4.05 (0.89-11.75)	1.05	0.511
Remission	51	3.86 (0.02-20.28)		
Differential expression compared to controls				
Entire cohort	83	3.95 (0.02-27.52)	3.95	< 0.0001
M4-5	36	3.94 (0.49-26.26)	3.94	< 0.0001
non-M4-5	44	4.05 (0.02-27.52)	4.05	0.0004
Control	29	1 (0.10-4.02)	1	***
WBC subgroups ($\times 10^9$/L)				
≥ 100	14	5.56 (0.49-26.26)	1.42	0.222
< 100	69	3.93 (0.02-27.52)		
Age subgroups (M)				
≤ 12	6	5.61 (2.11-26.26)	1.43	0.280
> 12	77	3.93 (0.02-27.52)		
Sex subgroups				
Male	43	3.93 (0.02-27.52)	0.98	0.986
Female	40	4.00 (0.03-19.55)		

[a]Median expression relative to normal bone marrow, determined by RT-qPCR; [b]Median fold expression of specific subgroup compared to all other patients; [c]Determined by Mann-Whitney test, significant *p*-values (< 0.05) in bold italics; [d]CN: cytogenetically normal, without cytogenetic aberrations; [e]Other: Other karyotype, with miscellaneous cytogenetic aberrations; [f]Negative: No positive results were detected; ***No calculable *p*-value due to little cases.

To examine the association of miR-155 expression level with outcome of AML patient, we divided 63 pure chemotherapy cases into two groups based on miR-155 level, namely low miR-155 expression (≤ M) and high miR-155 expression (> M) subgroups. Subsequent survival analysis detected a statistically negative association of miR-155 expression with overall survival (OS) between these two subgroups (Log Rank *p* = 0.011) (Figure 2A), with the median follow-up of 60.4 weeks and the follow-up rate of 97%.

The Expression level of miR-25 is Higher in AML Patients Carrying C-Kit Mutation and Lower in M3 Subtype Comparing With the Rest

Although no differential expression of miR-25 appeared between the entire cohort of pediatric

