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Reduced expression of miR-3653 in glioma and its correlations with clinical progression and patient survival

Y. CHEN, Z.-H. LI, X. LIU, G.-X. LIU, H.-M. YANG, P.-F. WU

Department of Emergency, The First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine, Guiyang, Guizhou, China

Abstract. – OBJECTIVE: Growing evidence in recent years have demonstrated that the dysregulation of microRNAs (miRNAs) strongly affected the biological development and progression of human tumors, including glioma. There have been few studies on the clinical significance of miRNAs in glioma. The aim of our study is to explore the expression pattern and the value of miR-3653 in the prognosis of glioma patients.

PATIENTS AND METHODS: qRT-PCR assays were performed to the miR-3653 expression level in 168 cases of glioma tissues and matched normal tissues. The correlations of miR-3653 expression level with the clinicopathological factors in glioma patients were analyzed. The associations between miR-3653 expression and survival of glioma patients were investigated by the Kaplan-Meier analysis and the log-rank test. The prognostic value of miR-3653 was estimated *via* univariate and multivariate analysis.

RESULTS: We presented that miR-3653 level in glioma tissues is notably reduced compared to matched non-cancerous brain tissues ($p<0.01$). Clinical research revealed that the lower miR-3653 expression was associated with larger tumor size and lymph node metastasis, lower KPS ($p=0.028$), and advanced WHO grade (p=0.019). Moreover, the clinical data further suggested that the low expression of miR-3653 predicted a worse 5-year overall survival in glioma patients. Finally, the multivariate analysis confirmed that low miR-3653 expression (HR=2.682, 95% CI: 1.148-4.281, $p=0.021$) was a significant independent predictor of poor survival in glioma.

CONCLUSIONS: Our findings suggested that miR-3653 could serve as a valuable prognostic indicator in glioma. Further researches are required to explore the potential function and mechanism of miR-3653 in glioma.

Key Words:
MiR-3653, Glioma, Prognosis.

Introduction

Human gliomas are the most prevalent brain neoplasms in adults and are characterized by high morbidity and mortality rates, with an annual incidence of ~6/100,000 worldwide[1,2]. The grade III and IV (malignant) gliomas, such as glioblastoma accounting for approximately 60-70% of malignant gliomas, are aggressive and lethal brain tumors[3]. Up to date, the standard therapy in clinical practice consists of surgical resection followed by radiotherapy and chemotherapy (temozolomide frequently used)[4]. The targeted therapy was also commonly used for the treatment of glioma patients[5]. However, the clinical outcome of glioma patients in China is still quite poor with a median survival rate of only about ten to fifteen months after diagnosis[6,7]. The potential reasons causing shorter overall survival and low level of quality of life of glioma patients are rapid cell growth, distant metastasis, and primary understanding of its molecular mechanisms[8,9]. Thus, finding out accurate prognostic markers would help gliomas prognosis estimations to further guide the treatment of glioma patients. MiRNAs are a class of endogenous, small (containing ~ 23 nucleotides) noncoding RNA molecules, which function as cellular regulators in the modulation of genes expression at the post-transcriptional level[10]. The regulator mechanism of miRNAs in biological activity remains largely unclear and it has been confirmed that they bind to mRNA' 3' UTR, which leads to the degradation or translated suppression of mRNA[11-13]. Growing evidence from various cells experiments indicated that miRNAs especially functional miRNA play important regulator roles in the modulation and control of cellular progress, such as proliferation, cellular metabolism, differentiation, and apopto-

Corresponding Author: Zheng-huan Li, MD; e-mail: lzzz776154@sina.com

sis of cells *via* various complex pathways[14,15]. Of note, the research of tumor biology provides a large number of evidence that miRNAs can act as oncogenes or tumor suppressors in the biological progress depending on the specific target genes of miRNAs and the type of neoplasm, which also suggests that some miRNAs can serve as useful biomarkers in cancer detection[16-18]. Although several investigations determining the expression pattern of miRNAs expression using RT-PCR and microarray analysis in glioma have been conducted, there is little information available regarding the function and molecular structure of specific miRNA in glioma.

MiR-3653, located on chromosome 6, was a newly discovered miRNA. Previously, the expression profiles of miR-3653 in several tumors were investigated[19,20]. Functionally, anti-oncogenic roles of miR-3653 have been confirmed by Zhang et al[21] in hepatocellular carcinoma. In addition, it was also shown that miR-3653 up-regulation was associated with increased metastatic risk in pancreatic neuroendocrine tumors, suggesting that miR-3653 may contribute to the progression of this tumor[22]. These findings indicated that the function of miR-3653 in tumors is controversial according to the type of tumors. Up to date, the research of miR-3653 in tumors is limited and the expression and clinical significance of this miRNA in glioma have not been investigated. In this study, we aimed to determine whether miR-3653 was abnormally expressed in glioma patients and explore its prognostic value.

