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Downregulation of plasma miR-124 expression is a predictive biomarker for prognosis of glioma

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Abstract. – OBJECTIVE: MicroRNAs (miRNAs) have been widely identified as potential biomarkers for predicting prognosis of glioma. The objective of the study was to examine the clinical role of plasma miR-124 expression in glioma.

PATIENTS AND METHODS: MiR-124 expression in plasma samples from 64 cases glioma patients and 40 normal healthy controls was examined by quantitative reverse transcription PCR (qRT-PCR). The correlation of miR-124 expression with clinicopathological feathers or prognosis of glioma patients was assessed. Univariate and multivariate Cox analysis were used to analyze the risk factors of prognosis. The receiver operating characteristic (ROC) curve was established, and the area under the ROC curve (AUC) was calculated to evaluate the difference of miR-124 expression in glioma patients.

RESULTS: We showed that miR-124 expression was significantly downregulated in plasma samples of glioma patients compared with normal healthy controls ($p<0.05$). Plasma miR-124 expression significantly associated with Karnofsky Performance Status (KPS) score ($p<0.05$) and WHO grade ($p<0.05$) in glioma plasma. Patients with low miR-124 expression had worse disease free survival (DFS) and overall survival (OS) time than patients with high miR-124 expression. Univariate and multivariate analysis implied that low miR-124 expression was an independent maker of disease free survival (DFS) and overall survival (OS) in glioma.

CONCLUSIONS: Our findings implied that plasma miR-124 expression may serve as an independent predictor of poor prognosis.

Key Words:
MircroRNA, miR-124, Glioma, Plasma, Prognosis.

Introduction

Glioma is the most malignant and incurable primary brain tumor. Although therapeutic methods including surgery, radio-therapy and chemotherapy have advanced in the past years, the median survival of patients is 12-15 months[1]. Deregulations of oncogenes and tumor suppressors have been found in glioma progression[2]. Thus, to identify early diagnostic biomarkers and therapeutic strategies for glioma is needed. MicroRNAs (miRNAs) are a class of non-coding RNAs that are 22 nucleotides in length. MiRNAs function as post-transcriptional regulators of genes expression by targeting 3' untranslated region (UTR) (3'UTR) of their target mRNAs, which lead to protein translation inhibition or mRNA degradation[3,4]. MiRNAs have been increasingly recognized as oncogenes or tumor suppressors to be involved in human glioma. For instances, microRNA-141-3p promotes glioma cell growth and temozolomide resistance by directly targeting p53[5]. MicroRNA-625 inhibits the proliferation and increases the chemosensitivity of glioma by directly targeting AKT2[6]. MiR-216b inhibits glioma cell migration and invasion through suppression of FOXM1[7]. MiR-124 is reported to function as a tumor suppressor in glioma. For example, miR-124 could suppress the migration and invasion of glioma cells *in vitro* via regulating Capn4 expression[8]. MicroRNA-124-3p regulates cell proliferation, invasion, apoptosis, and bioenergetics by targeting PIM1 in astrocytoma[9]. However, the clinical value of plasma miR-124 expression in glioma remains unknown. We found that miR-124 expression was significantly downregulated in plasma samples of glioma patients compared with normal healthy controls. Plasma miR-124 expression was associated with WHO grade and KPS score of patients. Patients with low miR-124 expression had worse prognosis. Thus, our findings implied that plasma miR-124 expression may be an independent prognostic maker of glioma.

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Patients and Methods

Patient Samples

A total of 64 glioma patients who received surgery and 40 health controls were enrolled from Department of Neurosurgery, Beijing Boai Hospital (Beijing, China) between January 2012 and April 2014. Plasma samples of glioma patients were obtained in pre-operation and plasma samples from healthy controls were also obtained. Plasma samples were stored at -80°C until further RNA analysis. All of patients had not received any therapy. This study was approved by Research Ethics Committee of Boards of Beijing Boai hospital, China Rehabilitation Research Center (Beijing, China). Written informed consent was obtained from all the patients who were selected. The blood samples were collected and centrifuged for 10 min at 1500 g within 2 h after collection, and the supernatant was removed to RNase-free tubes and further centrifuged for 10 min at 12,000 g and 4°C to remove cells and debris.

