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MiR-599 as a potential biomarker for prognosis of glioma

X.-Y. ZHU[1], G.-X. LI[2], Z.-L. LIU[2]

[1]Department of Pathology, [2]Department of Laboratory, Linyi People's Hospital, Linyi, Shandong, China

Abstract. – OBJECTIVE: MicroRNAs (miRNAs) have been demonstrated to play a central role in development and progression in various cancers including glioma. This study aimed to determine the expression level of miRR-599 in glioma and to further investigate its clinical significance.

PATIENTS AND METHODS: The expression levels of miR-599 were evaluated in 184 paired of glioma and normal tissues by Real-time RT-PCR. The correlation between tumor miR-599 expression and the clinical and pathological features was analyzed using x2 test. Overall survival and progression-free survival curves were obtained using the Kaplan-Meier method. Cox regression analysis was conducted to analyze the prognostic value of miR-599.

RESULTS: We observed that miR-599 expression was significantly downregulated in glioma compared with the adjacent noncancerous tissues ($p < 0.01$). Correlation analysis revealed that miR-599 downregulation was significantly correlated with KPS ($p = 0.000$), WHO grade ($p = 0.003$) and recurrence ($p = 0.039$). Moreover, Kaplan-Meier analysis confirmed that low miR-599 expression was related to decreased and overall survival ($p = 0.002$) and progression-free survival ($p = 0.001$). Then, multivariate analysis revealed that miR-599 was an independent prognostic indicator for overall survival ($p = 0.006$) and progression-free survival ($p = 0.003$).

CONCLUSIONS: Our data, for the first time, demonstrated that miR-599 expression in glioma could be a useful prognostic marker.

Key Words:
miR-599, Glioma, Prognosis.

Introduction

Gliomas, the most common primary malignant brain tumors in adults, are derived from glial cells that surround and support neurons in the brain[1,2]. In China, the incidence of glioma increased in the past decade[3]. In recent years, glioma accounts for 30% of all brain tumors and 80% of all malignant brain tumors[4]. So far, surgery, followed by radiotherapy and chemotherapy, was the standard treatment[5]. Despite the great progress in therapeutic technologies, the survival rates of patients with high-grade gliomas are <10% at 5 years[6,7]. The poor outcome of glioma patients has been associated with the appearance of metastasis and recurrence[8]. Thus, it is important to develop novel prognostic markers, which might improve the current prognostic models and guide therapeutic strategies. MiRNAs are a family of small non-coding RNA molecules that are 18-25 nucleotides in length and regulate gene expression by targeting 3′-UTR of target mRNA[9]. miRNAs have been shown to play an important role in the regulation of cell differentiation, proliferation and apoptosis[10]. In recent years, more and more evidence indicated that miRNAs were involved in the development and progression of various tumors[11]. Importantly, a number of studies have confirmed that some miRNAs expression was abnormal in different types of tumors, such as lung cancer[12], cervical cancer[13] and glioma[14]. Indeed, growing evidence showed that miRNAs play tumor suppressors or oncogenes in different tumors. Recently, dysregulation of miR-599 was found in some different tumors. However, the effects seem not to be consistent. Upregulation of miR-599 promoted non-small cell lung cancer cell invasion[15]. On the contrary, miR-599 served as tumor suppressor in hepatocellular carcinoma[16] and breast cancer[17]. Zhang et al[18] firstly reported that miR-599 was lowly expressed in glioma. However, to our best knowledge, its prognostic value has not been reported.

Patients and Methods

Patients and Tissue Samples

A total of 184 patients were analyzed in this study and underwent resection of their primary gliomas at Linyi People's Hospital between 2007 and 2011. None of the patients received preope-

Corresponding Author: Zhong-Lu Liu, MD; e-mail: lz777gl@126.com

rative chemotherapy or radiotherapy before surgery. All samples were diagnosed in accordance with the World Health Organization criteria. There were 99 males and 85 females, aged from 29 to 72 years old, with a median age of 51 years. Follow-up data were conducted using hospital medical records and telephone interviews. The clinicopathological information of the patients is shown in Table I. The experimental protocol was approved by the hospital Ethics Committee and informed consent was obtained from all patients.

