Gene 640 (2018) 79–85

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

High bone marrow *miR-19b* level predicts poor prognosis and disease recurrence in *de novo* acute myeloid leukemia

Ting-juan Zhang[a,c,d,1], Jiang Lin[b,c,d,1], Jing-dong Zhou[a,c,d], Xi-xi Li[a,c,d], Wei Zhang[a,c], Hong Guo[b,c], Zi-jun Xu[b,c,d], Yang Yan[d], Ji-chun Ma[b,c,*], Jun Qian[a,c,d,**]

[a] Department of Hematology, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu, People's Republic of China
[b] Laboratory Center, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu, People's Republic of China
[c] The Key Lab of Precision Diagnosis and Treatment of Zhenjiang City, Zhenjiang, Jiangsu, People's Republic of China
[d] School of Medicine, Jiangsu University, Zhenjiang, Jiangsu, People's Republic of China

ARTICLE INFO

Keywords:
miR-19
Prognosis
Acute myeloid leukemia (AML)

ABSTRACT

Oncogenic role of *miR-19* family has been identified in human cancers especially in lymphoid malignancies. However, to date, little studies investigated the role of *miR-19* family in myeloid malignancies. Herein, we examined *miR-19a/b* expression and explored its clinical significance in *de novo* acute myeloid leukemia (AML). The detection of *miR-19a/b* expression was performed by real-time quantitative PCR in bone marrow mononuclear cells of 113 patients and 42 healthy donors. Both *miR-19a/b* levels were significantly increased in AML patients in contrast to controls. Patients with *miR-19a/b* overexpression were more frequently occurred in female, and had an older age. Moreover, cases with *miR-19a* overexpression had a higher frequency of *U2AF1*, *C-KIT* and *CEBPA* mutations, whereas *miR-19b* overexpressed cases harbored *U2AF1* and *IDH1/2* mutations. There was no significant association of *miR-19a* overexpression with complete remission (CR) rate and overall survival (OS) among whole-cohort AML, non-M3 AML, and cytogenetically normal AML (CN-AML). However, although *miR-19b* overexpression was not correlated with CR rate, patients with *miR-19b* overexpression presented significantly shorter OS in whole-cohort AML and a trend in non-M3 AML and CN-AML patients. Importantly, our data also showed that *miR-19a/b* expression level at CR phase was lower than diagnosis time, and was returned to primary level even higher when at relapse phase. Our findings revealed that *miR-19a/b* overexpression were frequent events in *de novo* AML patients. Moreover, up-regulation of *miR-19b* expression was associated with poor prognosis and disease recurrence in AML.

1. Introduction

Acute myeloid leukemia (AML) is a genetically and molecularly heterogeneous disorder characterized by uncontrolled proliferation and blocked maturation of abnormal myeloid precursors (Estey and Döhner, 2006). Genetic analyses can identify recurrent chromosomal aberrations and gene mutations that are crucial for disease pathogenesis, response to therapy, and prognosis (Marcucci et al., 2011). Moreover, aberrant expression of cancer-related genes has advanced our understanding of leukemogenesis and is still required for risk-adapted treatment (Estey and Döhner, 2006; Marcucci et al., 2011). For example, ectopic expression of *WT1*, *BAALC*, *MN1*, *EVI1*, and *ERG* is associated with clinical outcome of AML especially in those without cytogenetic abnormalities (Baldus et al., 2007). Recently, aberrant expression of microRNAs in AML has also been attracted great attention (Marcucci et al., 2008; Liao et al., 2017). Dysregulation of microRNAs (miRNAs) post-transcriptionally regulating gene expression by targeting 3′-UTR of their mRNAs contributes to carcinogenesis and is closely associated with the diagnosis and prognosis of human cancers including AML (Lu et al., 2005).