Patients and Methods

Patients and Tissue Samples

A total of 168 paired glioma samples and the matched adjacent normal brain tissues were surgically excised at The First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine between March 2011 and May 2014. Tissue samples were immediately frozen in liquid nitrogen for further use. All patients with glioma were diagnosed based one the guidelines of the guidelines of the International Union Against Cancer (IUAC). The criteria for the inclusion/exclusion of patients are: (1) age 10-65; (2) only received surgery, without other system therapy; (3) without other tumors, autoimmune obstacle, and infection diseases. All the patients' clinical data which are shown in Table I were obtained from The First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine. Informed consent was obtained from all glioma patients. The study was approved by the Ethics

Table I. Clinicopathological features associated with miR-3653 expression in 168 glioma patients.

Clinicopathological features	Total	miR-3653 expression		*p*-value
		High	Low	
Age (years)				0.538
< 45	85	40	45	
≥ 45	83	43	40	
Gender				0.290
Male	115	60	55	
Female	43	23	30	
Family history of cancer				0.285
Yes	70	38	32	
No	98	45	53	
Tumor location				0.729
Supratentorial	101	51	50	
Infratentorial	67	32	35	
Tumor size (cm)				0.089
< 3	100	44	56	
≥ 3	68	39	29	
KPS				0.028
< 90	63	38	25	
≥ 90	105	45	60	
WHO grade				0.019
I-II	102	43	59	
III-IV	66	40	26	

Committee of The First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine (2017-DY-2018).

RNA Extraction and qRT-PCR Analyses

The total RNA in glioma tissues and matched normal tissues were extracted with the mirVana PARIS kit (Ambion, Foster City, CA, USA) according to the manufacturer's instructions. The quality and concentrations of total RNA were measured by Nanodrop 2000c (Thermo Fisher Scientific, Beijing, China). RNA was reverse-transcribed by performing the PrimeScript RT Reagent Kit (Invitrogen, Carlsbad, CA, USA) and qPCR was carried out to detect the expression of miR-3653 *via* the use of the SYBR Premix Ex Taq (TaKaRa, Otsu, Shiga, Japan). Each cycle consisted of 94°C for 3 minutes, 94°C for 30 seconds, 58°C for 30 seconds, and 70°C for 45 seconds. The expression levels of miR-3653 were normalized to GAPDH. The fold changes were also calculated by relative quantification ($2^{-\Delta\Delta Ct}$) method. The primer sequences are shown as the following: glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward sense 5'-TGTAGTTGAGGTCAATGAAGGG-3' and reverse sense 3'-ACATCGCTCAGACACCATG-3'; miR-3653, forward sense 5'-TCTCCCGAGAGACATATTT-3' and reverse sense 5'-GATGAGAAGGTATGAATCA-3'. All qPCR experiments were conducted in triplicate.

Statistical Analysis

The Statistical analysis was performed using the SPSS statistical software package (standard version 18.0, SPSS Inc., Chicago, IL, USA). The differences between the groups were analyzed using the Student's *t*-test, the Chi-square test, and the Fisher's exact tests. Overall survival was calculated, and the survival curves were plotted using the Kaplan-Meier method with the differences between groups calculated using the log-rank tests. Univariate and multivariate Cox regression models were used to control for possible confounding factors. The differences were considered statistically significant at $p<0.05$.

Results

MiR-3653 Expression is Downregulated in Glioma Tissues

To explore whether miR-3653 was a potential functional miRNA associated with the progression of glioma, we collected 168 paired glioma clinical specimens and performed RT-PCR. As presented in Figure 1, we observed that miR-3653 was significantly upregulated in most glioma samples compared to adjacent normal brain tissues ($p<0.01$), suggesting that miR-3653 may be involved in the regulation of miR-3653 progress.

MiR-3653 Downregulation Associates With Advanced Clinicopathological Features of Glioma Patients

Based on the relative expression of miR-3653 in glioma tissues *vs.* normal tissues in all 168 patients as a cutoff, all patients were divided into high miR-3653 expression group (n=83), and low miR-3653 expression group (n=85). Then, we performed the Chi-square test to explore the possible influence of miR-3653 on clinical progression of glioma. As shown in Table I, the data showed that the tissue of the miR-3653 level was dramatically correlated with several clinicopathological parameters, including KPS ($p=0.028$) and WHO grade ($p=0.019$). However, several other parameters were found not to be significantly associated with miR-3653 expression in glioma patients ($p>0.05$).

