Int J Clin Exp Med 2019;12(5):5527-5535
www.ijcem.com /ISSN:1940-5901/IJCEM0074680

Original Article

Dysregulation and clinical implication of microRNA-183, microRNA-182 and microRNA-96 in childhood acute myeloid leukemia

Jian Hu, Haixiao Qi, Wendi Zhou, Wenxia Kuai, Xingzhen Sun, Ze Hong

Department of Pediatrics, Huai'an First People's Hospital, Nanjing Medical University, 6 Beijing Road West, Huai'an 223300, Jiangsu, P. R. China

Received September 3, 2017; Accepted July 5, 2018; Epub May 15, 2019; Published May 30, 2019

Abstract: Objective: microRNA (miR)-183-182-96 cluster function as potential biomarkers for early diagnosis and accurate prognosis as well as therapeutic targets of multiple human cancers. Here, we aimed to investigate the expression features of members in the miR-183-182-96 cluster and their clinical significance in childhood acute myeloidleukemia (AML). Methods: Expression levelsof miR-183, miR-182 and miR-96 in peripheral blood and bone marrow samples of patients with childhood AML and healthy controls were detected by quantitative PCR. Associations of their expression with clinicopathological features and patients' prognosis were further evaluated. Results: The expression levels of miR-183, miR-182 and miR-96 in bone marrow and patients' sera of childhood AML were all significantly higher than those in the corresponding normal controls (all $P<0.001$). Interestingly, receiver-operating-characteristic (ROC) curve analysis showed the high diagnostic efficiencies of serum miR-183, miR-182 and miR-96 with the area under the ROC curve (AUC) of 0.905, 0.904 and 0.905, respectively. Moreover, high serum miR-183, miR-182 and miR-96 levels were significantly associated with French-American-British classification subtype M7 (all $P=0.02$), unfavorable karyotypes (all $P=0.01$), and shorter relapse-free and overall survivals (all $P=0.001$). Further Multivariate Cox regression analysis identified serum miR-183, miR-182 and miR-96 levels as independent prognostic factors for both relapse-free and overall survivals. Conclusion: Our data suggest that the upregulation of miR-183, miR-182 and miR-96 may play pivotal roles in the development and progression of patients with childhood AML. Serum miR-183, miR-182 and miR-96 may serve as novel prognostic biomarkers for this malignancy.

Keywords: Childhood acute myeloid leukemia, microRNA-183, microRNA-96, microRNA-182, serum, real-time quantitative PCR, prognosis

Introduction

MicroRNAs (miRNAs) represent a group of small, endogenous, non-coding RNA molecules with 18-25 nucleotides in length [1]. Functionally, miRNAs can negatively regulate the expression of genes post-transcriptionally or translationally by base-pairing to the 3' untranslated region (UTR) of the target mRNAs [2]. MiRNAs have indicated to be involved in various cellular processes, including development, growth, proliferation, differentiation, cell cycle, mobility and death, via their interactions with one or more target genes [3, 4]. Growing evidence reveal that miRNAs may exhibit either oncogenic or tumor suppressive functions in various human cancer types [5, 6]. The aberrant expression of miRNAs may be involved into cancer diagnosis, classification, therapy and patients' prognosis [7, 8]. As a heterogeneous disease, acute myeloid leukemia (AML) is caused by various cytogenetic and molecular abnormalities, and is featured by a combination of differentiation arrest and proliferative advantage of myeloid progenitors [9]. Different from adult AML, childhood AML has distinct characteristics, leading to different response to the same therapy and clinical outcome [10]. Although the development of current therapeutic strategies, the five-year survival of childhood AML is approximately 60%, and the relapse is still main reason of therapy failure [11], implying that it is urgently required to improve our understanding on the molecular mechanisms

Table 1. Association of serum miR-183, miR-182 and miR-96 levels with clinical characteristics of 106 childhood acute myeloid leukemia patients

Clinical variables	No. of patients (%)	miR-183-high (≥2.67)	P	miR-182-high (≥2.22)	P	miR-96-high (≥3.15)	P
Gender							
Male	58 (54.7)	29 (50.00)	0.3	29 (50.00)	0.3	29 (50.00)	0.3
Female	48 (45.3)	25 (52.08)		26 (54.17)		24 (50.00)	
Age (year)							
>6	40 (37.7)	20 (50.00)	0.2	20 (50.00)	0.2	20 (50.00)	0.2
≤6	66 (62.3)	34 (51.52)		35 (53.03)		33 (50.00)	
Leukocyte (/μL)							
>10,000	66 (62.3)	34 (51.52)	0.2	35 (53.03)	0.2	33 (50.00)	0.2
≤10,000	40 (37.7)	20 (50.00)		20 (50.00)		20 (50.00)	
French-American-British classification							
M0	3 (2.8)	0 (0)	0.02	0 (0)	0.02	0 (0)	0.02
M1/M2	62 (58.5)	20 (32.26)		19 (30.65)		21 (33.87)	
M3	10 (9.4)	6 (60.00)		6 (60.00)		6 (60.00)	
M4/M5	21 (19.8)	16 (76.19)		16 (76.19)		16 (76.19)	
M7	10 (9.4)	10 (100.0)		10 (100.0)		10 (100.0)	
Extramedullary disease							
Absent	80 (75.5)	38 (47.50)	0.08	37 (46.25)	0.08	39 (48.75)	0.08
Present	26 (24.5)	12 (46.15)		12 (46.15)		12 (46.15)	
Cytogenetics							
Favorable	35 (33.0)	8 (22.86)	0.01	8 (22.86)	0.01	8 (22.86)	0.01
Intermediate	52 (49.1)	26 (50.00)		25 (48.08)		27 (51.92)	
Unfavorable	19 (17.9)	18 (94.74)		18 (94.74)		18 (94.74)	
Day 7 response to treatment							
Favorable	65 (61.3)	33 (50.77)	0.2	33 (50.77)	0.2	33 (50.77)	0.2
Unfavorable	41 (38.7)	21 (51.22)		22 (53.66)		20 (48.78)	

