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Down-expression of miR-373 predicts poor prognosis of glioma and could be a potential therapeutic target

S.-Y. JING[1], S.-Q. JING[2], L.-L. LIU[2], L.-F. XU[2], F. ZHANG[2], J.-L. GAO[2]

[1]Department of Neurosurgery, Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science & Technology, Wuhan, China.
[2]Department of Neurosurgery, The First Hospital of HeBei Medical University, Shijiazhuang, Hebei, China.

Shi-yuan Jing and Shan-quan Jing contributed equally to this work.

Abstract. – OBJECTIVE: MicroRNAs (miRNAs) are epigenetic regulators of gene expression, and their deregulation plays an important role in human cancer, including glioma. The main objective of this work was to investigate the expression level of miR-373 and its clinical significance in glioma.

PATIENTS AND METHODS: The expression levels of miR-373 in glioma tissues and non-neoplastic brain tissues were measured by the qRT-PCR assay. Patients were divided into two groups based on the median miR-373 expression. The probability of differences in overall and progression-free survival as a function of time was ascertained by use of the Kaplan-Meier method. Cox regression analysis of factors potentially associated with survival was conducted to identify independent factors.

RESULTS: In clinical gastric cancer samples, we found that miR-373 expression was significantly down-regulated in glioma tissues compared with non-neoplastic brain tissues ($p<0.01$). Reduced expression of miR-373 was associated with serum WHO grade ($p=0.015$) and KPS score ($p=0.001$). Kaplan-Meier analysis indicated that patients with low level of miR-373 expression had poorer overall survival (OS) and progression-free survival (PFS). Multivariate survival analysis verified that miR-373 expression level was an independent predictor of both OS and PFS for glioma patients.

CONCLUSIONS: Our study showed miR-373 was associated to progression in glioma, and suggested it as a potential predictive factor for the prognosis of glioma.

Key Words:
MiR-373, Glioma, Prognosis.

Introduction

Human gliomas are the most common malignant brain tumors, which most commonly occur in the central nervous system of both children and adults[1,2]. Despite the use of conventional therapeutic modalities, such as surgery, chemotherapy, and radiotherapy, the therapeutic efficacy is still poor, and especially in patients with poorly differentiated glioma[3]. Therefore, it is of critical importance to identity novel biomarkers that can predict the prognosis and provide new therapeutic targets.

It is well known that miRNAs are small non-coding RNA molecules consisting of 19-23 nucleotides that can modulate gene expression post-transcriptionally[4,5]. Increasing evidences show that miRNAs were involved in the various pathological processes, such as cell proliferation, angiogenesis, invasion, and metastasis[6,7]. Recent studies showed that miRNAs could play oncogene or tumor suppressor roles in the etiology and pathogenesis of cancer by targeting tumor suppressors or oncogenes[8]. For instance, Sun et al[9] showed that miR-126 can inhibit NSCLC cells proliferation *in vitro* and inhibit tumor growth *in vivo* by targeting EGFL7. Xia et al[10] found that Overexpressing miR-1908 promotes migration and invasion of glioblastoma cells by suppressing PTEN tumor suppressor pathway. Fan et al[11] reported that Overexpression of miR-98 inhibits cell invasion in glioma cell lines via downregulation of IKKε. Those data suggested miRNAs potentially represented prognostic markers.

MiR-373 are transcribed from same genes located on chromosome 19q13.4[12]. Previous studies[14] revealed that miR-373 migth be an independent prognostic marker for cancer, such as oral carcinomas[13] and T cell lymphoma. Wei et al[15] showed that over-expression of miR-373 could inhibit glioma cell migration and invasion, suggesting that miR-373 may serve as a tumor suppressor in glioma. However, the relationship

Corresponding Author: Feng Zhang, MD; e-mail: glj02ll@126.com

remains unclear between miR-373 expression level and prognosis of glioma patients. Thus, we aimed to explore the expression and clinical significance in glioma.