Association Between MiR-3653 Expression and Survival of Glioma Patient

To further study the value of miR-3653 in the clinical outcome of patients with glioma, we used the Kaplan-Meier assays and the log-rank tests.

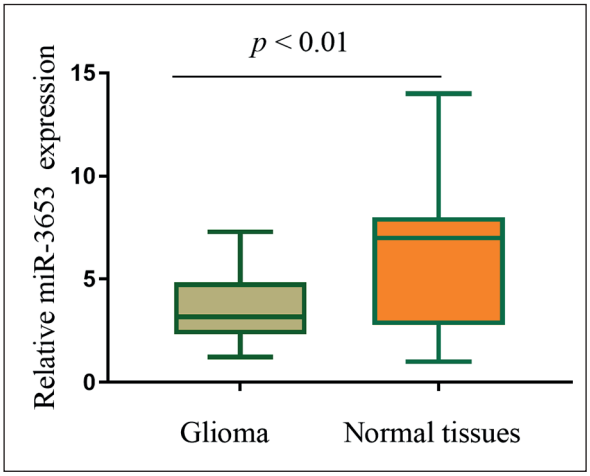

Figure 1. The expressions of miR-3653 in human glioma tissues and matched normal brain tissues were detected by qRT-PCR. MiR-3653 was found to be significantly upregulated in glioma tissues, compared with that in the matched tumor adjacent tissues.

As shown in Figure 2, patients in the low miR-3653 expression group presented with significantly worse overall survival compared with those in the high miR-3653 expression group (p=0.008). Moreover, univariate analysis was performed to identify possible prognostic factors and the results showed that KPS (HR=3.548, 95% CI: 1.428-4.587, p=0.008), WHO grade (HR=3.672, 95% CI: 1.582-4.892, p=0.004), and miR-3653 expression (HR=3.029, 95% CI: 1.328-4.667, p=0.011) were prognostic factors for glioma patients (Table II). More importantly, our data revealed that miR-3653 expression level (HR=2.682, 95% CI: 1.148-4.281, p=0.0021) was an independent prognostic factor for overall survival of glioma patients, in addition to KPS and WHO grade (Table II).

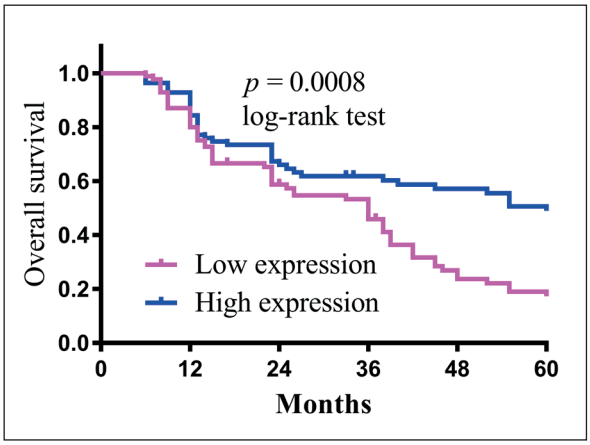

Figure 2. Overall survival curves for two groups defined as low and high expression of miR-3653 in GC patients. Patients in the high miR-3653 expression group had better 5-year overall survival than those in the low miR-3653 expression group (p=0.008, log-rank test).

Discussion

Glioma remains among the deadliest human tumors despite medical and surgical improvements over the past decades. Early diagnosis and predication of outcome using various methods are very important for the clinical management in the treatment of glioma patients[23,24]. For a long time, the tumor stages system was used for finishing this task. In addition, several tumor-related proteins involved in the progression of cancers were also reported to be a potential biomarker in the prognosis of glioma patients[25,26]. However, only a few have practical value in the clinical setting due to the limited specificity and sensitivity of these prognostic factors. With the development of epigenetic inheritance, many ncRNAs which were previously considered to be "transcriptional noise" were identified as important regulators in the development and progression of various tumors, including glioma. Among ncRANs, miRNAs become a hotspot of research. Many functional miRNAs were identified to be dysregulated in various tumors and have clinical value as novel screening and management biomarkers for tumors[27,28]. In this study, we identified a new glioma-related miRNA.