RNA Extraction and Quantitative Real-time PCR

Total RNA was isolated from tissues using TRIzol reagent and reverse transcribed by M-MLV reverse transcriptase kit (Invitrogen, Carlsbad, CA, USA). Quantitative PCR was performed using ABI 7500 Sequence Detection System (Life Technologies, Grand Island, NY, USA). GAPDH gene was used as an internal control for normalization. Data were collected and analyzed using the $2^{-\Delta\Delta Ct}$ method for quantification of the relative miR-599 expression levels. The primers were used as follow: miR-599, sense 5'- GUUGUGUCAGUUUAUCAAAC-3'; antisense, 5'-CTCCATATCGCACTTTAATCTCTAACT-3'; GAPDH, sense 5'- TGC ACC ACCAAC TGC TTA-3'; antisense, 5'-GGATGCAGGGATGATGTT C-3'.

Statistical Analysis

SPSS 17.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. The Student's *t*-test was applied to determine the statistical significance of the observed differences between groups. Pearson's x^2 test was used to compare the expression level of miR-599 and clinical variables. Survival curves were plotted by the Kaplan-Meier method and compared by the log-rank test. Multivariate analysis was performed using the Cox proportional hazard model. $p < 0.05$ was considered to be statistically significant.

Results

miR-599 is Downregulated in Glioma

To investigate whether miR-599 was dysregulated in glioma, we collected glioma tissues and matched normal tissues to detect the expression levels of miR-599 by PCR. As shown in Figure 1, we observed that levels of miR-599 were lower in glioma tissues than in corresponding non-neoplastic brain tissue ($p < 0.01$). These results indicate that miR-599 might be critical in glioma.

Correlation of miR-599 Expression and Clinical Parameters in Glioma

To explore the association between miR-599 expression and the clinicopathological features of glioma patients, the expression levels of miR-599 glioma tissues were categorized as low or high in relation to the mean value. As shown in Table I, miR-599 downregulation was significantly correlated with KPS ($p = 0.000$), WHO grade ($p = 0.003$) and recurrence ($p = 0.039$). However, there was no association between miR-599 levels and other clinical features, such as age, gender, tumor location, necrosis and tumor size (all $p > 0.05$).

Prognostic Value of miR-599 in Glioma

In order to explore the prognostic value of miR-599 expression in glioma, we analyzed 599 glioma patients with complete clinical follow-up. The results of Kaplan-Meier analysis revealed that patients with low expression of miR-599 had significantly shorter overall survival (OS) than the high expression group ($p = 0.0002$; log rank test, Figure 2). Furthermore, we also found that patients with lower levels of miR-599 had poorer progression free survival (PFS) than those with higher levels of miR-599 (Figure 3, $p = 0.0001$). Subsequently, the clinicopathological factors were further examined using multivariate analysis. As shown in Table II, the data suggested that miR-599 was an independent prognostic indicator for OS ($p = 0.006$) and PFS ($p = 0.003$).

Discussion

Glioma is a complex disease, involving various changes in gene expression[19]. Despite

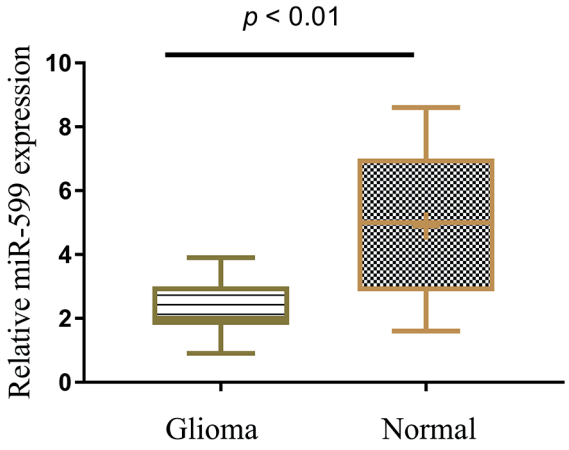

Figure 1. MiR-599 was significantly downregulated in glioma tissues compared with adjacent tissues ($p < 0.01$). MiR-599 expression levels were calculated by the $2^{-\Delta CT}$ method and normalized to GADPH.

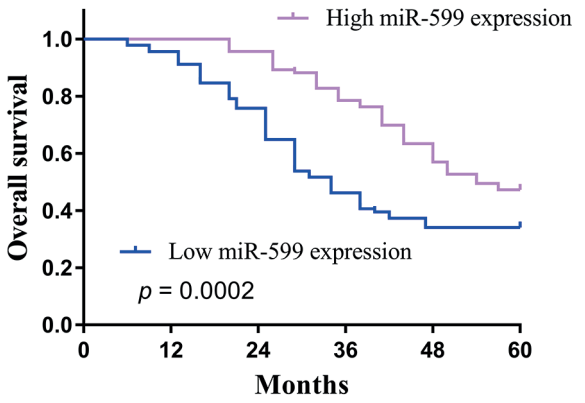

Figure 2. Kaplan-Meier analyses of OS were performed for all 184 patients with glioma according to miR-599 expression (miR-599-High *vs.* -Low).