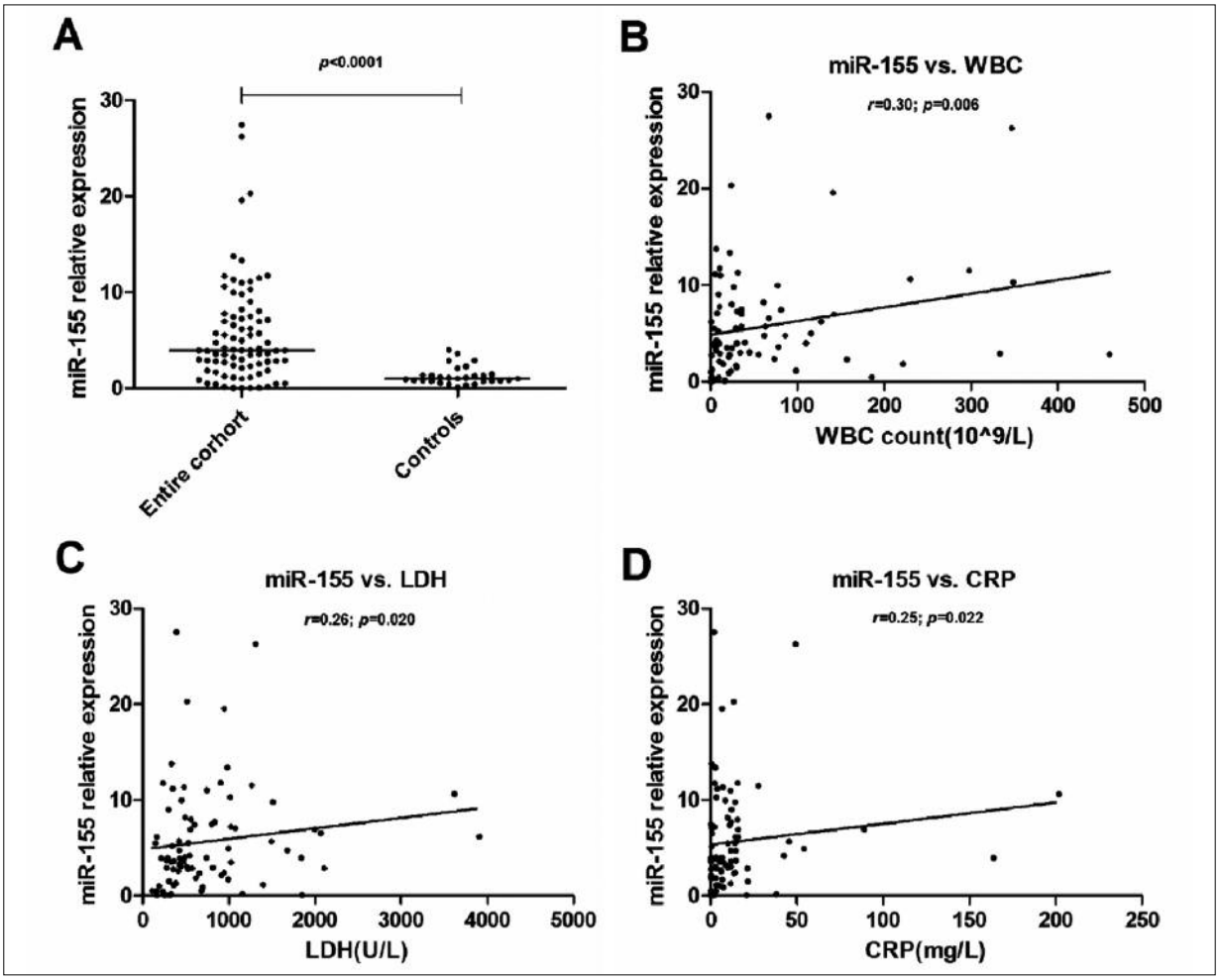

Figure 1. Significant overexpression of miR-155 compared to controls ***(A)***, and positively correlation existed in miR-155 expression with WBC counts ***(B)***, serum LDH ***(C)*** and CRP ***(D)*** level of initial peripheral blood.

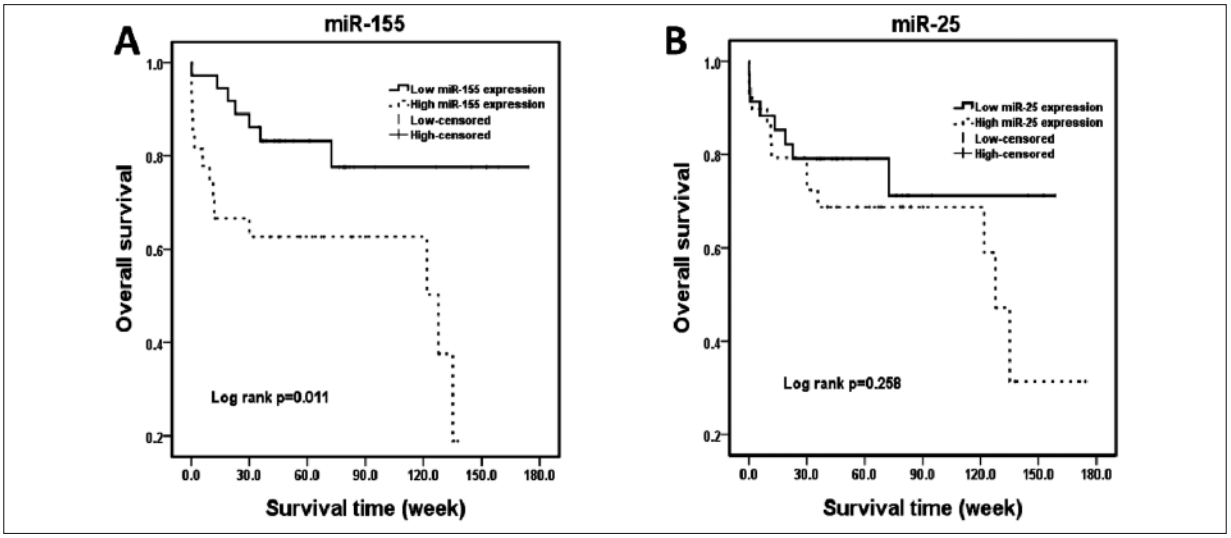

Figure 2. Survival analysis performed in single chemotherapy group. The overall survival rate is statistically higher in low miR-155 expression group compared to high miR-155 expression group ***(A)***, but not miR-25 ***(B)***.

