Circulating microRNA-125b and microRNA-130a expression profiles predict chemoresistance to R-CHOP in diffuse large B-cell lymphoma patients

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Abstract. Numerous studies have reported the aberrant expression profiles of microRNAs (miRNAs) in diffuse large B-cell lymphoma (DLBCL), although very few of these studies were concerned with chemoresistance to R-CHOP in DLBCL patients. This study was designed to assess the correlation between circulating miRNA expression and chemoresistance and prognosis in DLBCL patients. At the start of the study, we demonstrated that miRNA expression levels in serum were significantly associated with those in formalin-fixed, paraffin-embedded tissues, which indicated that circulating miRNAs may be powerful, non-invasive biomarkers reflecting miRNAs levels isolated from tumor tissue. Then from eight potential drug-resistant miRNAs which were deregulated in DLBCL and which had been reported to be associated with drug resistance in other carcinomas, we screened out the circulating miR-125b and miR-130a, which may related to R-CHOP resistance. Dynamic monitoring of the levels of circulating miR-125b and miR-130a further demonstrated that they were involved in recurrence, progression and chemoresistance in DLBCL patients. Finally, we demonstrated that high miR-125b indicated poor prognosis, as patients with higher miR-125b levels had a shorter overall survival. To our knowledge, this is the first study demonstrating that miR-125b and miR-130a are associated with the risk of chemoresistance in DLBCL patients, and that dynamic monitoring of the levels of circulating miR-125b and miR-130a predicts the therapeutic response and disease status of DLBCL patients.

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Introduction

Diffuse large B-cell lymphoma (DLBCL), the most common type of non-Hodgkin lymphoma, is a heterogeneous group of tumors with an aggressive clinical course (1). The application of R-CHOP as the first-line treatment regimen has led to complete remission for 75-80% of patients (2). However, ~20% of patients are not sensitive to this immunochemotherapy, and these patients then relapse following the first-line regimen and tend to respond poorly to additional chemotherapy lines. Studies suggest that abnormalities in signaling pathways including NF-YB, SFPQ, MYBL2, BIM/APAF-1, AEG-1, BCL-2, XIAP, DR4 and ABCA1 are involved in primary or acquired chemoresistance and poor prognosis (3-9). Therefore, the identification of chemoresistance-related biomarkers may achieve a better prediction of chemotherapy efficacy for DLBCL patients, and could be used for the diagnosis of poor outcome cases, providing an advanced treatment strategy.

microRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression at the post-transcriptional level through sequence complementation with target mRNAs to repress transcription or induce mRNA degradation (10). Previous studies have determined a link between the expression of miRNAs and cancer pathogenesis, and miRNAs have revealed themselves to be significant biomarkers for tumor diagnosis, invasion, metastasis and evaluation of prognosis in certain types of tumors (11-14). However, chemoresistance-related miRNA expression profiles in DLBCL are reported less often. In this study, we aim to identify a series of serum miRNAs that may be involved in drug resistance in a cohort of 56 DLBCL patients treated with R-CHOP, then focus on monitoring the changes in these chemoresistance-related miRNAs dynamically. The correlation between serum and tumor tissues of miRNA expression levels was also assessed at the start of the study. Our results may help to elucidate potential chemoresistance-related miRNAs for DLBCL so as to provide guidance for a rational therapeutic schedule for clinical use.

Materials and methods

Patients. A total of 56 DLBCL patients undergoing treatment in Shandong Cancer Hospital, China, between May 2010 and

June 2011 were recruited in this study. A summary of the DLBCL patient details is provided in Table I. All patients had a histologically confirmed diagnosis of DLBCL according to the 2008 World Health Organization classification. All cases demonstrated positive staining for CD20. Patients who had localized extranodal invasion only, central nervous system involvement, previous immunosuppressive treatments, or who were associated with HIV or HBV infections were excluded. Formalin-fixed, paraffin-embedded (FFPE) tissues and blood samples were collected before patients were treated. In addition, blood samples from twenty age-and gender-matched healthy subjects were also collected. The study was approved by the Medical Ethics Committee of Shandong Cancer Hospital for Clinical Research, and was conducted in accordance with the Declaration of Helsinki. Written informed consent for use of biomaterials was obtained from all patients and donors.

Treatment and follow-up. All patients received R-CHOP chemotherapy as the first-line treatment and this was administered for 6-8 cycles in most cases. The R-CHOP regimen consisted of rituximab 375 mg/m^2 on day 0, cyclophosphamide 600 mg/m^2 on day 1, vincristine 1.4 mg/m^2 on day 1, epirubicin 60 mg/m^2 on day 1 and prednisolone 60 mg/m^2 on days 1-5. If patients were defined as having no response or progression during the treatment, or relapsing after achievement of complete remission or unconfirmed complete remission within 3 months of the end of treatment, it was considered that treatment failure and chemotherapy resistance had occurred. Such patients went on to receive second-line regimens. Responses were evaluated according to the Revised International Workshop criteria (15).

All follow-up data were updated in October 2014. Patients who succumbed for other reasons (treatment toxicity unrelated to the disease, for example) or who had insufficient data available were excluded, leaving 56 patients in the final analysis. Tumor response was re-evaluated after every two cycles (after cycles 2, 4, 6 and 8) of chemotherapy. Then periodic examination was performed every 3 months for the first year and every 6 months for the next years. Blood counts, a blood biochemical examination, an electrocardiogram, thoracic and abdominal computed tomography CT scans and a bone marrow biopsy were performed every time. Five milliliters of peripheral blood was obtained at every periodic examination to monitor serum miRNA levels dynamically. The blood samples were allowed to stand for ~1 h at room temperature. The samples were centrifuged at 2,000 x g for 15 min, then centrifuged again at 12,000 x g for 10 min at 4˚C. The resultant serum was aliquoted into Eppendorf tubes (Eppendorf, Hamburg, Germany) and stored at -80˚C until further use.