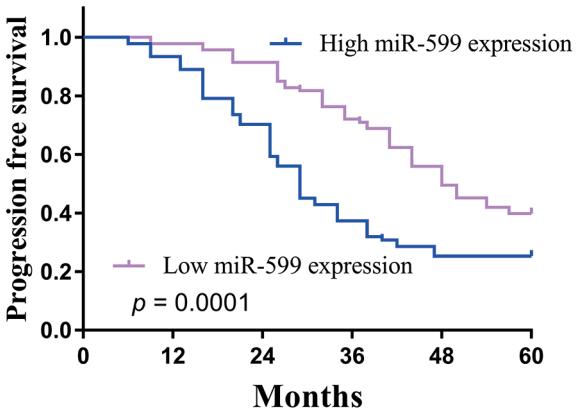

Figure 3. Kaplan-Meier analyses of PFS were performed for all 184 patients with glioma according to miR-599 expression (miR-599-High *vs.* -Low).

Table I. Association of miR-599 with clinicopathological characteristics of glioma patients.

	miR-599 expression (n)			
Variable	**n**	**Low**	**High**	***p***
Age (years)				0.568
≤50	111	53	58	
>50	73	38	35	
Gender				0.109
Male	99	45	54	
Female	85	46	39	
Tumor location				0.599
Supratentorial	164	80	84	
Infratentorial	20	11	9	
Necrosis				0.587
Yes	29	13	16	
No	155	78	77	
KPS				0.000
< 90	87	56	31	
≥ 90	97	35	62	
Tumor size (cm)				0.116
≤ 3	133	61	72	
> 3	51	30	21	
WHO grade				0.003
I-II	105	42	63	
III-IV	79	49	30	
Recurrence				0.039
Yes	87	50	37	
No	97	41	56	

advances in standard therapy, including surgical resection followed by radiation and chemotherapy, to our disappointment, satisfactory therapeutic prognosis has not been realized[20]. Given the poor survival rate in glioma patients, it is urgent to identify novel biomarkers, which can accurately predict outcome for glioma patients and guide treatment. Although many miRNAs have been well studied, the biological function of miR-599 in disease remains largely unknown. MiR-599 is located at 8q22.2 on human genome. Previous studies indicated a critical role for miR-599 in several cancer initiation, promotion, and progression. For instance, miR-599 was reported to be downregulated and suppressed the migration and invasion by targeting BRD4 in human breast cancer[17]. Tian et al[16] found that miR-599 inhibited hepatocellular carcinoma cells proliferation, migration and invasion by targeting MYC. These findings revealed miR-599 as a tumor suppressor in hepatocellular carcinoma and breast cancer. However, recent findings by Tian et al[15] showed that miR-599 acted as a potential tumor promoter and promoted non-small cell lung cancer cell invasion via SATB2. The different results indicated miR-599 could serve as tumor suppressor or tumor promoter according to the types of cancer. Importantly, Zhang et al[18] reported that miR-599 was down-regulated in glioma tissues compared with adjacent normal brain tissues. Furthermore, *in vitro* and *in vivo* assay showed that up-regulation of miR-599 suppressed proliferation and invasion by down-regulating periostin expression. However, to our best, the clinical significance of miR-599 in glioma has not been reported. In the present study, we found that miR-599 expression level was significantly lower in glioma tissues compared with non-neoplastic brain tissues. Furthermore, our data showed that miR-599 expression was significantly associated with KPS, WHO grade and

Table II. Multivariate Cox's hazards model analysis for prognostic factors associated with OS and PFS.

	Overall survival			Progression-free survival		
	Hazard ratio	95% CI	*p*	Hazard ratio	95% CI	*p*
Age	1.155	0.733-3.231	0.318	1.352	0.523-2.894	0.278
Gender	2.131	0.643-4.553	0.214	1.774	0.452-3.994	0.177
Tumor location	2.544	0.673-5.226	0.179	2.032	0.477-4.328	0.155
Necrosis	2.329	0.823-3.779	0.264	1.893	0.674-3.231	0.167
KPS	2.783	1.423-5.447	0.001	3.139	1.773-6.832	0.001
Tumor size	1.423	0.822-2.673	0.143	1.673	0.772-3.321	0.097
WHO grade	2.732	1.323-5.579	0.006	2.933	1.563-6.131	0.003
Recurrence	2.673	1.131-3.973	0.021	2.932	1.423-3.784	0.027
miR-599 expression	2.931	1.632-5.872	0.006	3.137	1.932-6.773	0.003

recurrence, suggesting that miR-599 might be involved in the carcinogenesis of glioma. More importantly, we proved that decreased miR-599 expression contributed to poor OS and PFS. In the subsequent Cox regression analysis, we confirmed that miR-599 expressions were independent predictors for OS and PFS of patients with glioma. Because of limit of the number of patients, further study of a larger case population is necessary to further confirm our results.