RNA Extraction and Quantitative Reverse-Transcriptase Polymerase Chain Reaction (qRT-PCR)

A total of 500 μL plasma sample was used to extract RNA using the miRcute miRNA isolation kit (Tiangen biotech C, LTD. Beijing, China) according to the manufacturer's protocol. RNA was quantified using the Nano Drop 1000 (NanoDrop, Wilmington, DE, USA). The cDNA was synthesized using miScript II RT kit (Tiangen Biotech., Ltd. Beijing, China) according to the manufacturer's instructions. QRT-PCR analysis was performed using miScript SYBR Green PCR Kit (Tiangen Biotech., Ltd. Beijing, China) according to manufacturer's instructions using an Applied Biosystems 7500 Sequence Detection system (Applied Biosystems, Foster City, CA, USA; Thermo Fisher Scientific, Waltham, MA, USA). The fold change of miR-124 expression was calculated using $2^{-\Delta\Delta CT}$ methods. U6 mRNA expression was used as an internal control. Primer sequences were as follows: miR-124-forward: 5'-TGGGTTCGGTGGTCAAGTC-3';miR-124-reverse:5'-CGCTCTGGTAGTGCTGGGA-3'.

Statistical Analysis

The statistical analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). All data are expressed as the mean ± standard deviation (SD). Differences between groups were analyzed by the Student *t*-test or the x^2-test. Survival plots were performed by using the Kaplan-Meier method and the log-rank test. Univariate and multivariate Cox regression analysis were performed to evaluate relative risks factors of DFS and OS. $p<0.05$ were identified as statistically significant.

Results

Plasma miR-124 Expression is Downregulated in Glioma Patients

To investigate the clinical significance of miR-124 expression, we detected plasma samples from 64 cases of glioma patients and 40 cases normal healthy controls by quantitative reverse transcription PCR (qRT-PCR). The plasma miR-124 expression was significantly downregulated compared to normal healthy controls (p <0.05) (Figure 1). These findings indicated that downregulation of plasma miR-124 may serve as an important biomarker in glioma prognosis.

Association Between Plasma miR-124 Expression and the Clinicopathological Parameters of Glioma

To further assess the clinical role of plasma miR-124 expression in glioma, we analyzed the association between plasma miR-124 expression and the clinicopathological parameters in glioma patients. According to the mean expression of miR-124 expression, the patients were divided into two groups (high miR-124 expression group and low miR-124 expression group). Low plasma

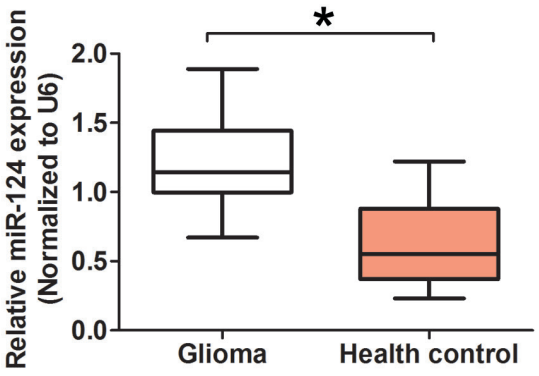

Figure 1. MiR-124 expression was detected in plasma samples from 64 cases of glioma patients and 40 cases normal healthy controls by quantitative reverse transcription PCR (qRT-PCR). U6 was used as an internal control, *p < 0.05.

Table I. Correlation between plasma miR-124 expression and clinical factors

Clinical factors	Total (n=64)	miR-124 expression		p-value
		Low (n=34)	High (n=30)	
Gender				0.975
Male	34	18	16	
Female	30	16	14	
Age				0.071
≤ 55	29	19	10	
> 55	35	15	20	
Tumor size				0.443
≤ 3 cm	33	16	17	
> 3 cm	31	18	13	
Tumor location				0.251
Parenchyma	45	26	19	
Ventricle	19	8	11	
Karnofsky Performance Status				0.003*
≤ 80	30	10	20	
> 80	34	24	10	
Resection range				0.380
Total resection	44	25	19	
Local resection	20	9	11	
WHO grade				0.033*
I-II	38	16	22	
III-IV	26	18	8	

*$p < 0.05$.

miR-124 expression was significantly associated with higher KPS score ($p = 0.003$) and WHO grade ($p = 0.033$) (Table I). However, there was no significant association between plasma miR-124 expression and other clinical factors including gender, age, tumor size, and so on (all $p > 0.05$, Table I).

Plasma miR-124 Expression Serves as a Prognostic Predictor of Glioma

To further assess whether plasma miR-124 expression could predict prognosis of glioma patients, we performed Kaplan-Meier method and log rank test. The results showed that patients with low plasma miR-124 expression had significantly shorter disease free survival (DFS) (log rank=12.894, $p < 0.001$) (Figure 2A). Furthermore, we found that patients with low plasma miR-124 expressing also had significantly shorter overall survival (OS) (log rank=14.596, $p < 0.001$, Figure 2B). In addition, univariate and multivariate Cox analysis were used to examine whether plasma miR-124 expression was an independent risk factor for prognosis in glioma patients (Table II-III). The results demonstrated that plasma miR-124 expression was an independent prognostic factor for DFS [HR, 2.421; 95% CI, 1.621-4.120; $p<0.05$] and OS time [HR, 2.289; 95% CI, 1.245-4.066; $p = 0.004$] patients. In addition, the receiver operating characteristic (ROC) curve analysis revealed that the value of miR-124 in predicting the DFS or OS risk of glioma was high, the area under the ROC curve (AUC) was 0.851 for DFS and 0.857 for OS, and 95% confidence interval (CI) was 0.764-.938 for DFS or 0.773-0.942 for OS, respectively (Figure 3A-B). These results indicated that plasma miR-124 expression could serve as a prognostic maker of glioma patients.