RNA isolation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total miRNA was extracted from the FFPE using the RecoverAll™ Total Nucleic Acid isolation kit (Ambion, Austin, TX, USA), and from the peripheral blood using a miRcute miRNA isolation kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's instructions. Total miRNA quality and quantity were assessed using the NanoDrop 1000 (Thermo Scientific, Wilmington, DE, USA). Then miRNA was reverse transcribed using a miRcute miRNA First-Strand cDNA synthesis kit (Tiangen), and qPCR was performed using a miRcute miRNA qPCR detection kit (Tiangen). The forward primers for amplification are shown in Table II and the common reverse primers were provided with the kit. Twenty microliters of the reaction product was incubated at 94˚C for 2 min, then 40 cycles at 94˚C for 20 sec and 60˚C for 35 sec were performed with ABI PRISM 7000HT (Applied Biosystems, Foster City, CA, USA). Each sample was run in triplicate. The miRNA expression value was expressed relative to that of U6 and the $2^{-\Delta CT}$ method was used in the analysis of PCR data [ΔCt = mean Ct (miRNA of interest) - mean Ct (U6)].

Statistical analysis. The statistical differences in mean values were tested using the Mann-Whitney U test. Pearson's correlation analysis was carried out to assess the correlation of miRNAs expression levels between the serum samples and FFPE tissue samples. Receiver operating characteristic (ROC) curves were constructed and the area under the ROC curve (AUC) was calculated to evaluate the specificity and sensitivity of the diagnostic and predictive values of miRNA levels. Overall survival (OS) was calculated as the time from diagnosis to the date of mortality or last contact. The OS of different groups of DLBCL patients was calculated using the Kaplan-Meier method and compared by the log-rank test. The percentage of patients alive at the median follow-up time and 95% confidence intervals were noted. All tests were two-sided, and $P \leq 0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed with GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Characteristics of enrolled patients. The baseline characteristics of the 56 patients are summarized in Table I. The median follow-up time for all patients was 37 months (range, 2-50 months). Among these patients, 29 patients (52%) experienced disease progression during or after treatment with R-CHOP, of which 5 progressed during the treatment and 24 progressed after the treatment. Twenty-seven patients (48%) succumbed due to disease progression. The chemotherapy response of the 56 patients was as follows: 21 patients experienced drug resistance to R-CHOP, of which 7 had primary resistance and 14 had secondary resistance. In addition, 35 patients remained sensitive to chemotherapy from beginning to end.

Correlation of miRNA expression levels in serum and FFPE tissue. To elucidate the correlation of miRNA expression levels between serum and FFPE tissue, we analyzed the expression levels of eight miRNAs in the 56 DLBCL patients prior to treatment. The expression levels of miR-155, miR-200c, miR-29c, miR-130a, miR-145, miR-451 and miR-21 ($P=0.035$, $P=0.006$, $P=0.001$, $P=0.019$, $P=0.025$, $P=0.001$ and $P=0.004$, respectively) were significantly higher in FFPE than those in serum. The expression of miR-125b was also higher in FFPE, although this was not significant ($P=0.067$; Fig. 1). Furthermore, Pearson's correlation analysis revealed that the serum miR-155, miR-200c, miR-29c, miR-130a, miR-145, miR-451, miR-125b and miR-21 expression levels were significantly

Table I. Clinical characteristics of 56 patients with diffuse large B-cell lymphoma.

Clinical characteristics	Number (%)
Age (range)	23-74
Median age	54.7
<55	26 (46)
≥55	30 (54)
Gender	
Male	32 (57)
Female	24 (43)
B symptoms	
Yes	15 (29)
No	41 (71)
Extranodal site involvement	
Yes	38 (68)
No	18 (32)
Ann Arbor stage	
I	6 (10)
II	13 (24)
III	21 (38)
IV	16 (28)
International prognostic index	
0-2	16 (28)
≥3	40 (72)
Disease progression	
Yes	29 (52)
No	27 (48)
Chemotherapy response	
Drug sensitivity	35 (62)
Drug resistance	21 (38)

Table II. Forward primer sequences.

Name	Sequences (5'-3')
miR-155	TTAATGCTAATCGTGATAGCCTG
miR-200c	TGGCAATAATACTGCCGGGTAAG
miR-29c	CCGGTACTAGCACCATTTGAAATCG
miR-130a	TTCGGCAGTGCAATGTTAAGC
miR-145	ATCCGCGTCCAGTTTTCCCAGGA
miR-451	CGTTAAACCGTTACCATTACTTCG
miR-125b	GGGCACTCCCTGAGCCCTAAC
miR-21	CGCATCCGGTAGCTTATCAGACTGA
U6	CGGCTTCGGCAGCACATATAAC

associated with their expression levels in FFPE tissues. (r=0.68, r=0.589, r=0.688, r=0.57, r=0.603, r=0.763, r=0.709 and r=0.806, respectively; Fig. 2).

Dysregulated expression profiles of eight miRNAs in DLBCL serum. The expression of eight miRNAs was compared in the serum of the 56 DLBCL patients and 20 healthy controls, and all of the miRNAs were observed to be diversely dysregulated in DLBCL serum. The levels of miR-155, miR-200c, miR-130a, miR-125b and miR-21 were significantly upregulated (P=0.004, P=0.001, P=0.019, P=0.04 and P=0.006, respectively), whereas the levels of miR-29c, miR-451 and miR-145 were downregulated (P=0.049, P=0.011 and P=0.018, respectively) when compared with healthy controls (Fig. 1).