of this malignancy, as well as to develop novel and efficient treatment. Recent studies have identified various miRNA expression profiles which constitute progress in diagnosis, classification, prognosis and therapy of childhood AML [12, 13]. For example, Danen-van et al. [14] found the aberrant expression of miR-29a, miR-155, miR-196a, and miR-196b in clinically relevant cytogenetic and molecular subgroups of childhood AML; Our previous findings also indicated that the increased expression of miR-375, miR-29a and miR-100 were involved into the development and progression of childhood AML, and the two miRNAs might function as independent prognostic factors of this disease [15-17].

The miR-183-182-96 cluster, including miR-183, miR-182 and miR-96, is located within a 5-kb region on human chromosome 7q32.2 [18]. Members in this cluster have high homogeneity with the same transcription direction from telomere to centromere, and exert the similar biological functions [19]. Growing evidence revealed the increased expression of miRNAs in this cluster in various human cancer types, such as glioblastoma, melanoma, breast cancer, lung cancer, liver cancer, colon cancer, ovarian cancer, endometrial cancer, prostate cancer and bladder cancer, as well as their potentials as cancer biomarkers [20-29]. However, the involvements of miR-183, miR-182 and miR-96 in childhood AML have not been fully elucidated. To address this problem, this study detected the expression levels of miR-183, miR-182 and miR-96 in peripheral blood and bone marrow samples of patients with childhood AML and healthy controls by real-time quantitative PCR. Then, receiver-operating characteristic (ROC) curve analysis, Kaplan-Meier method, and Cox regression analysis