Patients and Methods

Patients and Tissue Samples

The present study included 170 patients with surgically resected and pathologically confirmed glioma, who were treated at The First Hospital of HeBei Medical University between March 2008 and January 2011. None of the patients had received radiotherapy or chemotherapy before surgery. The diagnosis in all cases was confirmed by histological examination of tissue sections stained with hematoxylin and eosin (H&E). Clinical staging was performed according to the 2007 World Health Organization (WHO) classification of tumors of the central nervous system. In the follow-up period, overall survival was measured from diagnosis to death or last follow-up. Survival data for patients that are listed as <30 days were omitted from the survival analysis. This study was approved by the Institutional Review Board of the First Hospital of HeBei Medical University. Informed consent was obtained from all patients included in this study.

qRT-PCR

Total RNA was extracted from tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was randomly primed from 2 μg of total RNA using the Omniscript Reverse Transcription Kit (Qiagen, Suzhou, Jiangsu, China). Expression levels of mature miRNAs were amplified using SYBR Green quantitative RT-PCR (qRT-PCR) on an ABI7300 real-time PCR machine (Applied Biosystems, Foster City, MA, USA). We assessed the RNA expression according to relative quantification using the $2^{-\Delta\Delta Ct}$ method to determine the fold change in the expression. The expression level of U6 was used as an internal control for mRNA expression. The involved primers were as follows: miR-373 forward: ACCTTACCAGCCCACTCTTA, miR-373 reverse: CCAAGGCCTCCTACATCAAAG; U6 forward: CTCGCTTCGGCAGCACA, U6 reverse: AACGCTTCACGAATTTGCGT.

Statistical Analysis

SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The association between PCR results and clinicopathological variables was assessed by chi-square test or Fisher exact test. Kaplan-Meier analysis was adopted to evaluate the effects of miR-373 expression on the OS and PFS of patients with glioma. A multivariable analysis of several independent prognostic factors was performed by Cox's proportional hazards regression model. All statistical tests were 2-tailed and $p < 0.05$ was regarded as significant.

Results

MiR-373 Expression was Down-regulated in Glioma Tissues

To evaluate the relevance of miR-373 in glioma, we first performed RT-qPCR to determine the miR-373 expression in glioma tissues. As shown in Figure 1, miR-373 expression was significantly downregulated in glioma tissues as compared to non-neoplastic brain tissues ($p < 0.01$).

Correlation Between miR-373 Expression and Clinicopathologic Features in Patients with Glioma

The associations between the combined expression of miR-373 and the clinicopathologic characteristics of patients with glioma were statistically analyzed as shown in Table I. The results showed that decreased miR-373 expression was associated with the WHO grade and higher KPS score (p=0.011 and p=0.001, respectively). However, there were no significant associations

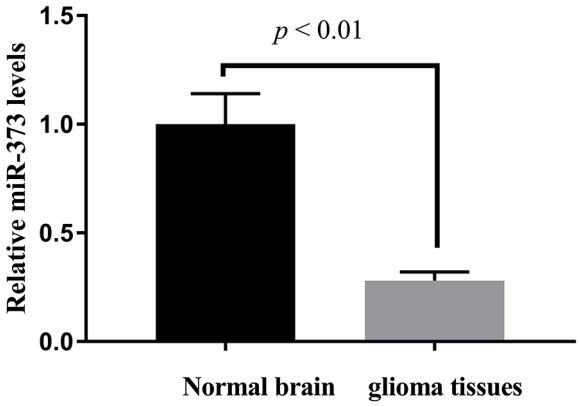

Figure 1. Expression of miR-373 in glioma tissues and matched normal tissues was determined by qRT-PCR. The expression levels of miR-373 were significantly decreased in glioma tissues compared to adjacent non-neoplastic tissues ($p < 0.01$).

Table I. Correlation of clinicopathological features of glioma with miR-373 expression levels.

Characteristics	All cases	miR-373 expression High (n, %)	Low (n, %)	*p*-value
Age				0.816
<55 years	65	31 (47.7)	34 (52.3)	
≥55 years	105	52 (49.5)	53 (50.5)	
Sex				0.117
Male	113	60 (53.1)	53 (46.9)	
Female	57	23 (40.4)	34 (59.6)	
Tumor size				0.283
<5 cm	85	45 (53)	40 (47)	
≥5 cm	85	38 (44.7)	47 (55.3)	
Surgery				0.700
GTR	66	31 (47)	35 (53)	
PR	104	52 (50)	52 (50)	
WHO grade				0.015
I-II	105	59 (56.2)	46 (43.8)	
III-IV	65	24 (37)	41 (28)	
KPS score				0.001
<90	76	26 (34.2)	50 (65.8)	
≥90	94	57 (60.6)	37 (39.4)	

between miR-373 expression and other clinical features including age, sex, tumor size and surgery (all $p>0.05$).