AML patients and controls ($p > 0.05$, Table III), we still find some meaningful results similar to miR-155. MiR-25 was highly expressed in C-Kit subgroup (1.28-fold expression, M = 0.91, $p = 0.047$), and was expressed with low level in M3 subgroup, as compared to other patients, respectively (0.50-fold expression, $p = 0.020$) (Table III). Yet no significantly statistical differences for miR-25 expression were verified among cytogenetic subgroups ($p > 0.05$). Additionally, as for other clinical factors (i.e., WBC, HB, or PLT count of PB, and serum LDH or CRP values, as well as age and sex), we still fail to find pronounced correlations between subgroups ($p > 0.05$). Also, no statistical association with outcome was found in subsequently survival analysis for the expression of miR-25 in chemotherapy group (n = 63, Log Rank $p = 0.258$) (Figure 2B).

Table III. MiR-25 relative expression among clinical/cytogenetic/molecular subgroups of AML.

	MiR-25			
	n	Median expression (rang)[a]	Fold expression vs. rest[b]	*p*-value[c]
Cytogenetic abnormalities				
11q23 (MLL)	8	1.09 (0.52-2.33)	1.42	0.208
inv (16)	9	0.95 (0.19-1.54)	1.29	0.671
t (8;21)	22	0.82 (0.21-4.10)	1.11	0.634
t (15;17)	11	0.6 (0.15-4.10)	0.83	0.317
CN[d]	18	0.85 (0.27-3.25)	1.12	0.509
Other[e]	11	0.80 (0.16-5.61)	1.06	0.466
Molecular abnormalities				
C-kit	9	0.91 (0.62-4.10)	1.28	0.047
CEBPA	10	0.66 (0.32-3.25)	0.86	0.704
FLT3-ITD	5	0.48 (0.27-0.96)	0.63	0.176
FLT3-TKD	2	0.43 (0.40-0.46)	0.57	***
Negative[f]	35	0.74 (0.15-2.33)	0.99	0.688
FAB Subtypes				
M1	4	1.24 (0.53-2.05)	1.70	0.204
M2	26	0.82 (0.16-4.10)	1.18	0.444
M3	12	0.41 (0.15-2.67)	0.50	0.020
M4-5 (Mononuclear cell group)	36	0.77 (0.16-5.61)	1.05	0.520
NCCN 2015 Prognosis Group				
Favorable prognosis	40	0.72 (0.15-2.67)	0.89	0.268
Intermediate prognosis	29	0.90 (0.14-4.10)	1.25	0.202
Poor prognosis	14	0.86 (0.40-1.57)	1.17	0.694
One Regimen Response (day 26)				
non-Remission	15	0.62 (0.27-4.10)	0.78	0.624
Remission	51	0.80 (0.18-2.67)		
Differential expression compared to controls				
Entire cohort	83	0.74 (0.14-5.61)	0.74	0.393
M4-5	36	0.77 (0.16-5.61)	0.77	0.717
non-M4-5	44	0.73 (0.14-4.10)	0.73	0.287
Control	29	1 (0.14-4.01)	1.00	***
WBC Subgroups ($\times 10^9$/L)				
≥ 100	14	0.60 (0.16-5.61)	0.75	0.316
< 100	69	0.80 (0.14-4.10)		
Age Subgroups (M)				
≤ 12	6	0.99 (0.16-5.61)	1.37	0.295
> 12	77	0.73 (0.14-4.10)		
Sex Subgroups				
Male	43	0.85 (0.16-5.61)	1.26	0.234
Female	40	0.67 (0.14-2.20)		

[a]Median expression relative to normal bone marrow, determined by RT-qPCR; [b]Median fold expression of specific subgroup compared to all other patients; [c]Determined by Mann-Whitney test, significant *p*-values (< 0.05) in bold italics; [d]CN: cytogenetically normal, without cytogenetic aberrations; [e]Other: Other karyotype, with miscellaneous cytogenetic aberrations; [f]Negative: No positive results were detected; ***No calculable p-value due to little cases.

Positive Relationship is Presented Between the Expression of miR-155 and miR-25, as well as miR-155 and miR-196b Described Earlier

In this study, we carried out correlation analysis between miR-155 and miR-25, as well as miR-196b. The results delimitated a forcefully positive relationship not only between miR-155 and miR-25 ($r = 0.64$, $p < 0.0001$) (Figure 3A), but also involved miR-196b, which has been confirmed as diagnostic and prognostic reminders in our previous research. As a result, moderate positive correlation was found between miR-196b and miR-155 ($r = 0.25$, $p = 0.023$) (Figure 3B).