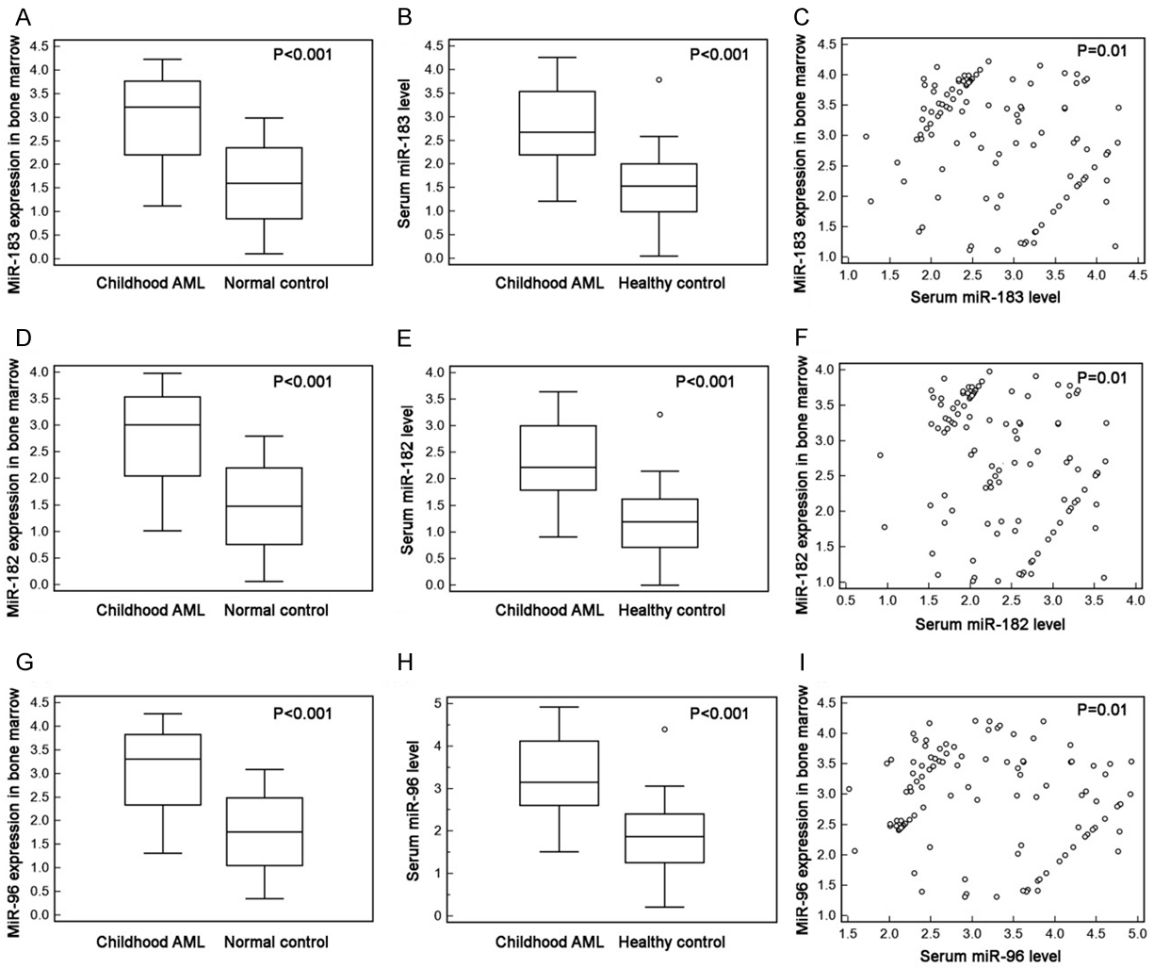

Figure 1. Expression levels of miR-183, miR-182 and miR-96 in bone marrow and sera of patients with childhood AML. A: Expression levels of miR-183 in bone marrow of patients with childhood AML and normal controls; B: Expression levels of miR-183 in sera of patients with childhood AML and healthy controls; C: Expression levels of miR-183 in the bone marrow of childhood AML patients were closely correlated with those in patients' sera; D: Expression levels of miR-182 in bone marrow of patients with childhood AML and normal controls; E: Expression levels of miR-182 in sera of patients with childhood AML and healthy controls; F: Expression levels of miR-182 in the bone marrow of childhood AML patients were closely correlated with those in patients' sera; G: Expression levels of miR-96 in bone marrow of patients with childhood AML and normal controls; H: Expression levels of miR-96 in sera of patients with childhood AML and healthy controls; I: Expression levels of miR-96 in the bone marrow of childhood AML patients were closely correlated with those in patients' sera.

were performed to evaluate the diagnostic and prognostic relevance of serum miR-183, miR-182 and miR-96 in childhood AML.

Materials and methods

Patients and tissue samples

In thisstudy, we used the same patient corhot with our previous studies [15, 16]. Our study was approved by Huai'an First People's Hospital Ethics Committee. Prior informed consent was obtained from the patients for the collection of specimens in accordance with the guidelines of Huai'an First People's Hospital, China. All specimens were handled and made anonymously according to the ethical and legal standards. Please see detail information on patients and tissues samples in Supplementary File 1. The clinical characteristics of 106 patients with AML were summarized in **Table 1**.

Real-time quantitative RT-PCR

Expression levels of miR-183, miR-182 and miR-96 in bone marrow mononuclear cells and

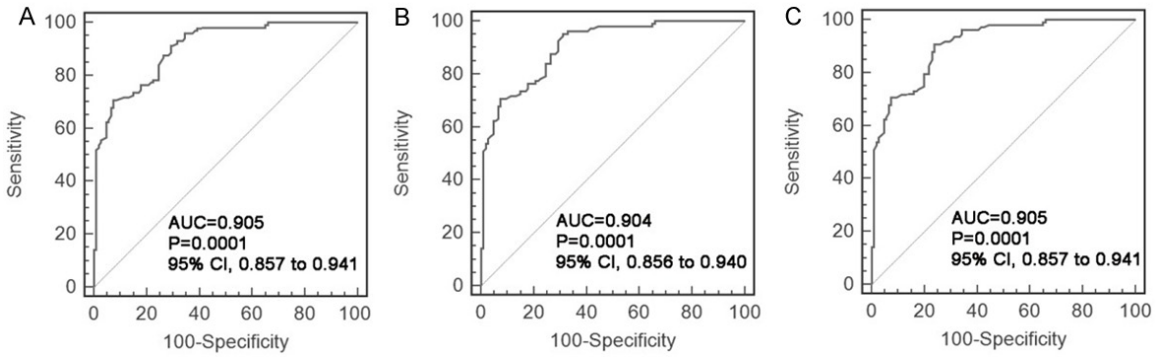

Figure 2. Diagnostic values of serum miR-183 (A), miR-182 (B) and miR-96 (C) levels in patients with childhood AML. The sensitivities and specificities of serum miR-183, miR-182 and miR-96 levels in screening childhood AML patients from healthy controls were all 68.87% (95% CI, 59.10-77.50%) and 95.28% (95% CI, 89.30-98.40%), respectively, when the optimal diagnostic cut-off points of serum miR-183, miR-182 and miR-96 levels were selected as 1.84, 1.49 and 2.22.

sera of patients with AML were detected by real-time quantitative RT-PCR according to the protocols described in our previous studies [15, 16]. RNU6B was used as an internal control. The primer sequences were as following: for miR-183: forward 5'-CTA TGG CAC TGG TAG AAT TCA CT-3', reverse 5'-TCG TAT CCA GTG CAG GGT C-3'; for miR-182: forward 5'-GCA ATG GTA GAA CTC ACA CT-3', reverse 5'-AAC ATG TAC AGT CCA TGG ATG-3'; for miR-96: forward 5'-GCC CGC TTT GGC ACT AGC ACA TT-3', reverse 5'-GTG CAG GGT CCG AGG T-3'; for RNU6B: forward 5'-CGC TTC GGC AGC ACA TAT AC-3', reverse 5'-TTC ACG AAT TTG CGT GTC AT-3'. Relative quantification of target miRNA expression was evaluated using the comparative cycle threshold (CT) method. The raw data were presented as the relative quantity of target miRNAs, normalized with respect to RNU6B. Each sample was examined in triplicate.