Expression status of eight miRNAs associated with chemoresistance. A total of 20 DLBCL patients were enrolled in the preliminary experiment, of which 10 were chemotherapy-resistant cases and 10 were chemotherapy-sensitive cases. There were no significant differences between the two groups in terms of gender, age, international prognostic index (IPI) and stage. We investigated the pretreatment concentrations of serum miR-155, miR-200c, miR-29c, miR-130a, miR-145, miR-451, miR-125b and miR-21 expression levels, which have been reported in certain other tumor types to be associated with chemotherapy resistance (Table III) (9,14,16-29). We observed that only the expression of miR-130a and miR-125b was correlated significantly with the response to chemotherapy. miR-130a and miR-125b were upregulated in the drug-resistant group compared with the chemotherapy-sensitive group (P=0.026 and P=0.013, respectively; Fig. 3).

To further verify the discriminating power of miR-130a and miR-125b identified in the preliminary marker selection stage, the levels of the two miRNAs were measured in a cohort of 56 DLBCL patients comprising 21 drug-resistant cases and 35 drug-sensitive cases, which also included the 20 cases from the preliminary experiment. In line with the results of the preliminary evaluation of 20 patients, miR-130a and miR-125b were significantly elevated in the serum of the chemoresistant cases (P=0.028 and P=0.005, respectively; Fig. 4A and C). To verify the sensitivity and specificity of miR-130a and miR-125b, we used the the ROC curves to demonstrate the relative separation of the chemoresistance and chemosensitivity groups with the AUCs. These were identified to be 0.689 for miR-130a (95% CI, 0.524-0.854; P=0.028), and 0.741 for miR-125b (95% CI, 0.595-0.887; P=0.005; Fig. 4B and D). At the cut-off value of 3.275/3.07 for miR-130a and miR-125b, the sensitivity was 61.1/77.8% and the specificity was 75.0/62.5%, respectively.

miR-130a and miR-125b are involved in recurrence, progression and chemoresistance of DLBCL. To directly test the correlation between miR-130a and miR-125b expression and patients' response to chemotherapy, the levels of the two high-risk miRNAs were dynamically analyzed. Fig. 5 reveals the miR-130a and miR-125b continuous-time analysis profiles for six DLBCL patients. In case 1 and case 6, where no recurrence or progression was observed during the three-year follow-up, serum miR-130a and miR-125b expression decreased significantly at 2 months after R-CHOP chemotherapy and returned to normal levels at 12 months after the treatments. Case 2 relapsed following a long-term remission, then achieved remission again with the R-CHOP chemotherapy. In this case, the serum miR-130a and miR-125b expression decreased after the first treatment, but increased at 12 months, then decreased

Table III. Eight selected miRNAs involved in chemoresistance.

miRNA	Expression	Targets	Drug resistance	Tissue type	References
miR-125b	Up	E2F3	5-FU	Breast cancer	Wang *et al* (16)
	Up	ABCC4	Multidrug	Hepatocellular carcinoma	Borel *et al* (9)
miR-451	Down	ABCB1	Irinotecan	Colon cancer	Bitarte *et al* (17)
miR-29c	Down	Bcl-2 Mcl-1	Cisplatin	Nasopharyngeal carcinoma	Zhang *et al* (18)
miR-200c	Up	AKT	Cisplatin	Esophageal cancer	Hamano *et al* (19)
	Down	TrkB Bmi1	Doxorubicin	Breast cancer	Kopp *et al* (20)
	Down	Bmi1	Cisplatin	Melanoma	Liu *et al* (21)
	Down		Paclitaxel	Ovarian cancer	Cittelly *et al* (14)
miR-155	Up	FOXO3a	Doxorubicin	Breast cancer	Kong *et al* (22)
miR-145	Down	Oct4 Sox2	TMZ	Glioblastoma	Yang *et al* (23)
miR-21	Up	STAT3	5-FU	Glioblastoma	Ren *et al* (24)
	Up	PTEN	CHOP	Diffuse large B-cell lymphoma	Bai *et al* (25)
miR-130a	Down	XIAP	Cisplatin	Ovarian cancer	Zhang *et al* (26)
	Down	Met	Gefitinib	Lung cancer	Zhou *et al* (27)
	Up	MDR1/P-glycoprotein	Cisplatin	Ovarian cancer	Yang *et al* (28)
	Up	RUNX3 Wnt	Cisplatin	Hepatocellular carcinoma	Xu *et al* (29)