Discussion

MiRNAs plays an important role in leukemogenesis, and its expression signatures have been performed to classify cancers since 2005[21]. However, the relationship between miRNAs and pediatric AML remains dismal. This study is aimed at exploring the latent significances of miR-155 and miR-25 in *de novo* childhood AML. Our results showed that high expression level of miR-155 occurred in initial pediatric AML, and strongly linked to initial WBC/LDH/CRP, cytogenetic/ molecular abnormalities, as well as clinical outcome. Moreover, we also found significantly positive correlation of miR-155 with miR-25 and miR-196b described earlier.

MiR-155 is located on chromosome 21q21.3. It has been found to be a gene involving proliferation, apoptosis, differentiation, as well as inflammation and immunity[22]. Overexpression of miR-155 in hematopoietic stem cells could lead to amplification of myeloid population in mouse[14]. It has been validated that high expressed miR-155 in serum could be as non-invasive and diagnostic biomarker of AML[23]. In the present study, we displayed pronounced overexpression of miR-155 in entire group of newly diagnosed pediatric AML compared to controls. This is consistent with the research previously, and it can be preliminarily deduced that miR-155 in bone marrow might be also a promising biomarker for earlier diagnosis of pediatric AML. In AML adult, higher miR-155 level was found in patients carrying FLT3-ITD compared to "Negative" mutation group[24], and linked to significantly low rate of complete remission (CR) and short OS in adult AML[25]. The bad influence of FLT-ITD on outcome of AML has also been firmly established[26]. We also determined the higher miR-155 level in FLT3-ITD (n = 5) versus "Negative" mutation group, but for small sample size. As for C-Kit mutation with the highest expression of miR-155 in our results, there were researches to show that C-Kit mutation was strongly associated with poor prognosis in pediatric AML[27,28]. Instead, it has been found patients with FLT3-TKD mutations owned relatively favorable outcomes in non-promyelocytic AML[29], while M3 subtype was conferred superior outcome due to the introduction of all-trans retinoic acid (ATRA)[30]. In this work, we still found the lowest expression level of miR-155 existed in favorable prognosis group and *t* (15; 17)/M3 as well as to FLT3-TKD mutation subgroup compared with the rest. Thus, from what have been men-

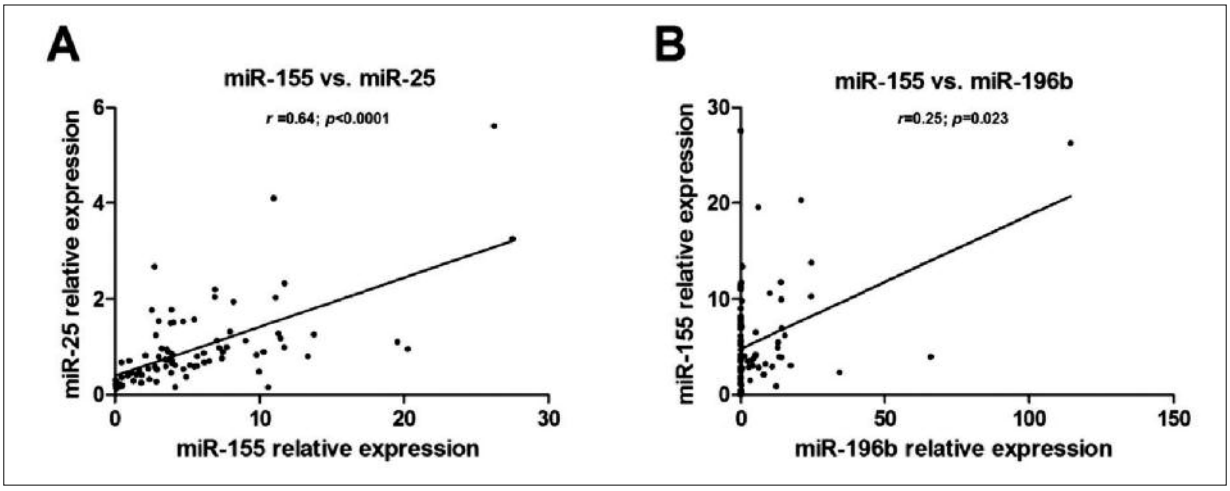

Figure 3. Positively correlation existed in miR-155 expression with ***(A)*** miR-25 expression, and ***(B)*** miR-196b expression.