Statistical analysis

The software of SPSS version 11.0 for Windows (SPSS Inc, IL, USA) was used for statistical analysis. Data were shown as mean ± standard deviation (SD). Group differences were compared using two-tailed Student's *t* test. The correlation of a certain miRNA expression between bone marrow mononuclear cells and patients' sera was determined by Spearman Correlation analysis. ROC curve and the area under the ROC curve (AUC) were applied to evaluate the diagnostic values of serum miR-183, miR-182 and miR-96 levels in childhood AML. Associations of serum miR-183, miR-182 and miR-96 levels with clinicopathological features of childhood AML were performed using the χ^2 test for categorical variables. The Kaplan-Meier survival curves were used to determine any significant relationships between the serum levels of miR-183, miR-182 and miR-96 expression and the status of the patients with respect to relapse-free survival (RFS) or overall survival (OS). Multivariate Cox regression analysis was also performed to determine whether the serum levels of miR-183, miR-182 and miR-96 expression were independent prognostic factors of childhood AML. *P* values less than 0.05 were considered to be statistically significant.

Results

Up-regulation of miR-183, miR-182 and miR-96 in patients with childhood AML

Expression levels of miR-183 (in bone marrow, AML vs. normal: 2.95±0.91 vs. 1.57±0.87, P<0.001, **Figure 1A**; in serum, AML vs. normal: 2.82±0.77 vs. 1.46±0.73, P<0.001, **Figure 1B**), miR-182 (in bone marrow, AML vs. normal: 2.76±0.88 vs. 1.45±0.83, P<0.001, **Figure 1D**; in serum, AML vs. normal: 2.36±0.69 vs. 1.14±0.65, P<0.001, **Figure 1E**) and miR-96 (in bone marrow, AML vs. normal: 3.05±0.88 vs. 1.73±0.83, P<0.001, **Figure 1G**; in serum, AML vs. normal: 3.33±0.86 vs. 1.79±0.82, P<0.001, **Figure 1H**) in bone marrow and patients' sera were all significantly higher than those in bone marrow and sera of healthy controls.

In addition, Spearman's correlation analysis showed the close correlations of miR-183

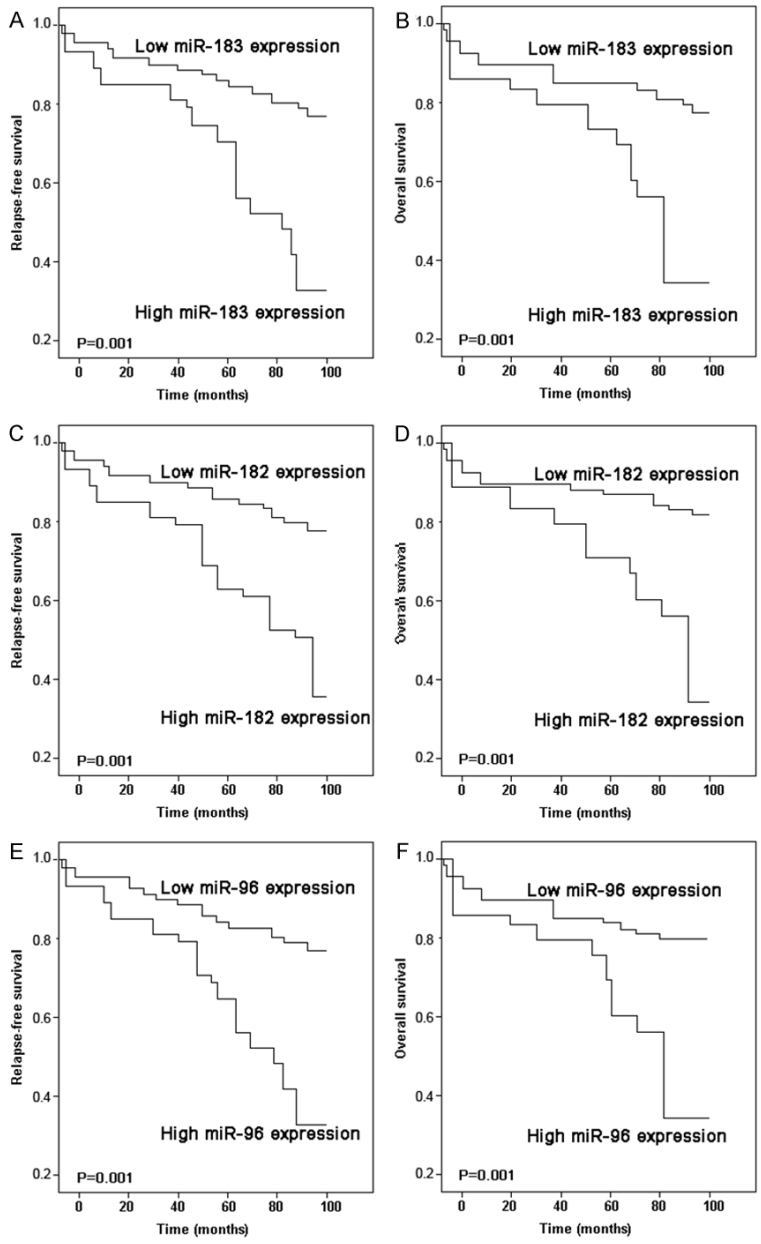

Figure 3. Kaplan-Meier curves of relapse-free survival (RFS) and overall survival (OS) of patients with childhood AML stratified by the serum miR-183 (A and B), miR-182 (C and D) and miR-96 (E and F) levels. The median values of serum miR-183 (2.67), miR-182 (2.22) and miR-96 (3.15) levels were used as cutoff points to divide all 106 patients with childhood AML into miR-183-low/high (n=52/54), miR-182-low/high (n=51/55) and miR-96-low/high (n=53/53) expression groups. Childhood AML patients with high serum miR-183, miR-182 and miR-96 levels had shorter RFS and OS than those with low serum miR-183, miR-182 and miR-96 levels (all P=0.001).