Discussion

Glioma is the most lethal primary brain tumor. In spite of surgery and radiochemotherapy have advanced, the median survival for patients is low[10]. Some noninvasive, clinically applicable detection and prognostic biomarkers urgently need for glioma. Circulating miRNAs of plasma or serum have been identified as potential biomarkers for detection, identification, and classification of cancers including glioma[11]. Specific miRNAs have potential clinical value for diagnosing and predicting prognosis in glioma patients. For example, plasma levels of miR-128

Table II. Cox regression analysis of prognostic factors for DFS in glioma patients.

	Univariate Cox analysis		Multivariate Cox analysis	
Factor	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***
Gender	0.881 (0.432-1.211)	0.877		
Sex	1.081 (0.551-1.869)	0.545		
Tumor size (mm)	1.122 (0.634-1.987)	0.477		
Tumor location	0.938 (0.522-1.876)	0.678		
Karnofsky Performance Status	2.477 (1.675-4.099)	0.001*	1.988 (1.255-3.332)	0.001*
Resection range	1.091 (0.698-1.764)	0.496		
WHO grade	2.066 (1.452-4.186)	0.001*	1.897 (1.211-3.212)	0.002*
Low miR-124 expression	2.899 (1.766-4.544)	0.001*	2.421 (1.621-4.120)	0.001*

*$p < 0.05$.

Table III. Cox regression analysis of prognostic factors for OS in glioma patients.

	Univariate Cox analysis		Multivariate Cox analysis	
Factor	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***
Gender	0.941 (0.484-1.765)	0.792		
Sex	1.133 (0.556-1.991)	0.466		
Tumor size (mm)	1.052 (0.438-1.865)	0.517		
Tumor location	0.885 (0.611-1.643)	0.846		
Karnofsky Performance Status	2.518 (1.511-4.288)	0.001*	2.244 (1.255-3.862)	0.001*
Resection range	0.786 (0.342-1.669)	0.912		
WHO grade	2.155 (1.612-3.255)	0.001*	1.934 (1.441-2.895)	0.001*
Low miR-124 expression	3.112 (1.814-4.899)	0.001*	2.289 (1.245-4.066)	0.001*

*$p < 0.05$.

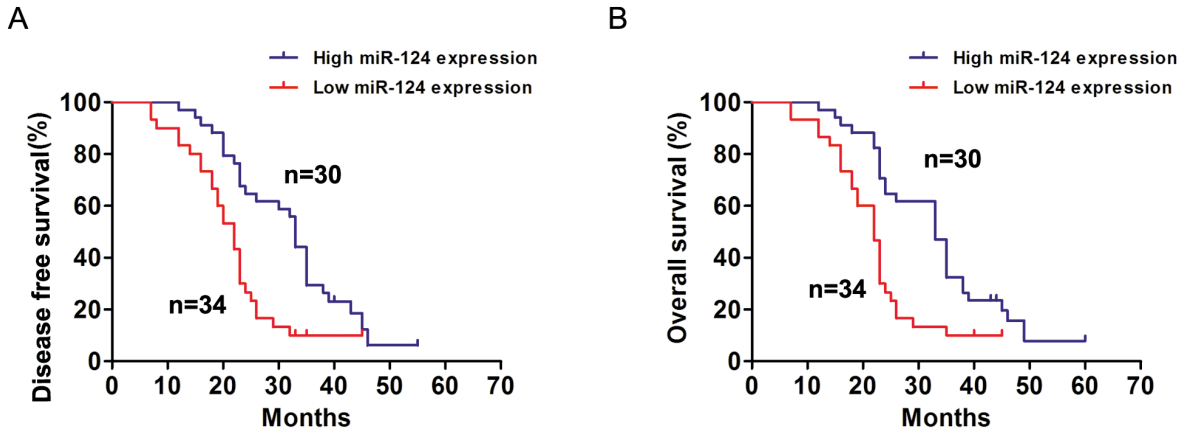

Figure 2. Plasma miR-124 expression serves as a prognostic predictor of glioma. ***(A)*** Low plasma miR-124 expression showed a poor DFS compared to high plasma miR-124 expression in glioma patients. ***(B)*** Low plasma miR-124 expression showed a poor OS compared to high plasma miR-124 expression in glioma patients.