tioned above, we can infer clearly that miR-155 might play key role in the adverse prognosis of childhood AML, although no statistic relationship was found between the expression of miR-155 and CR after one course in present study. Besides, positively associations were explored between miR-155 expression and WBC count of PB, LDH and CRP value of serum. It is generally known that initial serum LDH and CRP values are associated with burden of solid tumor[31], while primary WBC count is one of risk factors in de novo childhood AML[32]. These data all should serve as reminders that high expression of miR-155 predict inferior prognosis in childhood AML. The adverse correlativity was also further supported by Kaplan-Meier Survival analysis in current study, which pointed out a statistically negative association between OS and high expression of miR-155 in pure chemotherapy group.

MiR-25 is a member of the miR-106b-25 cluster (i.e., miR-106b, miR-93 and miR-25) located on chromosome 7q22.1[33]. It has been found to be related to OS of adult AML[34]. However, so far no researches about miR-25 aimed at pediatric AML. In this study on pediatric AML, we failed to find significantly differential expression of miR-25 presented in newly diagnosed AML compared with controls, though the level of miR-25 increases regularly in diverse solid tumor[15,35]. We confirmed its statistically highest expression in C-Kit subgroup and the lowest level in M3 subgroup compared to normal BM control. As before, the prognostic significance of C-Kit mutation and FAB-M3 subgroup has been discussed in pediatric AML. Moreover, it was proved that miR-25 expression levels increased with that of miR-155 in present study. All these findings suggest that an underlying connection between high level and unfavorable prognosis might exist not only in miR-155, but also in miR-25 which might even be involved in leukemogenesis in pediatric AML. It has yet to in-depth research to find out more information about miR-25.

This study also indicated the positive correlation between miR-155 and miR-25/miR-196b. The coordinately increased expressions of miR-155 and miR-196b have been demonstrated in chronic hepatitis C (CHC) patient patients and strictly associated with the disease progression[36]. Taking all these together with our previous research results that a high expression of miR-196b was not merely diagnostic but closely prognostic biomarkers of childhood AML[16], the present findings of a positive correlation between miR-155 and miR-196b suggest the potential implication of miR-155 and miR-25 to poor outcome of AML children.

Our results reveal the association between miR-155 and childhood AML, and confirm the important application value in diagnosis and prognosis. Although studies have shown that miR-155 is closely related to remission in adult AML, we indeed found no significant difference for miR-155 expression between remission group and non-remission group after the first induction therapy, even in the subgroup of available patients with cytogenetically normal AML (CN-AML) ($n = 15$, $p > 0.05$). It is different from the previously mentioned research on adult AML[25]. Meanwhile, we have not been able to find significant difference of miR-25 expression between AML children and controls, which is also inconsistent with the previous researches on solid tumors and adult AML[34,35]. Causes of these disagreements might be due to not only the smaller scale of samples, and highly heterogeneity of childhood leukemia. Further study on functions and more careful evaluation in a larger scale cohort of pediatric AML and particular subgroup analysis is needed. In addition, adequate time is desperately needed for dynamic following-up to determine the long-term effects of miR-155 and co-expressed miR-25.

Conclusions

This pilot study explored the differential expression of miR-155 in Chinese children population with primary AML, and its underlying significances in clinic. Overexpressed miR-155 in *de novo* AML cases and the relevance to poor outcome hint us the pivotal roles of miR-155 for early diagnosis and prognosis. Moreover, the functions of correlated miR-25 gene remain to be in-depth investigation in pediatric AML.