The *miR-17-92* cluster, located on chromosome 13 (13q31.3), is a polycistronic gene encoding six miRNAs (*microRNA-17*, *microRNA-18a*, *microRNA-19a*, *microRNA-19b*, *microRNA-20a*, and *microRNA-92*) (Fuziwara and Kimura, 2015). The *miR-17-92* with roles in the

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; CR, complete remission; FAB, French-American-British; HRMA, high-resolution melting analysis; RQ-PCR, real-time quantitative PCR; ROC, receiver operating characteristic; AUC, area under the ROC curve; OS, overall survival; CN-AML, cytogenetically normal; HCC, hepatocellular carcinoma

* Correspondence to: J.C. Ma, Laboratory Center, Affiliated People's Hospital of Jiangsu University, 8 Dianli Rd., 212002 Zhenjiang, People's Republic of China.
** Correspondence to: J. Qian, Department of Hematology, Affiliated People's Hospital of Jiangsu University, 8 Dianli Rd., 212002 Zhenjiang, People's Republic of China.
E-mail addresses: majichun606@sohu.com (J.-c. Ma), qianjun0007@hotmail.com (J. Qian).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.gene.2017.10.034
Received 26 June 2017; Received in revised form 26 August 2017; Accepted 11 October 2017
Available online 12 October 2017
0378-1119/

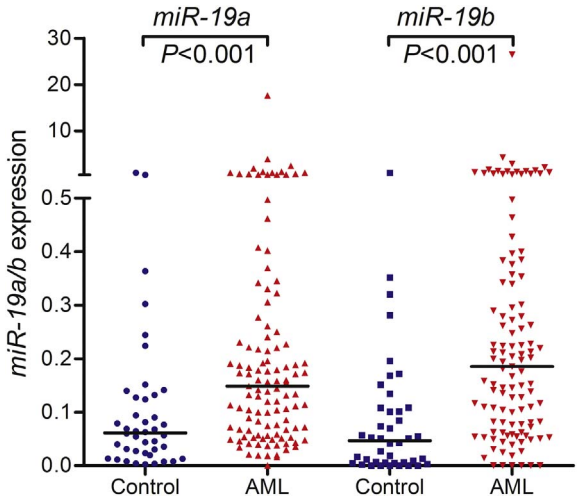

Fig. 1. Bone marrow *miR-19a/b* expression level in controls and AML patients. The distributions of the *miR-19a/b* expression level were presented with scatter plots. The median levels of *miR-19a/b* expression were shown with horizontal line. Both *miR-19a/b* expression were significantly increased in AML patients (Spearman test).

development of tumors and other diseases has been well studied. Due to its overexpression in numerous types of tumors, as well as its extensive roles in promoting transformation in hematologic malignancies, *miR-17-92* has been recognized as an oncogenic miRNA cluster (Mogilyansky and Rigoutsos, 2013). Recent studies identified *microRNA-19a* and *microRNA-19b* (*microRNA-19a/b*, *miR-19a/b*) were the key oncogenic components of *miR-17-92* cluster (Olive et al., 2009). Up-regulation of *miR-19a/b* has been reported in T/B-cell lymphoma as well as multiple myeloma, which was thought to be the result of tumor suppressor gene *PTEN* down-regulation (Chen et al., 2011; Xi et al., 2015; Mu et al., 2009; Wang et al., 2013). Moreover, elevated expression of *miR-19a/b* was associated with prognosis in those patients with T-cell lymphoblastic lymphoma and multiple myeloma (Xi et al., 2015; Hao et al., 2015). However, up to now, little studies investigated the *miR-19a/b* expression or its role in myeloid malignancies. The current study was aimed to investigate *miR-19a/b* expression and its clinical significance in patients with *de novo* AML.

2. Materials and methods

2.1. Patients

A total of 155 participants including 113 *de novo* AML patients and 42 healthy donors were enrolled in the current study approved by the Ethics Committee and Institutional Review Board of the Affiliated People' Hospital of Jiangsu University, China. Patients with antecedent hematological diseases or therapyrelated AML were excluded. After written informed consents were signed, bone marrow (BM) was collected from 113 patients at diagnosis time, 40 patients at complete remission (CR) time, and 24 patients at relapse time. The diagnosis was made according to the morphologic and cytochemical criteria of the French-American-British (FAB) classification (Bennett et al., 1976). All the patients received chemotherapy as reported in our previous literature (Zhou et al., 2017).