and miR-342-3p were positively correlated with histopathological grades of glioma[12]. The plasma levels of miR-185 in glioblastoma multiform patients with operation and chemo-radiation almost revived to normal levels and low plasma miR-185 levels are correlated with poor survival in glioma patients[13]. Circulating miR-182 in glioma patients is higher than that in healthy controls, and is an independent prognostic indicator for OS and DFS[14]. Plasma miR-199a-3p expression is significantly lower in glioma patients when compared with healthy controls. Glioma patients

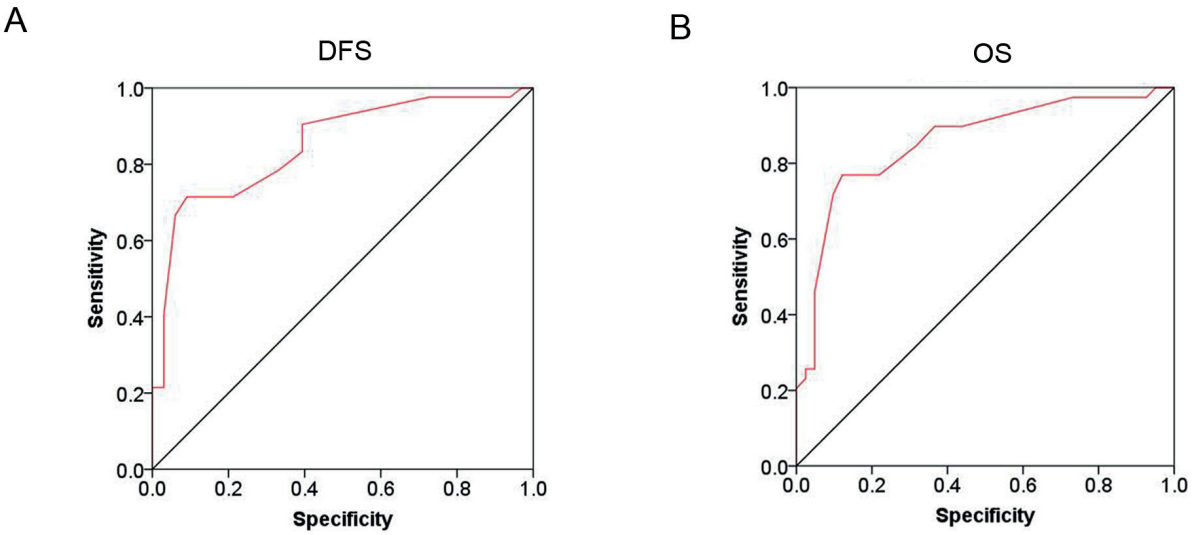

Figure 3. ROC curves of miR-124 in the plasma samples of glioma and the control group. The value of miR-124 in predicting the risk of glioma is high, the area under the ROC curve (AUC) was ***(A)*** 0.851 for DFS and 95% confidence interval (CI) was 0.764-.938 and ***(B)*** 0.857 for OS, and 95% confidence interval (CI) was 0.773-0.942.

with low miR-199a-3p expression have distinctly shorter overall survival than patients with high miR-199a-3p expression[15]. These findings implied that the plasma microRNAs could be used as a promising novel biomarker for the diagnosis and prognosis of glioma.

In previous study, miR-124 is involved in glioma progression. For instances, miR-124 is downregulated in glioma tissues and loss of brain-enriched miR-124 microRNA enhances stem-like traits and invasiveness of glioma cells[16]. MiR-124 suppresses glioblastoma growth and potentiates chemosensitivity by inhibiting AURKA[17]. MiR-124 upregulation inhibits the growth of C6 glioma cells by targeting Smad4 directly[18]. In the study, our results showed that plasma miR-124 expression was significantly downregulated compared to normal healthy controls. Furthermore, plasma miR-124 expression was significantly associated with WHO grade and KPS score. However, there were no significant associations between plasma miR-124 expression and other clinical factors including age, gender, tumor size and so on. Kaplan-Meier method and log rank test showed that patients with low plasma miR-124 expressing had significantly shorter DFS and OS. Univariate and multivariate Cox analysis showed plasma miR-124 was an independent risk factor for prognosis in glioma patients. In addition, the receiver operating characteristic (ROC) curve analysis revealed that the value of miR-124 in predicting the DFS or OS risk of glioma was high and the area under the ROC curve (AUC) was 0.851 for DFS and 0.857 for OS. Thus, these results indicated that plasma miR-124 could be used as a promising novel biomarker for the diagnosis and prognosis of glioma.

Conclusions

We showed that plasma miR-124 was lower in glioma. Lower plasma miR-124 expression may serve as a promising predictor for the detection of glioma. Furthermore, plasma miR-124 downregulation may be potentially associated with unfavorable prognosis of glioma.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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