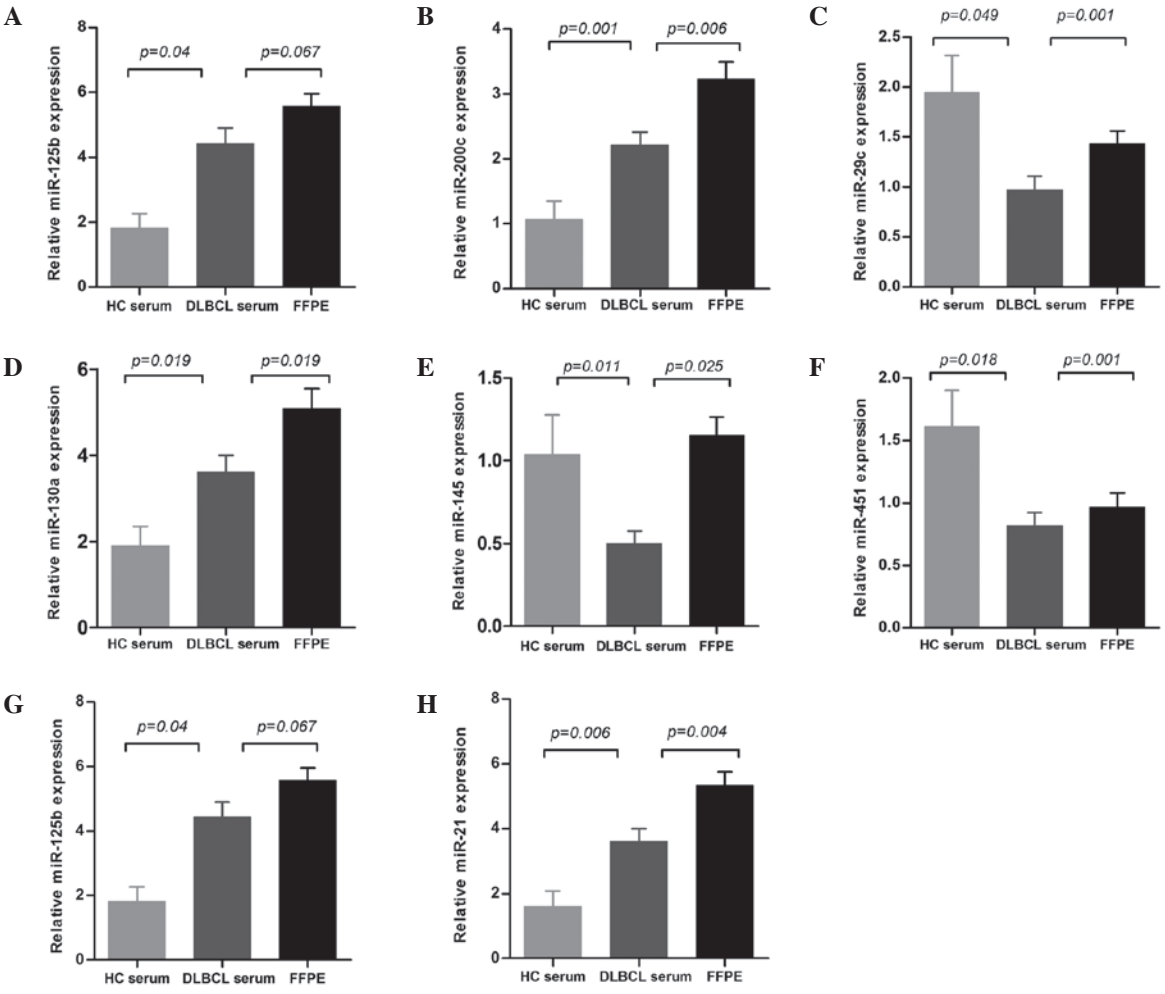

Figure 1. miRNA expression profiles in 20 healthy controls and 56 diffuse large B-cell lymphoma (DLBCL) patients before treatment measured by reverse transcription-quantitative polymerase chain reaction. (A-H) miR-155, miR-200c, miR-29c, miR-130a, miR-145, miR-451, miR-125b and miR-21 levels in healthy control serum, DLBCL serum and formalin-fixed, paraffin-embedded (FFPE) tissue, respectively. P-values were calculated using the Mann-Whitney U test.

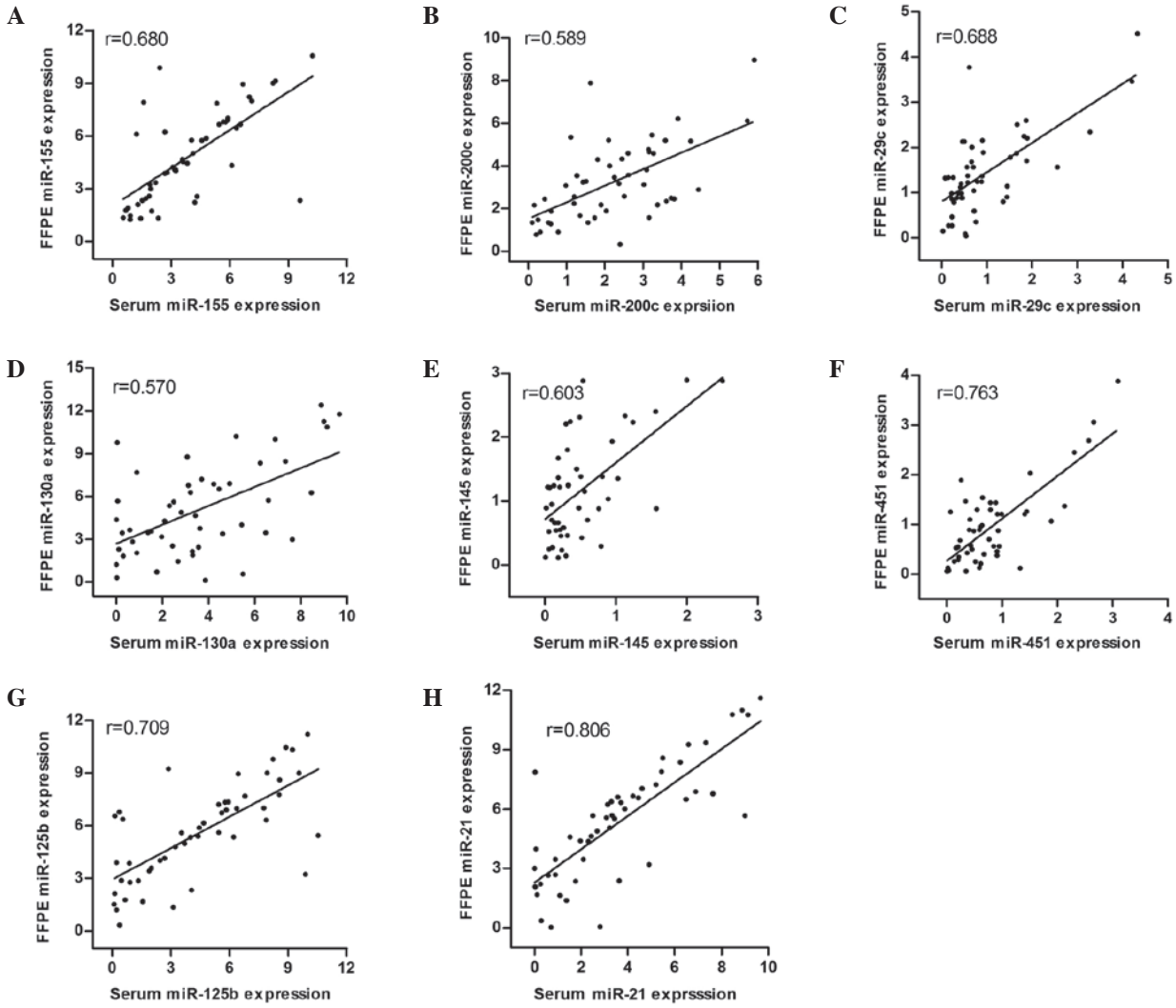

Figure 2. Expression values of miR-155, miR-200c, miR-29c, miR-130a, miR-145, miR-451, miR-125b and miR-21 (A-H, respectively) in serum are correlated with those in formalin-fixed, paraffin-embedded (FFPE) samples of 56 diffuse large B-cell lymphoma patients before treatment (P=0.01). Linear regression lines are shown. The correlation was assessed by Pearson's correlation analysis.