Acknowledgements

This work was supported by grants from National Natural Science Foundation of China (No. 81370627 and NO. 81170513) and a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions. The authors would like to thank Hongjie Shen, Qing Yan, Naichao Yang for their assistance, and Zixing Chen for helpful discussion and critical reading of the manuscript.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Dash A, Gilliland DG. Molecular genetics of acute myeloid leukaemia. Best Pract Res Clin Haematol 2001; 14: 49-64.
2) Manola KN. Cytogenetics of pediatric acute myeloid leukemia. Eur J Haematol 2009; 83: 391-405.
3) Kaspers GJ, Creutzig U. Pediatric acute myeloid leukemia: International progress and future directions. Leukemia 2005; 19: 2025-2029.
4) Harrison CJ, Hills RK, Moorman AV, Grimwade DJ, Hann I, Webb DK, Wheatley K, de Graaf SS, van den Berg E, Burnett AK, Gibson BE. Cytogenetics of childhood acute myeloid leukemia: United kingdom medical research council treatment trials AML 10 and 12. J Clin Oncol 2010; 28: 2674-2681.
5) Burnett A, Wetzler M, Lowenberg B. Therapeutic advances in acute myeloid leukemia. J Clin Oncol 2011; 29: 487-494.
6) Li L, Xiao ZJ. Research update on pharmacogenomics in acute leukemia - review. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2008; 16: 704-711.
7) Pui CH, Carroll WL, Meshinchi S, Arceci RJ. Biology, risk stratification, and therapy of pediatric acute leukemias: An update. J Clin Oncol 2011; 29: 551-565.
8) Moore AS, Kearns PR, Knapper S, Pearson AD, Zwaan CM. Novel therapies for children with acute myeloid leukaemia. Leukemia 2013; 27: 1451-1460.
9) Chen CZ. Micrornas as oncogenes and tumor suppressors. N Engl J Med 2005; 353: 1768-1771.
10) Garzon R, Garofalo M, Martelli MP, Briesewitz R, Wang L, Fernandez-Cymering C, Volinia S, Liu CG, Schnittger S, Haferlach T, Liso A, Diverio D, Mancini M, Meloni G, Foa R, Martelli MF, Mecucci C, Croce CM, Falini B. Distinctive microrna signature of acute myeloid leukemia bearing cytoplasmic mutated nucleophosmin. Proc Natl Acad Sci U S A 2008; 105: 3945-3950.
11) Mi S, Lu J, Sun M, Li Z, Zhang H, Neilly MB, Wang Y, Qian Z, Jin J, Zhang Y, Bohlander SK, Le Beau MM, Larson RA, Golub TR, Rowley JD, Chen J. Microrna expression signatures accurately discriminate acute lymphoblastic leukemia from acute myeloid leukemia. Proc Natl Acad Sci U S A 2007; 104: 19971-19976.
12) Zhang W, Zang J, Jing X, Sun Z, Yan W, Yang D, Shen B, Guo F. Identification of candidate mirna biomarkers from mirna regulatory network with application to prostate cancer. J Transl Med 2014; 12: 66.
13) Yan W, Xu L, Sun Z, Lin Y, Zhang W, Chen J, Hu S, Shen B. Microrna biomarker identification for pediatric acute myeloid leukemia based on a novel bioinformatics model. Oncotarget 2015; 6: 26424-26436.
14) O'Connell RM, Rao DS, Chaudhuri AA, Boldin MP, Taganov KD, Nicoll J, Paquette RL, Baltimore D. Sustained expression of microrna-155 in hematopoietic stem cells causes a myeloproliferative disorder. J Exp Med 2008; 205: 585-594.
15) Birks DK, Barton VN, Donson AM, Handler MH, Vibhakar R, Foreman NK. Survey of microrna expression in pediatric brain tumors. Pediatr Blood Cancer 2011; 56: 211-216.
16) Schotte D, Lange-Turenhout EA, Stumpel DJ, Stam RW, Buijs-Gladdines JG, Meijerink JP, Pieters R, Den Boer ML. Expression of miR-196b is not exclusively MLL-driven but is especially linked to activation of HOXA genes in pediatric acute lymphoblastic leukemia. Haematologica 2010;95:1675-1682.
17) Subspecialty Group of Hematology Diseases, the Society of Pediatrics, Chinese Medical Association. Suggestion of diagnosis and treatment of acute myelocytic leukemia in childhood. Chinese Journal of Pediatrics 2006; 44: 877-878.
18) Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative pcr and the 2(-delta delta c(t)) method. Methods 2001; 25: 402-408.
19) Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellstrom-Lindberg E, Tefferi A, Bloomfield CD. The 2008 revision of the world health organization (who) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 2009; 114: 937-951.
20) NCCN: NCCN clinical practice guidelines in oncology (NCCN guidelines®). Acute myeloid leukemia version 1. 2015 [eb/ol]. National Comprehensive Cancer Network. Inc. 2014. http://www.nccn.org/
21) Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. Microrna expression profiles classify human cancers. Nature 2005; 435: 834-838.
22) Joyce CE, Novina CD. Mir-155 in acute myeloid leukemia: not merely a prognostic marker? J Clin Oncol 2013; 31: 2219-2221.
23) Zhi F, Cao X, Xie X, Wang B, Dong W, Gu W, Ling Y, Wang R, Yang Y, Liu Y. Identification of circulating micrornas as potential biomarkers for detecting acute myeloid leukemia. PloS One 2013; 8: e56718.
24) Zhu YD, Wang L, Sun C, Fan L, Zhu DX, Fang C, Wang YH, Zou ZJ, Zhang SJ, Li JY, Xu W. Distinctive microrna signature is associated with the diagnosis and prognosis of acute leukemia. Med Oncol 2012; 29: 2323-2331.
25) Marcucci G, Maharry KS, Metzeler KH, Volinia S, Wu YZ, Mrozek K, Nicolet D, Kohlschmidt J, Whitman SP, Mendler JH, Schwind S, Becker H, Eisfeld AK, Carroll AJ, Powell BL, Kolitz JE, Garzon R, Caligiuri MA, Stone RM, Bloomfield CD. Clinical role of micrornas in cytogenetically normal acute myeloid leukemia: Mir-155 upregulation independently identifies high-risk patients. J Clin Oncol 2013; 31: 2086-2093.