(Spearman's correlation: r=0.68, P=0.01, **Figure 1C**), miR-182 (Spearman's correlation: r=0.65, P=0.01, **Figure 1F**) and miR-96 (Spearman's correlation: r=0.68, P=0.01, **Figure 1I**) expression levels in the bone marrow with the serum expression in AML patients, respectively. Therefore, we investigated the clinical significance of the three miRNAs in childhood AML patients using their serum levels in the next sections.

Diagnostic values of serum miR-183, miR-182 and miR-96 levels in patients with childhood AML

ROC curve analysis was performed to evaluate the diagnostic values of serum miR-183, miR-182 and miR-96 levels in childhood AML according to their expression in patients and healthy controls. As shown in **Figure 2**, the AUC values for serum miR-183, miR-182 and miR-96 were respectively 0.905 (P=-0.0001; 95% CI, 0.857 to 0.941), 0.904 (P=0.0001; 95% CI, 0.856 to 0.940) and 0.905 (P=0.0001; 95% CI, 0.857 to 0.941). Moreover, the sensitivities and specificities of serum miR-183, miR-182 and miR-96 levels in screening childhood AML patients from healthy controls were all 68.87% (95% CI, 59.10-77.50%) and 95.28% (95% CI, 89.30-98.40%), respectively, when the optimal diagnostic cut-off points of serum miR-183, miR-182 and miR-96 levels were selected as 1.84, 1.49 and 2.22.

Associations of serum miR-183, miR-182 and miR-96 levels with various clinical characteristics of patients with childhood AML

The median values of serum miR-183 (2.67), miR-182 (2.22) and miR-96 (3.15) levels were used as cutoff points to divide all 106 patients with childhood AML into miR-183-low/high (n=52/54), miR-182-low/high (n=51/55) and

Table 2. Univariate analysis of the impact of variables on relapse-free survival and overall survival in childhood AML patients

Variable	No. of patients	Relapse-free survival		Overall survival	
		Median (months ± S.D.)	P	Median (months ± S.D.)	P
Cytogenetics					
Favorable	35	Not reached	<0.001	Not reached	<0.001
Intermediate	52	12.61±1.82		22.90±2.12	
Unfavorable	19	3.14±0.53		9.81±1.73	
French-American-British classification					
M1-M6	96	14.26±2.19	0.01	36.26±3.81	0.008
M7	10	7.92±1.44		20.72±2.52	
MiR-183 expression					
Low	52	5.56±0.73	0.01	20.39±1.28	0.01
High	54	10.92±0.82		36.67±2.85	
MiR-182 expression					
Low	51	5.81±0.56	0.01	20.28±1.39	0.01
High	55	10.89±0.72		36.87±2.36	
MiR-96 expression					
Low	53	5.96±0.55	0.01	20.58±1.48	0.01
High	53	10.94±0.82		36.55±2.39	

miR-96-low/high (n=53/53) expression groups. **Table 1** summarized the associations of serum miR-183, miR-182 and miR-96 levels with various clinical characteristics of patients with childhood AML. As a result, high serum miR-183, miR-182 and miR-96 levels were significantly associated with French-American-British classification subtype M7 (all P=0.02, **Table 1**) and unfavorable karyotypes (all P=0.01, **Table 1**). There were no significant associations between their serum levels and patients' gender and age, leukocyte count, extramedullary disease and day 7 response to treatment (all P>0.05, **Table 1**).

Prognostic values of serum miR-183, miR-182 and miR-96 levels in patients with childhood AML

Kaplan-Meier curve analyses revealed that childhood AML patients with high serum miR-183, miR-182 and miR-96 levels had shorter RFS and OS than those with low serum miR-183, miR-182 and miR-96 levels (all P=0.001, **Figure 3**). Moreover, univariate analyses found that RFS and OS of patients with childhood AML were both significantly associated with the French-American-British classification subtype M7 (P=0.01, **Table 2**), unfavorable cytogenetic abnormalities (P<0.001, **Table 2**), high serum miR-183 level (P=0.01, **Table 2**), high serum miR-182 level (P=0.01, **Table 2**) and high serum miR-96 level (P=0.01, **Table 2**). Furthermore, Cox proportional hazards multivariate analyses of the univariate predictors identified the cytogenetic abnormalities (P=0.01 and 0.009, respectively, **Table 3**) and the serum levels of miR-183 (both P=0.02, **Table 3**), miR-182 (both P=0.02, **Table 3**) and miR-96 (both P=0.02, **Table 3**) as independent prognostic factors for both RFS and OS.