again. In case 3, R-CHOP was switched for the second-line regimen at 3 months due to primary refractory disease when using the first-line therapy. In this case the serum miR-130a and miR-125b expression was not significantly downregulated even following the change in the therapeutic schedule. Case 4, who relapsed at 18 months, received R-CHOP and the second-line regimen for salvage treatment, but failure occurred due to chemoresistance. In this case the serum miR-130a and miR-125b expression was re-elevated following the first treatment. Case 5 was administered the second-line regimen following a partial response, and in this case the serum miR-130a and miR-125b did not significantly decrease for 6 months.

Based on our observations, serum miR-130a and miR-125b changes always occur ahead of the clinical diagnosis of recurrence or progression. Patients who maintained miR-130a and miR-125b overexpression had a significantly increased probability of chemoresistance compared with those with none or only one high-regulated miRNA. In addition, miR-125b and miR-130a levels always remained high before treatment, then returned to normal levels gradually following R-CHOP treatment in drug-resistant cases. In contrast, in primary/secondary resistant cases, these miRNAs did not decrease significantly or increase again after remaining at normal levels for some time. Moreover, re-elevated levels of miR-125b and miR-130a were also observed in patients with recurrence or progression.

High levels of miR-130a and miR-125b are associated with poor prognosis. To calculate the potential prognostic impact of miR-130a and miR-125b in DLBCL, the patients were divided into two groups according to the median value of their expression levels of the two miRNAs. Using Kaplan-Meier survival analysis, we observed that DLBCL patients with high miR-125b but not miR-130a relative expression had a significantly shorter OS compared with those with a low expression. The three-year OS rates of DLBCL patients was 44% (95% CI, 30.25-41.72) in the high miR-125b group and 65% (95% CI, 19.08-33.31) in the low miR-125b group (log-rank test, P=0.048). The Kaplan-Meier curves according to miR-125b and miR-130a expression are shown in Fig. 6.

Furthermore, we used multivariate Cox regression analysis to examine the effect of various parameters (including miR-125b levels as well as age, gender, B symptoms, Ann Arbor