In this study, we firstly explored whether miR-3653 was dysregulated in glioma using RT-PCR, finding that miR-3653 expression was decreased in the majority of glioma tissues compared with the matched normal brain tissues. Previously, miR-3653 has been reported to be down-regulated in hepatocellular carcinoma and up-regulated in pancreatic tumors[21,22]. Our findings added the expression profiles of miR-3653 in glioma. Then, the clinical information was collected, and statistical assays revealed that glioma patients with higher expression of miR-3653 exhibited KPS and advanced WHO grade, suggesting that dysregulated miR-3653 may influence the clinical prognosis and tumor progression. In addition,

Table II. Prognostic factors for overall survival by univariate and multivariate analysis.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age	1.672 (0.722-2.319)	0.258	–	–
Gender	1.427 (0.895-1.889	0.562	–	–
Family history of cancer	1.448 (0.482-1.925)	0.329	–	–
Tumor location	1.285 (0.479-2.319)	0.148	–	–
Tumor size	2.148 (0.859-2.763)	0.092	–	–
KPS	3.548 (1.428-4.587)	0.008	3.182 (1.129-4.192)	0.014
WHO grade	3.672 (1.582-4.892)	0.004	3.045 (1.284-4.029)	0.018
MiR-3653 expression	3.029 (1.328-4.667)	0.011	2.682 (1.148-4.281)	0.021

with five years follow-up, the survival data of 168 glioma patients were collected and the results of the Kaplan-Meier assays showed that the overall survival of patients with low miR-3653 expression was significantly shorter than those with high miR-3653 expression. Moreover, using univariate and multivariate analyses, miR-3653 was further confirmed to be an independent factor predicting poor prognosis for glioma patients.

Previously, many studies reported that several miRNAs involved in the development of glioma served as a tumor suppressor or oncogenes based on the targeting genes. For instance, it was reported that miR-338 was down-regulated in glioma and its forced expression could inhibit cell proliferation and metastasis by targeting CTBP2[29]. MiR-1908 was shown to be overexpressed in glioma and its downregulation predicted a favorable prognosis and resulted in the suppression of ability of proliferation and migration of glioma cells by modulating SPRY4/RAF1 axis[30]. Zhang et al[21] firstly reported the suppressive roles of miR-3653 in hepatocellular carcinoma. They found that low expression of miR-3653 was associated with a poor outcome of hepatocellular carcinoma patients and its overexpression suppressed tumor cells growth and metastasis by targeting ITGB1. In this study, we also provided evidence that miR-3653 had prognostic value in glioma patients. However, due to funds and experiment condition, the functional studies of miR-3653 in glioma were not performed. In the future, whether miR-3653 also displayed tumor-suppressive roles and its potential targeting genes in glioma needed to be further explored. In addition, the relatively small number of glioma patients analyzed in this study may influence the accuracy of our results. Further investigations on more patients are needed to confirm our findings.

Conclusions

This study suggested for the first time that miR-3653 was down-regulated in glioma patients and correlates with unfavorable clinical outcome, thereby potentially representing a novel diagnostic and prognostic marker for glioma patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.
2) Omuro A, DeAngelis LM. Glioblastoma and other malignant gliomas: a clinical review. JAMA 2013; 310: 1842-1850.
3) Delgado-López PD, Corrales-García EM, Martino J, Lastra-Aras E, Dueñas-Polo MT. Diffuse low-grade glioma: a review on the new molecular classification, natural history and current management strategies. Clin Transl Oncol 2017; 19: 931-944.
4) Matsutani M. Chemoradiotherapy for brain tumors: current status and perspectives. Int J Clin Oncol 2004; 9: 471-474.
5) Miller JJ, Wen PY. Emerging targeted therapies for glioma. Expert Opin Emerg Drugs 2016; 21: 441-452.
6) Sturm D, Pfister SM, Jones DTW. Pediatric gliomas: current concepts on diagnosis, biology, and clinical management. J Clin Oncol 2017; 35: 2370-2377.
7) Alireza M, Amelot A, Chauvet D, Terrier LM, Lot G, Bekaert O. Poor prognosis and challenging treatment of optic nerve malignant gliomas: literature review and case report series. World Neurosurg 2017; 97: 751.e1-751.e6.
8) Davis ME. Epidemiology and overview of gliomas. Semin Oncol Nurs 2018; 34: 420-429.
9) Srinivasan VM, Ferguson SD, Lee S, Weathers SP, Kerrigan BCP, Heimberger AB. Tumor vaccines for malignant gliomas. Neurotherapeutics 2017; 14: 345-357.
10) Kappel A, Keller A. MiRNA assays in the clinical laboratory: workflow, detection technologies and automation aspects. Clin Chem Lab Med 2017; 55: 636-647.
11) Mishra S, Yadav T, Rani V. Exploring miRNA based approaches in cancer diagnostics and therapeutics. Crit Rev Oncol Hematol 2016; 98: 12-23.
12) Shukla V, Varghese VK, Kabekkodu SP, Mallya S, Satyamoorthy K. A compilation of Web-based research tools for miRNA analysis. Brief Funct Genomics 2017; 16: 249-273.
13) Alipoor SD, Adcock IM, Garssen J, Mortaz E, Varahram M, Mirsaeidi M, Velayati A. The roles of miRNAs as potential biomarkers in lung diseases. Eur J Pharmacol 2016; 791: 395-404.
14) Zempleni J, Baier SR, Howard KM, Cui J. Gene regulation by dietary microRNAs. Can J Physiol Pharmacol 2015; 93: 1097-1102.
15) Meijer HA, Smith EM, Bushell M. Regulation of miRNA strand selection: follow the leader? Biochem Soc Trans 2014; 42: 1135-1140.
16) Zhao C, Wang XB, Zhang YH, Zhou YM, Yin Q, Yao WC. MicroRNA-424 inhibits cell migration, invasion and epithelial-mesenchymal transition in hu-