Prognostic Value of miR-373 Expression for the Clinical Outcome of Glioma Patients

To further evaluate whether miR-373 levels were associated with glioma prognosis, we performed survival analysis. Kaplan-Meier survival analysis demonstrated that high miR-373 expression predicted significantly better OS ($p=0.001$) and PFS ($p=0.001$) (Figures 2 and 3). Next, Univariate and multivariate analyses were utilized to evaluate whether the miR-373 expression level and various clinicopathological features were independent prognostic parameters of glioma patient outcomes. As shown in Table II, according to a univariate analysis, WHO grade, KPS score, and miR-373 expression were statistically significant prognostic factors. Moreover, multivariate analysis indicated that miR-373 expression were independent prognostic indicators for the OS ($p<0.001$) and PFS ($p<0.001$) of patients with glioma.

Discussion

Gliomas account for approximately 30% of all brain and central nervous system tumors and 80% of all malignant brain tumors[16]. It is very important

Table II. Univariate and multivariate analyses for progression-free survival and overall survival by Cox regression model.

	Progression-free survival			Overall survival		
	Univariate analysis	Multivariate analysis		Univariate analysis	Multivariate analysis	
Parameters	*p*	HR 95 % CI	*p*	*p*	HR 95 % CI	*p*
Age	0.371	-	-	0.542	-	-
Sex	0.633	-	-	0.714	-	-
Tumor size	0.231	-	-	0.274	-	-
Surgery	0.428	-	-	0.515	-	-
WHO grade	0.003	1.237-4.582	0.006	0.002	1.364-5.213	0.008
KPS score	< 0.001	1.322-5.268	< 0.001	< 0.001	1.657-5.992	< 0.001
miR-373 expression	< 0.001	1.631-6.339	< 0.001	< 0.001	1.822-6.894	< 0.001

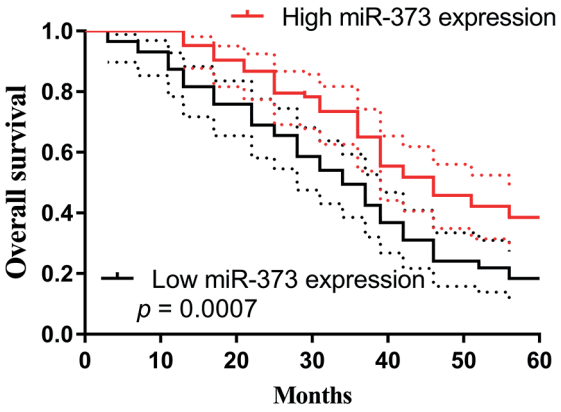

Figure 2. Kaplan-Meier analysis of the overall survival in 170 glioma patients in relation to miR-373 expression level.

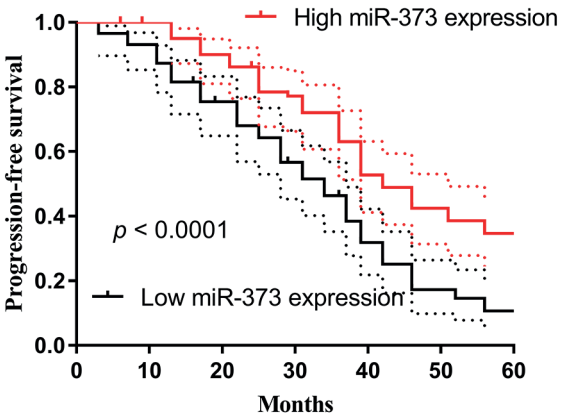

Figure 3. Kaplan-Meier analysis of the progression-free survival in 170 glioma patients in relation to miR-373 expression level.

to explore biomarkers to improve the detection and prognostic outcome of glioma. In the past ten years, researchers found that some protein and mRNA may be useful for pre-predicting five-years overall survival[17]. For instance, Fan et al[18] reported that high ADAM9 expression also correlated with poor clinical outcome in glioma patients MK et al[19] found that p16 mRNA expression can act as an independent prognostic biomarker in high-grade glioma. With the rapid development of sequencing technologies, identification of novel prognostic biomarkers has better elucidated disease mechanisms resulting in glioma. MiRANs have become research hotspots recently.