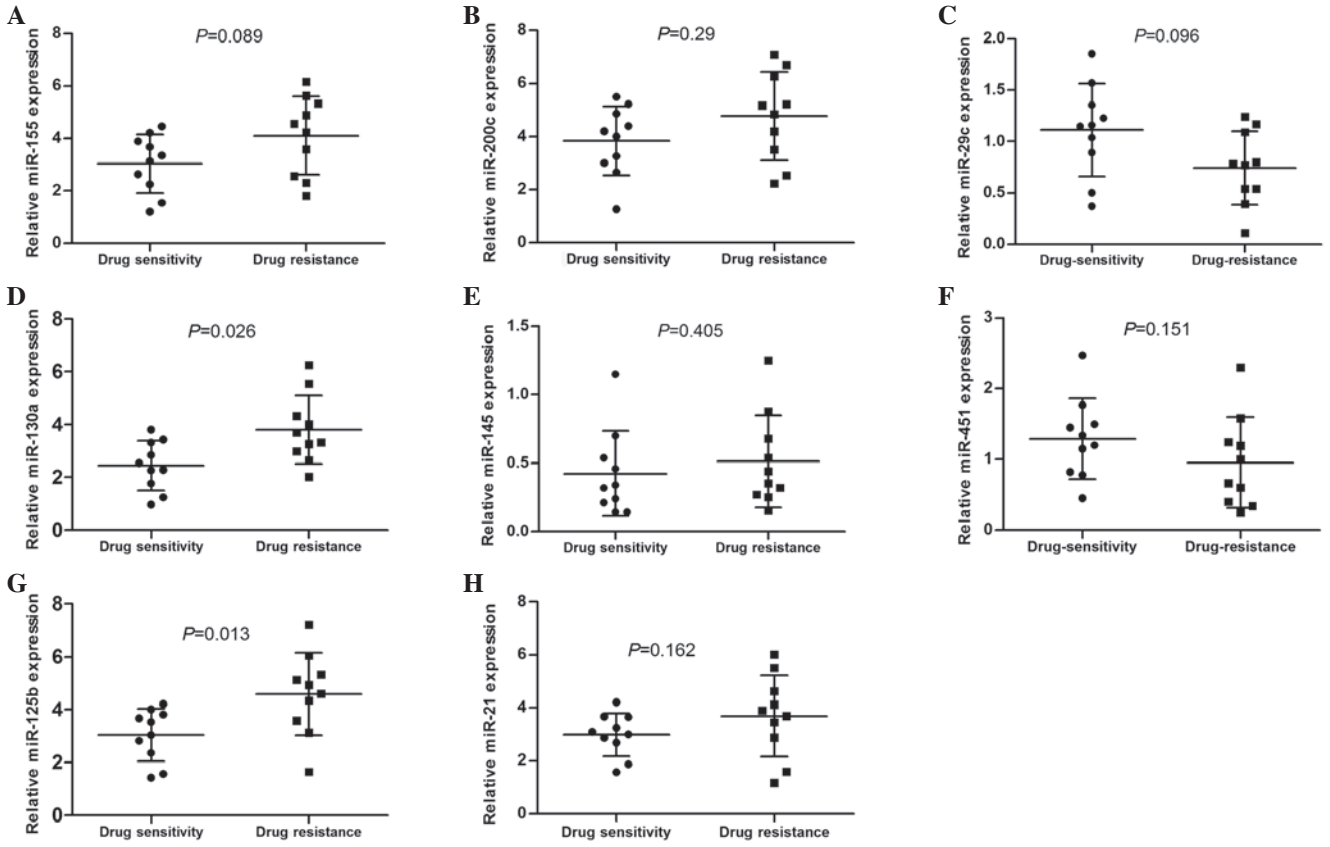

Figure 3. Validation of circulating miR-155, miR-200c, miR-29c, miR-130a, miR-145, miR-451, miR-125b and miR-21 (A-H, respectively) in drug resistance group (n=10) and drug sensitivity group (n=10) in 20 diffuse large B-cell lymphoma patients. Statistically significant differences were determined using the Mann-Whitney U test.

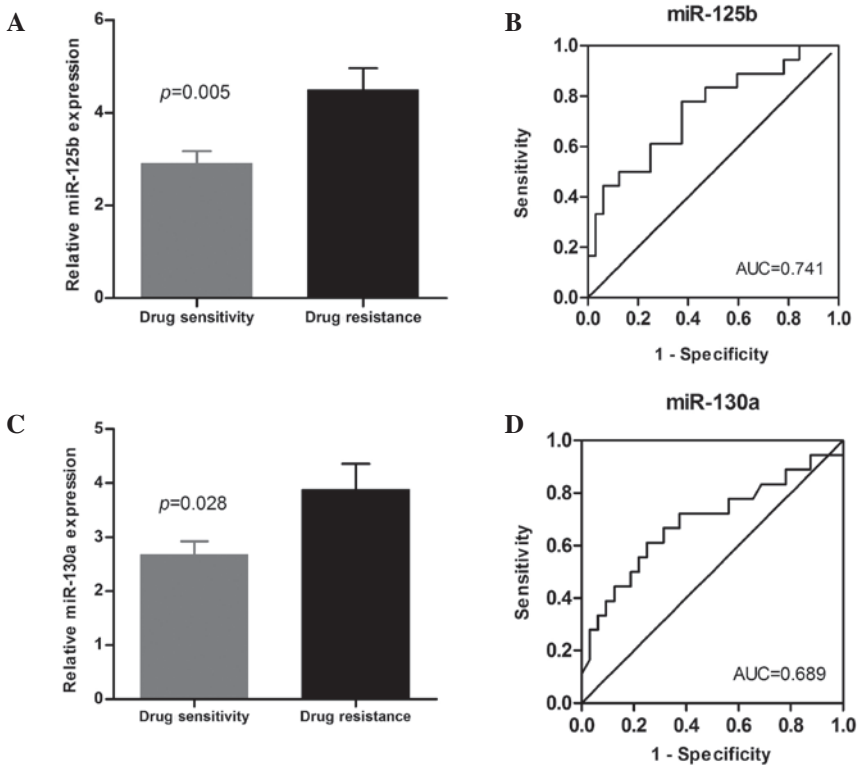

Figure 4. Validation of circulating miR-125b and miR-130a in a cohort of 56 diffuse large B-cell lymphoma patients. (A) miR-125b and (C) miR-130a expression levels were significantly higher in the drug resistance group (n=21) than in the drug sensitivity group (n=35). The receiver operating characteristic plot reflected strong separation between the two groups. (B) Cut-off value of 3.07 for miR-125b (sensitivity, 77.8%; specificity, 62.5%; 95% CI, 0.595-0.887; P=0.005). (D) Cut-off value of 3.275 for miR-130a (sensitivity, 61.1%; specificity, 75%; 95% CI, 0.524-0.854; P=0.028).

Table IV. Multivariate Cox regression analysis of various parameters involved in overall survival in diffuse large B-cell lymphoma.

Factor	Cox regression coefficient	Hazard ratio (95% CI)	P-value
miR-125b	0.399	1.491 (1.073-2.072)	0.017
miR-130a	0.207	1.230 (0.938-1.624)	0.135
IPI status	0.742	2.099 (1.068-4.126)	0.031

IPI, international prognostic index; CI, confidence interval.