Conclusions

Combing with the previous findings, our findings clearly indicated that low miR-599 expression is associated with poor prognosis in glioma patients. In the further, miR-599 potentially serves as a prognostic biomarker, and may be a therapeutic target in glioma.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Ohgaki H. Epidemiology of brain tumors. Methods Mol Biol 2009; 472: 323-342.
2) Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.
3) Yang P, Wang Y, Peng X, You G, Zhang W, Yan W, Bao Z, Wang Y, Qiu X, Jiang T. Management and survival rates in patients with glioma in China (2004-2010): a retrospective study from a single-institution. J Neurooncol 2013; 113: 259-266.
4) Camelo-Piragua S, Kesari S. Further understanding of the pathology of glioma: implications for the clinic. Expert Rev Neurother 2016; 16: 1055-1065.
5) Taal W, Bromberg JE, van den Bent MJ. Chemotherapy in glioma. CNS Oncol 2015; 4: 179-192.
6) Ohgaki H, Kleihues P. Epidemiology and etiology of gliomas. Acta Neuropathologica 2005; 109: 93-108.
7) Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO; Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005; 352: 987-996.
8) Touat M, Duran-Peña A, Alentorn A, Lacroix L, Massard C, Idbaih A. Emerging circulating biomarkers in glioblastoma: promises and challenges. Expert Rev Mol Diagn 2015; 15: 1311-1323.
9) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
10) Bushati N, Cohen SM. microRNA functions. Annu Rev Cell Dev Biol 2007; 23: 175-205.
11) Di Leva G, Croce CM. Roles of small RNAs in tumor formation. Trends Mol Med 2010; 16: 257-267.
12) Xiao P, Liu W, Zhou H. miR-429 promotes the proliferation of non-small cell lung cancer cells via targeting DLC-1. Oncol Lett 2016; 12: 2163-2168.
13) Li B, Yang XX, Wang D, Ji HK. MicroRNA-138 inhibits proliferation of cervical cancer cells by targeting c-Met. Eur Rev Med Pharmacol Sci 2016; 20: 1109-1114.
14) Li B, Liu YH, Sun AG, Huan LC, Li HD, Liu DM. MiR-130b functions as a tumor promoter in glioma via regulation of ERK/MAPK pathway. Eur Rev Med Pharmacol Sci 2017; 21: 2840-2846.
15) Tian W, Wang G, Liu Y, Huang Z, Zhang C, Ning K, Yu C, Shen Y, Wang M, Li Y, Wang Y, Zhang B, Zhao Y. The miR-599 promotes non-small cell lung cancer cell invasion via SATB2. Biochem Biophys Res Commun 2017; 485: 35-40.
16) Tian J, Hu X, Gao W, Zhang J, Chen M, Zhang X, Ma J, Yuan H. Identification a novel tumor-suppressive

hsa-miR-599 regulates cells proliferation, migration and invasion by targeting oncogenic MYC in hepatocellular carcinoma. Am J Transl Res 2016; 8: 2575-2584.

17) Wang Y, Sui Y, Zhu Q, Sui X. Hsa-miR-599 suppresses the migration and invasion by targeting BRD4 in breast cancer. Oncol Lett 2017; 14: 3455-3462.

18) Zhang T, Ma G, Zhang Y, Huo H, Zhao Y. miR-599 inhibits proliferation and invasion of glioma by targeting periostin. Biotechnol Lett 2017; 39: 1325-1333.

19) Ostrom QT, Gittleman H, Stetson L, Virk SM, Barnholtz-Sloan JS. Epidemiology of gliomas. Cancer Treat Res 2015; 163: 1-14.

20) Dellaretti M, Reyns N, Touzet G, Dubois F, Gusmão S, Pereira JL, Blond S. Diffuse brainstem glioma: prognostic factors. J Neurosurg 2012; 117: 810-814.