26) Abu-Duhier FM, Goodeve AC, Wilson GA, Gari MA, Peake IR, Rees DC, Vandenberghe EA, Winship PR, Reilly JT. Flt3 internal tandem duplication mutations in adult acute myeloid leukaemia define a high-risk group. Br J Haematol 2000; 111: 190-195.

27) Shimada A, Taki T, Tabuchi K, Tawa A, Horibe K, Tsuchida M, Hanada R, Tsukimoto I, Hayashi Y. KIT mutations, and not FLT3 internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): a study of the Japanese Childhood AML Cooperative Study Group. Blood 2006; 107: 1806-1809.

28) Manara E, Bisio V, Masetti R, Beqiri V, Rondelli R, Menna G, Micalizzi C, Santoro N, Locatelli F, Basso G, Pigazzi M. Core-binding factor acute myeloid leukemia in pediatric patients enrolled in the aieop aml 2002/01 trial: Screening and prognostic impact of c-kit mutations. Leukemia 2014; 28: 1132-1134.

29) Li W, Zhang L, Huang L, Mi Y, Wang J. Meta-analysis for the potential application of FLT3-TKD mutations as prognostic indicator in non-promyelocytic AML. Leuk Res 2012; 36: 186-191.

30) Masetti R, Vendemini F, Zama D, Biagi C, Gasperini P, Pession A. All-trans retinoic acid in the treatment of pediatric acute promyelocytic leukemia. Expert Rev Anticancer Ther 2012; 12: 1191-1204.

31) Abaza H, Ghanem A, Jmal A, Boussen H, Harzallah L, Rahal K, Guemira F. Importance of determination of c reactive protein (crp), carcinoembryonic antigen (cea) and lactic dehydrogenase (ldh) in colorectal cancer. Tunis Med 2010; 88: 409-413.

32) Meshinchi S, Arceci RJ. Prognostic factors and risk-based therapy in pediatric acute myeloid leukemia. Oncologist 2007; 12: 341-355.

33) Petrocca F, Vecchione A, Croce CM. Emerging role of mir-106b-25/mir-17-92 clusters in the control of transforming growth factor beta signaling. Cancer Res 2008; 68: 8191-8194.

34) Wang Y, Li Z, He C, Wang D, Yuan X, Chen J, Jin J. Microrna expression signatures are associated with lineage and survival in acute leukemias. Blood Cells Mol Dis 2010; 44: 191-197.

35) Li X, Yang C, Wang X, Zhang J, Zhang R, Liu R. The expression of mir-25 is increased in colorectal cancer and is associated with patient prognosis. Med Oncol 2014; 31: 781.

36) Grek M, Piekarska A, Bartkowiak J, Fendler W, Kuydowicz J, Wroblewski P, Paradowski M, Sidorkiewicz M. Coordinated increase of mirna-155 and mirna-196b expression correlates with the detection of the antigenomic strand of hepatitis c virus in peripheral blood mononuclear cells. Int J Mol Med 2011; 28: 875-880.