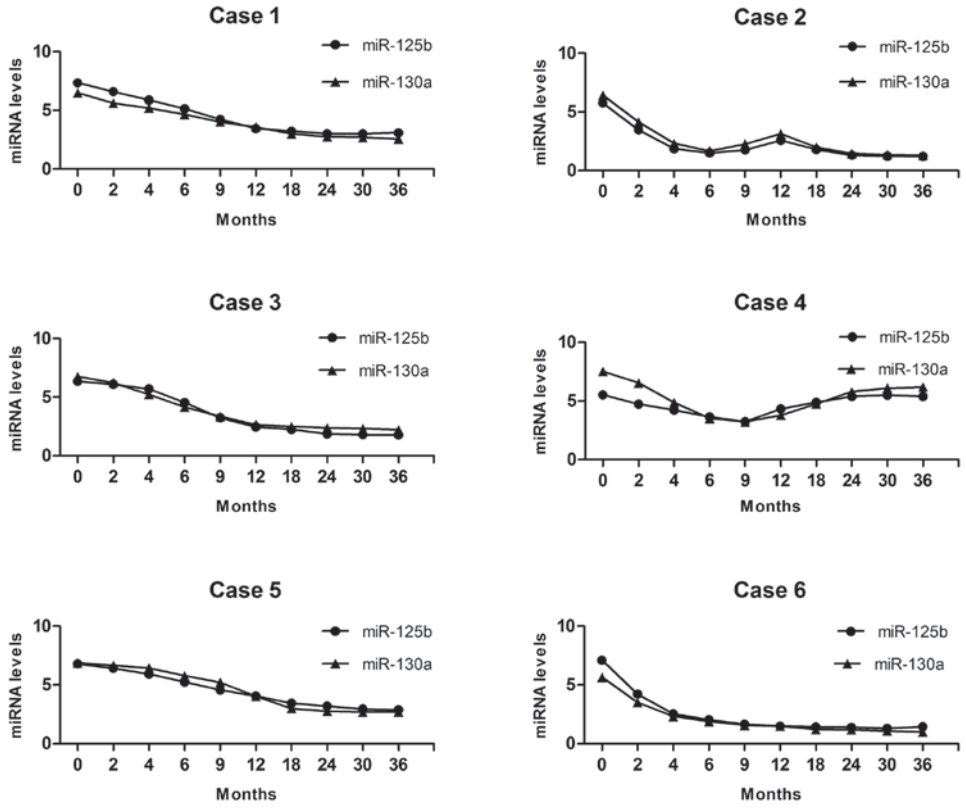

Figure 5. Dynamic changes in circulating miR-125b and miR-130a expression levels in six diffuse large B-cell lymphoma patients following chemotherapy.

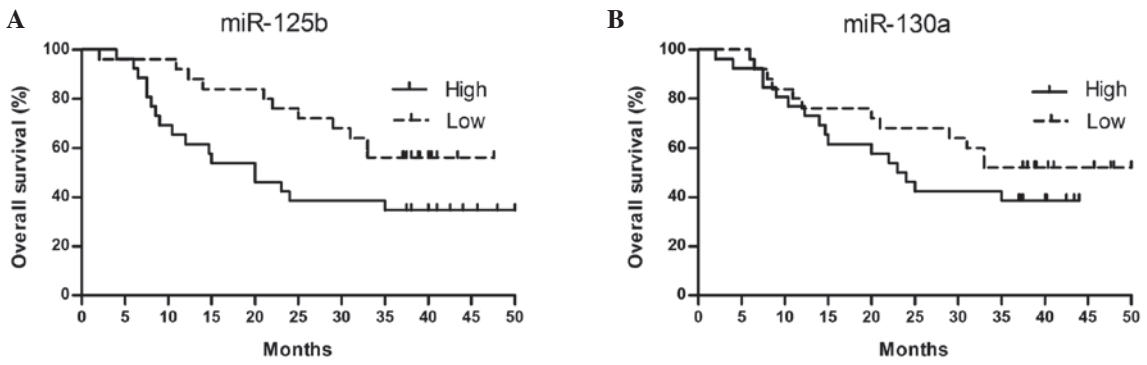

Figure 6. Overall survival curves of diffuse large B-cell lymphoma patients in miR-125b and miR-130a high and low expression groups. Kaplan-Meier survival curves were compared by log-rank test. (A) miR-125b overall survival rate, P=0.048; (B) miR-130a overall survival rate, P=0.29.

stage and IPI) that may affect prognosis. We observed that the upregulated miR-130a expression levels and high IPI scores were independent indicators for poor outcome (Table IV).

Discussion

A number of investigators have attempted to identify different

miRNAs to be used for diagnosis or to identify cancer patients with the poorest prognosis in order to design a more appropriate therapeutic strategy for them (30). Understandably, due to the ease and the non-invasive method of obtaining them, circulating miRNAs are becoming more attractive biomarkers compared with miRNAs isolated from tumor tissue, particularly in certain late-stage cancers. In addition, studies have demonstrated that miRNAs are stable in serum (30). Further studies have identified a significant correlation between serum and tumor tissues in certain solid tumors (31,32). However, for DLBCL, as a hematological malignant disease, no evidence for the clinical application of serum miRNA has been demonstrated up to the present day. In this study, we confirmed that miRNA expression levels in serum were significantly associated with their levels in FFPE tissues in DLBCL patients before treatment. We therefore propose that serum miRNAs levels might reflect tumor cell growth of DLBCL, although serum from a larger cohort of DLBCL patients should be tested.

Next, we demonstrated the overexpression of miR-155, miR-200c, miR-130a, miR-125b and miR-21 and underexpression of miR-29c, miR-451 and miR-145 in DLBCL serum. Notably, deregulation profiling of most of these miRNAs in DLBCL has been reported in a previous study (12), with the exception of miR-130a and miR-451. In this study, we first demonstrated the deregulated expression of circulating miR-130a and miR-451 in DLBCL. According to the results of other studies, miR-451 is a significant anti-oncomiRNA in colorectal carcinoma, and promoted PI3k/AKT and LKB1/AMPK activation by repressing the mitogen-activated protein kinase (MAPK) signaling pathway, and caused an decreased in self-renewal, tumorigenicity and vascular endothelial cell proliferation (33,34). In accordance with the above results, we also detected reduced miR-451 expression, which confirmed the function of miR-451 as a oncogene in DLBCL for the first time. miR-130a has been observed to play a critical role in various types of cancer. He *et al* (35) observed that miRNA-130a was upregulated in cervical cancer, which directly targeted Dicer mRNA, enhancing cellular growth, migration and invasion. In contrast, Li *et al* (36) detected that miR-130a was significantly downregulated in hepatocellular carcinoma. In the present study, we observed low miR-130a expression in DLBCL. The contradiction may be attributed to the small cohort of clinical experiments or the complex cell pathology and physiology in the different tumors.

Chemoresistance as a multifactorial progress has posed a critical issue in managing or preventing tumor progression. Particularly for DLBCL, the choice of a first-line treatment achieving complete remission without drug resistance is crucial. If the R-CHOP induction chemotherapy fails, then the prognosis remains poor even if another therapeutic schedule is used.