Discussion

In recent years, a number of miRNAs with promising biological and clinical relevance have been identified as diagnostic and prognostic markers of various human cancers. Expression status of miRNAs are stable in biofluids, such as serum and plasma, thus, it has been indicated that the detection of cancer-related miRNAs may be a potential and non-invasive tool for cancer detection and monitoring. In the current study, our data revealed that the expression levels of miR-183, miR-182 and miR-96 in bone marrow and patients' sera of childhood AML were all significantly higher than those in the corresponding normal controls. More importantly, ROC curve analysis showed the high diagnostic efficiencies of serum miR-183,

Table 3. Multivariate analysis of the impact of variables on relapse-free survival and overall survival in childhood AML patients

Variable	P	Hazard ratios (HR)	95% confidence intervals (CI)
Relapse-free survival			
Cytogenetics			
Unfavorable vs. Favorable/Intermediate	0.01	5.92	1.56~11.21
French-American-British classification			
M7 vs. M1-M6	0.06	2.33	0.62~5.02
MiR-183 expression			
High vs. Low	0.02	4.88	0.82~10.08
MiR-182 expression			
High vs. Low	0.02	4.96	0.86~10.11
MiR-96 expression			
High vs. Low	0.02	4.92	0.83~10.09
Overall survival			
Cytogenetics			
Unfavorable vs. Favorable/Intermediate	0.009	7.09	1.86~14.32
French-American-British classification			
M7 vs. M1-M6	0.06	2.52	0.66~5.17
MiR-183 expression			
High vs. Low	0.02	5.02	0.85~10.26
MiR-182 expression			
High vs. Low	0.02	5.11	0.89~10.81
MiR-96 expression			
High vs. Low	0.02	5.06	0.88~10.69

miR-182 and miR-96 with the AUC values near 1.0. In addition, high serum miR-183, miR-182 and miR-96 levels were dramatically associated with French-American-British classification subtype M7, unfavorable karyotypes, and shorter relapse-free and overall survivals. Further multivariate analysis identified serum miR-183, miR-182 and miR-96 levels as independent prognostic factors for both relapse-free and overall survivals. These findings imply the potentials of the miR-183-miR-182-miR-96 cluster in early diagnosis and prognosis of patients with childhood AML.

Accumulating studies reported that individual and multiple members of the miR-183-182-96 cluster may be aberrantly expressed and function as oncogenes in various human cancers [20-29]. Among them, miR-183 is implicated in regulating growth and progression of different malignancies. Its expression was found to be deregulated in osteosarcoma, breast cancer, gastric cancer, hepatocellular carcinoma, pancreatic adenocarcinoma, colorectal cancer and renal cancer, and the overexpression of this miRNA could promote the proliferation, invasion and migration of malignant cells [20, 22, 30-32]. Notably, miR-183 has been identified as a predictor of poor prognosis in pancreatic ductal adenocarcinoma [31]. Then, miR-182 is considered to be the most potent anti-apoptotic miRNA. It is also dysregulated in several human malignancies, including melanoma, breast cancer, lung cancer, hepatocellular carcinoma, gastric adenocarcinoma, colon cancer, ovarian carcinoma, bladder cancer and prostate cancer [21, 23-26, 33, 34]. It has been found that miR-182 may be directly involved in carcinogenesis, cancer cell motility and distant metastasis. Importantly, Wang et al. [34] identified the increased expression of miR-182 in colorectal carcinoma as an independent and tissue-specific prognostic factor. In addition to miR-183 and miR-182, miR-96 is another member of the miR-183-182-96 cluster, and is considered as a genuine onco-miRNA and also highly up-regulated during the disease progression, including breast cancer, lung cancer, hepatocellular carcinoma, colorectal cancer, endometrial cancer and prostate cancer [27-29]. Moreover, miR-96 downregulation was found to suppress invasion and promote apoptosis in lung cancer cells by targeting FOXO3 [27]; miR-96 overexpression may induce cell proliferation in breast cancer cells by targeting FOXO3a [28]; miR-96 upregulation was indicated to contribute to aggressive malignancy partly through suppressing CDKN1A protein expression in bladder cancer cells [29]. Similar to these previous studies, we here demonstrated the increased expression of miR-183, miR-182 and miR-96 in both bone marrow and patients' sera of childhood AML. Our results also con-

firmed the marked associations between the upregulation of miR-183, miR-182 and miR-96, and aggressive progression, as well as poor prognosis in this malignancy. However, a previous study of Zhao et al. [35] revealed that the expression of miR-96 was downregulated in newly diagnosed adult AML patients and associated with leukemic burden, as well as RFS and OS. These differences might be caused by the patients' heterogenecity and discrepancy between childhood and adult AML patients. Therefore, further studies based on large cohorts of clinical samples should be required.

In conclusion, our data offer the convincing evidence that the upregulation of three members of the miR-183-miR-182-miR-96 cluster may play pivotal roles in the development and progression of patients with childhood AML. Serum miR-183, miR-182 and miR-96 may serve as novel prognostic biomarkers for this malignancy.

Disclosure of conflict of interest

None.

Address correspondence to: Ze Hong, Department of Pediatrics, Huai'an First People's Hospital, Nanjing Medical University, 6 Beijing Road West, Huai'an 223300, Jiangsu, P. R. China. Tel: 86517-84952301; Fax: 86517-84952301; E-mail: hongze1278@163.com

References

[1] Yang C, Wu D, Gao L, Liu X, Jin Y, Wang D, Wang T and Li X. Competing endogenous RNA networks in human cancer: hypothesis, validation, and perspectives. Oncotarget 2016; 7: 13479-90.