2.2. Cytogenetic analysis and mutation detection

BM cells were harvested after 1 to 3 days of unstimulated culture. The metaphase cells were banded by trypsin-Giemsa technique and karyotyped according to the recommendations of the International System for Human Cytogenetic Nomenclature (ISCN). Genomic DNA was isolated using Gentra Puregene Blood Kit (Qiagen, Duesseldorf, Germany) according to the manufacturer's instructions. Hotspot mutations of *NPM1*, *C-KIT*, *DNMT3A*, *IDH1/2*, *N/K-RAS*, *U2AF1*, and *SRSF2* genes were detected using high-resolution melting analysis (HRMA) on the LightScanner platform (Idaho Technology Inc., Salt Lake City, Utah, USA) as reported (Lin et al., 2011a; Yang et al., 2013; Qian et al., 2012; Lin et al., 2014; Yang et al., 2016; Lin et al., 2012; Lin et al., 2011b), whereas *FLT3*-ITD and *CEBPA* mutations were detected by DNA sequencing (BGI Tech Solutions Co., Shanghai, China) (Wen et al., 2014; Wen et al., 2015). All positive samples were confirmed by DNA sequencing again.

2.3. RNA isolation and reverse transcription

Mononuclear cells were extracted from BM by Lymphocyte Separation Medium (TBD sciences, Tianjin, China). Based on the manufacturer's protocols, RNA was extracted using the mirVana miRNA

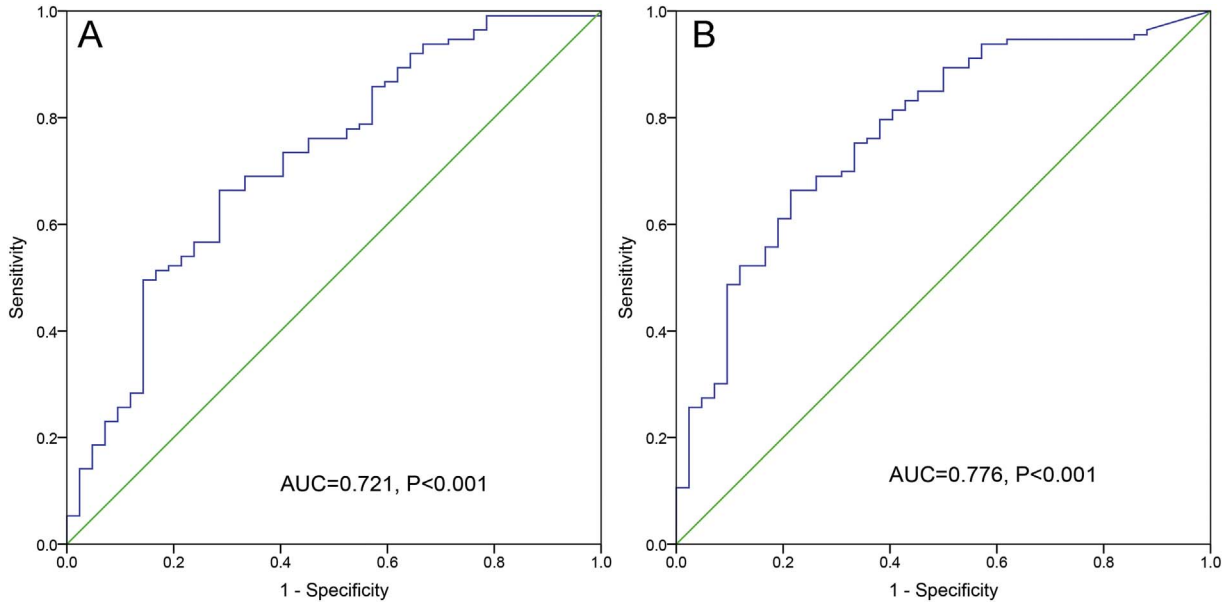

Fig. 2. Receiver operating characteristic (ROC) curve analysis of *miR-19a/b* expression for discriminating AML patients from controls. A: *miR-19a* expression with area under the ROC curve (AUC) value of 0.721 by ROC curve analysis (95% CI: 0.629–0.813); B: *miR-19b* expression with area under the ROC curve (AUC) value of 0.776 by ROC curve analysis (95% CI: 0.695–0.858).