A number of previous studies have revealed that miRNAs are involved in the progress of drug resistance in certain types of cancer (16-29). These miRNAs might play a crucial role in drug resistance by targeting their specific genes. In this study, RT-qPCR was used to identify and quantitate the expression levels of a series of miRNAs (namely miR-155, miR-200c, miR-29c, miR-130a, miR-145, miR-451, miR-125b and miR-21) to evaluate their low or high sensitivity to R-CHOP chemotherapy. Two miRNAs with differential expression were identified: the expression of miR-130a and miR-125b was not only notably upregulated in the drug resistant groups treated with R-CHOP, but also demonstrated a strong separation between the drug-resistance group and drug-sensitivity group. miR-125b is an essential oncomiR, restraining the tumor necrosis factor necrosis factor, alpha-induced protein 3 (TNFAIP3) to enhance the nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) signaling pathway, and is significantly overexpressed in DLBCL (37). Davoudi *et al* (38) reported that the NF-κB pathway cross-talks with the PI3k/AKT pathway to promote anti-apoptosis and multidrug resistance, and was correlated with the expression of the MDR1 gene. When targeting the NF-κB pathway by suppressing MDR1 gene expression, these authors observed significantly increasing apoptotic stimuli and decreasing drug resistance. In our study we also observed upregulated miR-125b, so it was speculated that NF-κB, as one of the most altered pathways in DLBCL (39), may mediate the miR-125b-induced chemoresistance occurring in DLBCL patients treated with the R-CHOP regimen. Zhang *et al* (26) reported that low miR-130a expression led to a significant upregulation in the XIAP mRNA levels, which further resulted in the development of ovarian cancer cell resistance to cisplatin. However, Yang *et al* (28) provided the adverse conclusion that low miR-130a restrains MDR1 mRNA/P-glycoprotein, which plays a repressive role in the ABC superfamily drug transporters and the drug resistance pathways of PI3k/AKT/PTEN/mTOR. Similarly, upregulated miR-130a expression was also detected in the drug-resistant group in the present study. miR-130a expression profiling in DLBCL has not previously been reported in the literature, and the apparently contradictory roles of miR-130a as oncomiRNA or anti-oncomiRNA in different tumors may be due to different circumstances or tissues. In addition, Bai *et al* (25) reported that the upregulation of miR-21 decreased the sensitivity of DLBCL cell lines to the CHOP regimen; however, these results were not reproduced in our study, so further research is required to confirm this finding.

Our further research focused on monitoring the changes of circulating miR-125b and miR-130a levels dynamically. According to our findings, patients with upregulated miR-130a and miR-125b had a significant probability of recurrence, progression and chemoresistance, whereas patients with downregulated miR-130a and miR-125b were inclined to achieve complete remission and chemosensitivity. In addition, we observed that the changes in miRNA expression occurred prior to clinical manifestations and image diagnosis. Therefore we deduced that miR-130a and miR-125b may constitute new biomarkers for the determination of treatment response and prognosis in DLBCL; in particular, assessment of the two high-risk miRNAs simultaneously would be effective. More notably, patients with miR-130a and miR-125b overexpression who were resistant to R-CHOP were more inclined to be refractory to other chemotherapy regimens; that is, miR-130a and miR-125b may be associated with multidrug resistance. However, further research is required to prove this theory. To our knowledge, only one similar investigation relating to colorectal cancer has been published which elaborates the implications of dynamically monitoring the levels of miRNAs for disease progression and

prognosis (40), and there are no other studies concerned with DLBCL. Thereby, our study is the first to demonstrate that dynamic monitoring of the levels of circulating miR-125b and miR-130a could reflect the therapeutic response and disease status of DLBCL patients, which may have considerable significance for DLBCL management.

Since the introduction of rituximab and hematopoietic stem cell transplantation in the treatment of DLBCL, a relatively effective therapeutic model has been established. Thereby, strategies including intensive immunochemotherapy have improved the outcome for a number of such patients. However, there is a certain risk that due to the inability to accurately differentiate DLBCL, such patients are not receiving the necessary intensive treatment. In addition, other patients at high risk who are not diagnosed in a timely manner miss out on being considered for immediate treatment. The IPI is one of the crucial prognostic indicators and is based on patient characteristics including age, lactate dehydrogenase levels and extranodal invasion status. Although IPI retains a predictive role in DLBCL patients treated with R-CHOP, to our knowledge, the application of IPI in the rituximab era has not been established. Sehn *et al* (41) stated that IPI was no longer capable of differentiating four risk groups in the rituximab era, and advocated a revised IPI (R-CHOP). Even so, the high-risk group still could not be discriminated accurately. For this reason, additional prognostic factors are required to determine a favorable or poor outcome in DLBCL patients so that appropriate therapeutic strategies may be selected. In the present study, we explored the feasibility of serum miR-125b and miR-130a, which are treatment response-associated miRNAs, as outcome predictors for DLBCL patients treated with R-CHOP. We observed that a low expression of miR-125b was associated with a long OS; furthermore, serum miR-125b signatures used as the prognostic indicator were independent of the IPI score. Our results suggest that high serum miR-125b levels are associated with a poor prognosis, and that serum miR-125b, a simple, accurate, non-invasive biomarker which may be easily measured in clinical practice, might be a significant prognostic marker in the rituximab era.

In summary, our data demonstrated for the first time that the expression of miR-125b and miR-130a has potential as a chemoresistance-related indicator to evaluate the risk of chemoresistance in DLBCL patients. In addition, it was also demonstrated for the first time that dynamically monitoring the levels of circulating miR-125b and miR-130a could reflect therapeutic response and disease status in DLBCL patients. Notably, miR-125b but not miR-130a was identified as an independent poor prognostic factor. The correlation between miRNA expression levels in serum and in FFPE tissues in DLBCL was also considered in our study. Our results may help to provide a new potential biomarker and therapeutic target for DLBCL. However, additional studies are required to elucidate the molecular mechanisms of miR-125b and miR-130a in chemoresistance and disease progression, and to characterize the expression of these miRNAs in a large cohort of DLBCL patients.

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