man glioma by targeting KIF23 and functions as a novel prognostic predictor. Eur Rev Med Pharmacol Sci 2018; 22: 6369-6378.

17) Park S, Eom K, Kim J, Bang H, Wang HY, Ahn S, Kim G, Jang H, Kim S, Lee D, Park KH, Lee H. MiR-9, miR-21, and miR-155 as potential biomarkers for HPV positive and negative cervical cancer. BMC Cancer 2017; 17: 658.

18) Xu DX, Guo JJ, Zhu GY, Wu HJ, Zhang QS, Cui T. MiR-363-3p modulates cell growth and invasion in glioma by directly targeting pyruvate dehydrogenase B. Eur Rev Med Pharmacol Sci 2018; 22: 5230-5239.

19) Yerukala Sathipati S, Ho SY. Identifying the miRNA signature associated with survival time in patients with lung adenocarcinoma using miRNA expression profiles. Sci Rep 2017; 7: 7507.

20) Gao D, Zhang Y, Zhu M, Liu S, Wang X. MiRNA expression profiles of HPV-infected patients with cervical cancer in the Uyghur population in China. PLoS One 2016; 11: e0164701.

21) Zhang L, Zhang T, Deng Z, Sun L. MicroRNA3653 inhibits the growth and metastasis of hepatocellular carcinoma by inhibiting ITGB1. Oncol Rep 2019; 41: 1669-1677.

22) Gill P, Kim E, Chua TC, Clifton-Bligh RJ, Nahm CB, Mittal A, Gill AJ, Samra JS. MiRNA-3653 is a potential tissue biomarker for increased metastatic risk in pancreatic neuroendocrine tumours. Endocr Pathol 2019; 30: 128-133.

23) Sasmita AO, Wong YP, Ling APK. Biomarkers and therapeutic advances in glioblastoma multiforme. Asia Pac J Clin Oncol 2018; 14: 40-51.

24) Gusyatiner O, Hegi ME. Glioma epigenetics: from subclassification to novel treatment options. Semin Cancer Biol 2018; 51: 50-58.

25) Westphal M, Lamszus K. Circulating biomarkers for gliomas. Nat Rev Neurol 2015; 11: 556-566.

26) Zacher A, Kaulich K, Stepanow S, Wolter M, Kohrer K, Felsberg J, Malzkorn B, Reifenberger G. Molecular diagnostics of gliomas using next generation sequencing of a glioma-tailored gene panel. Brain Pathol 2017; 27: 146-159.

27) Fang DZ, Wang YP, Liu J, Hui XB, Wang XD, Chen X, Liu D. MicroRNA-129-3p suppresses tumor growth by targeting E2F5 in glioblastoma. Eur Rev Med Pharmacol Sci 2018; 22: 1044-1050.

28) Liang RF, Li M, Yang Y, Wang X, Mao Q, Liu YH. Circulating miR-128 as a potential diagnostic biomarker for glioma. Clin Neurol Neurosurg 2017; 160: 88-91.

29) Liu DZ, Zhao H, Zou QG, Ma QJ. MiR-338 suppresses cell proliferation and invasion by targeting CTBP2 in glioma. Cancer Biomark 2017; 20: 289-297.

30) Chai Z, Fan H, Li Y, Song L, Jin X, Yu J, Li Y, Ma C, Zhou R. MiR-1908 as a novel prognosis marker of glioma via promoting malignant phenotype and modulating SPRY4/RAF1 axis. Oncol Rep 2017; 38: 2717-2726.