[2] Rupaimoole R, Calin GA, Lopez-Berestein G and Sood AK. MiRNA deregulation in cancer cells and the tumor microenvironment. Cancer Discov 2016; 6: 235-46.

[3] Kuninty PR, Schnittert J, Storm G and Prakash J. MicroRNA targeting to modulate tumor microenvironment. Front Oncol 2016; 6: 3.

[4] Huang H, Gu J, Yao S, Yao Z, Zhao Y, Xia Q, Ma J, Ling M, Yang S and Liu Y. MicroRNAs are related to Rituximab in combination with cyclophosphamide, doxorubicin, vincristine, and prednisone resistance in patients with diffuse large B-cell lymphoma. Cancer Transl Med 2015; 1: 11-15.

[5] Tiberio P, Callari M, Angeloni V, Daidone MG and Appierto V. Challenges in using circulating miRNAs as cancer biomarkers. Biomed Res Int 2015; 2015: 731479.

[6] Lan H, Lu H, Wang X and Jin H. MicroRNAs as potential biomarkers in cancer: opportunities and challenges. Biomed Res Int 2015; 2015: 125094.

[7] Osaki M, Okada F and Ochiya T. MiRNA therapy targeting cancer stem cells: a new paradigm for cancer treatment and prevention of tumor recurrence. Ther Deliv 2015; 6: 323-37.

[8] Hata A and Lieberman J. Dysregulation of microRNA biogenesis and gene silencing in cancer. Sci Signal 2015; 8: re3.

[9] Yan W, Xu L, Sun Z, Lin Y, Zhang W, Chen J, Hu S and Shen B. MicroRNA biomarker identification for childhood acute myeloid leukemia based on a novel bioinformatics model. Oncotarget 2015; 6: 26424-36.

[10] Khushboo Dewan and Kiran Agarwal. Acute lymphoblastic leukemia with normal platelet count. Cancer Transl Med 2015; 1: 168576.

[11] Forthun RB, Hinrichs C, Dowling TH, BruserudØ and Selheim F. The past, present and future subclassification of patients with acute myeloid leukemia. Curr Pharm Biotechnol 2016; 17: 6-19.

[12] Faulk K, Gore L and Cooper T. Overview of therapy and strategies for optimizing outcomes in de novo childhood acute myeloid leukemia. Paediatr Drugs 2014; 16: 213-27.

[13] Peloquin GL, Chen YB and Fathi AT. The evolving landscape in the therapy of acute myeloid leukemia. Protein Cell 2013; 4: 735-46.

[14] Danen-van Oorschot AA, Kuipers JE, Arentsen-Peters S, Schotte D, de Haas V, Trka J, Baruchel A, Reinhardt D, Pieters R, Zwaan CM and van den Heuvel-Eibrink MM. Differentially expressed miRNAs in cytogenetic and molecular subtypes of pediatric acute myeloid leukemia. Pediatr Blood Cancer 2012; 58: 715-21.

[15] Wang Z, Hong Z, Gao F and Feng W. Upregulation of microRNA-375 is associated with poor prognosis in pediatric acute myeloid leukemia. Mol Cell Biochem 2013; 383: 59-65.

[16] Bai J, Guo A, Hong Z and Kuai W. Upregulation of microRNA-100 predicts poor prognosis in patients with pediatric acute myeloid leukemia. Onco Targets Ther 2012; 5: 213-9.

[17] Zhu C, Wang Y, Kuai W, Sun X, Chen H and Hong Z. Prognostic value of miR-29a expression in pediatric acute myeloid leukemia. Clin Biochem 2013; 46: 49-53.

[18] Li P, Sheng C, Huang L, Zhang H, Huang L, Cheng Z and Zhu Q. MiR-183/-96/-182 cluster is up-regulated in most breast cancers and increases cell proliferation and migration. Breast Cancer Res 2014; 16: 473.

[19] Zhang QH, Sun HM, Zheng RZ, Li YC, Zhang Q, Cheng P, Tang ZH and Huang F. Meta-analysis of microRNA-183 family expression in human cancer studies comparing cancer tissues with noncancerous tissues. Gene 2013; 527: 26-32.
[20] Leung WK, He M, Chan AW, Law PT and Wong N. Wnt/β-Catenin activates MiR-183/96/182 expression in hepatocellular carcinoma that promotes cell invasion. Cancer Lett 2015; 362: 97-105.
[21] Wallis CJ, Gordanpour A, Bendavid JS, Sugar L, Nam RK and Seth A. MiR-182 is associated with growth, migration and invasion in prostate cancer via suppression of FOXO1. J Cancer 2015; 6: 1295-305.
[22] Gu C, Li X, Tan Q, Wang Z, Chen L and Liu Y. MiR-183 family regulates chloride intracellular channel 5 expression in inner ear hair cells. Toxicol In Vitro 2013; 27: 486-91.
[23] Kouri FM, Ritner C and Stegh AH. miRNA-182 and the regulation of the glioblastoma phenotype-toward miRNA-based precision therapeutics. Cell Cycle 2015; 14: 3794-800.
[24] Dambal S, Shah M, Mihelich B and Nonn L. The microRNA-183 cluster: the family that plays together stays together. Nucleic Acids Res 2015; 43: 7173-88.
[25] Song C, Zhang L, Wang J, Huang Z, Li X, Wu M, Li S, Tang H and Xie X. High expression of microRNA-183/182/96 cluster as a prognostic biomarker for breast cancer. Sci Rep 2016; 6: 24502.
[26] Bi DP, Yin CH, Zhang XY, Yang NN and Xu JY. MiR-183 functions as an oncogene by targeting ABCA1 in colon cancer. Oncol Rep 2016; 35: 2873-9.
[27] Chen Q, Yang L, Xiao Y, Zhu J and Li Z. Circulating microRNA-182 in plasma and its potential diagnostic and prognostic value for pancreatic cancer. Med Oncol 2014; 31: 225.
[28] Wei Q, Lei R and Hu G. Roles of miR-182 in sensory organ development and cancer. Thorac Cancer 2015; 6: 2-9.
[29] Rapti SM, Kontos CK, Papadopoulos IN and Scorilas A. High miR-96 levels in colorectal adenocarcinoma predict poor prognosis, particularly in patients without distant metastasis at the time of initial diagnosis. Tumour Biol 2016; 37: 11815-11824.
[30] Zhang Q, Ren W, Huang B, Yi L and Zhu H. MicroRNA-183/182/96 cooperatively regulates the proliferation of colon cancer cells. Mol Med Rep 2015; 12: 668-74.
[31] Zhou L, Zhang WG, Wang DS, Tao KS, Song WJ and Dou KF. MicroRNA-183 is involved in cell proliferation, survival and poor prognosis in pancreatic ductal adenocarcinoma by regulating Bmi-1. Oncol Rep 2014; 32: 1734-40.
[32] Ma Y, Liang AJ, Fan YP, Huang YR, Zhao XM, Sun Y and Chen XF. Dysregulation and functional roles of miR-183-96-182 cluster in cancer cell proliferation, invasion and metastasis. Oncotarget 2016; 7: 42805-42825.
[33] Sun J, Ji J, Huo G, Song Q and Zhang X. MiR-182 induces cervical cancer cell apoptosis through inhibiting the expression of DNMT3a. Int J Clin Exp Pathol 2015; 8: 4755-63.
[34] Wang S, Yang MH, Wang XY, Lin J and Ding YQ. Increased expression of miRNA-182 in colorectal carcinoma: an independent and tissue-specific prognostic factor. Int J Clin Exp Pathol 2014; 7: 3498-503.
[35] Zhao J, Lu Q, Zhu J, Fu J and Chen YX. Prognostic value of miR-96 in patients with acute myeloid leukemia. Diagn Pathol 2014; 9: 76.

Patients and tissue samples

This study was approved by Huai'an First People's Hospital Ethics Committee. Prior informed consent was obtained from the patients for the collection of specimens in accordance with the guidelines of Huai'an First People's Hospital, China. All specimens were handled and made anonymous according to the ethical and legal standards.

A total of 106 patients with pediatric AML, including 58 boys and 48 girls, were collected from the Department of Pathology, Huai'an First People's Hospital, China. All the patients were younger than 18 years of age (median 6 years). The diagnosis of AML was made according to a morphologic assessment of the Wright-Giemsa stained smears of the bone marrow aspirates along with special stains and immunophenotyping by flow cytometry. Laboratory investigation included conventional and molecular cytogenetic analyses. The median leukocyte count at diagnosis was 20,606/μL (range 420-352,906/μL). According to the French-American-British (FAB) classification, 3 patients had AML M0, 62 had M1/M2, 10 had M3, 21 had M4/M5 and 10 had M7. Among 26 patients with extramedullary disease, 21 patients had chloroma (scalp in 10 patients, orbit in 6 patients and skin in 5 patients), and 5 patients had a central nervous system involvement of leukemic cells. The clinical characteristic of 106 patients with AML was summarized in **Table 1**.

All patients with AML were treated with 10 days of induction chemotherapy, in which the dose of behenoyl 1-h-D-arabinofuranosylcytosine for the last 3 days was modified according to the bone marrow response on day 7. Discontinuation of the chemotherapy was allowed in patients who experienced sepsis with unstable vital signs before the completion of the induction regimen if at least 7 days of induction chemotherapy had been provided. If complete remission (CR) was not achieved after the primary induction chemotherapy regimen, an additional course of induction chemotherapy using high-dose 1-h-D-arabinofuranosylcytosine was given. Once CR had been achieved, patients with an appropriate stem-cell donor received consolidation chemotherapy until the hematopoietic stem-cell transplantation. An entire course of consolidation chemotherapy was given in patients without an appropriate stem-cell donor. All 106 patients with pediatric AML were receive follow-up analysis. The median follow-up duration was 35 months ranged from 10~86 months.

As normal controls, bone marrow was collected from 20 patients (12 boys and 8 girls; median age, 9 years; range, 3-18 years) with various diseases but with normal bone marrow morphology as demonstrated by cytological and histological analyses; Serum was collected from 50 healthy volunteers (30 boys and 20 girls; median age, 12 years; range, 5-18 years). Volunteers were all healthy, were not on medication, and had no signs or clinical symptoms of cancer, joint, liver, metabolic or endocrine diseases.