It was reported that miR-373 served as either oncogenes or antioncogenes and show variable expression in different types of human cancer. For instance, Chen et al[20] found that over-expression miR-373 induced EMT, migration, invasion and metastasis in breast cancer by directly inhibiting TXNIP. Wang et al[21] reported that the expression of miR-373 is upregulated in human cervical cancer tissues and cervical carcinoma cell lines. Further cells experiment showed that Ectopic over-expression of miR-373 promoted cell growth and tumorigenicity by targeting YOD1 gene in cervical cancer. Conversely, Eyking et al[22] showed that over-expression of miR-373 inhibited cell growth, migration, and invasion of colorectal cancer. In lung cancer, miR-373 also showed the potential anti-oncogene[23]. Recently, Lu et al[24] found that overexpression of miR-373 inhibited glioma cell growth and migration by regulating the activities of MMP2 and MMP9. However, the prognostic value of miR-373 in glioma has not been reported.

In the present paper, we investigated the clinical significance of miR-373 in glioma patients for the first time. We performed qRT-PCR and found that the expression levels of miR-373 were distinctly decreased in glioma tissues compared to adjacent normal tissues. We then investigated the correlation between decreased miR-373 levels and the clinicopathological features of glioma tissues. Then, a 60-month follow-up suggested that patients with low miR-373 expression had OS and PFS than those with high miR-373 expression. Moreover, using multivariate Cox regression analysis of potential prognostic factors for glioma survival, we identified miR-373 expression level was independently associate with the OS and PFS.

Conclusions

miR-373 may be potential prognostic markers and therapeutic targets for patients with glioma. The underlying molecular mechanisms of miR-373 involvement in the development and progression of glioma needs to be investigated in future studies.

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Conflict of interest

The authors declare no conflicts of interest.

References

1) Sathornsumetee S, Rich JN. New treatment strategies for malignant gliomas. Expert Rev Anticancer Ther 2006; 6: 1087-1104.
2) Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.
3) Yang QY, Guo CC, Chen ZP. Profile of nimotuzumab in the treatment of high-grade glioma. Onco Targets Ther 2015; 8: 819-825.
4) Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-233.
5) Guo E, Wang Z, Wang S. MiR-200c and miR-141 inhibit ZEB1 synergistically and suppress glioma cell growth and migration. Eur Rev Med Pharmacol Sci 2016; 20: 3385-3391.
6) Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006; 6: 857-866.
7) Rottiers V, Näär AM. MicroRNAs in metabolism and metabolic disorders. Nat Rev Mol Cell Biol 2012; 13: 239-250.
8) Peng T, Zhou L, Zuo L, Luan Y. MiR-506 functions as a tumor suppressor in glioma by targeting STAT3. Oncol Rep 2016; 35: 1057-1064.
9) Sun Y, Bai Y, Zhang F, Wang Y, Guo Y, Guo L. miR-126 inhibits non-small cell lung cancer cells proliferation by targeting EGFL7. Biochem Biophys Res Commun 2010; 391: 1483-1489.
10) Xia X, Li Y, Wang W, Tang F, Tan J, Sun L, Li Q, Sun L, Tang B, He S. MicroRNA-1908 functions as a glioblastoma oncogene by suppressing PTEN tumor suppressor pathway. Mol Cancer 2015; 14: 154.
11) Fan YH, Ye MH, Wu L, Lv SG, Wu MJ, Xiao B, Liao CC, Ji QK, Chai Y, Zhu XG. Overexpression of miR-98 inhibits cell invasion in glioma cell lines via downregulation of IKKε. Eur Rev Med Pharmacol Sci 2015; 19: 3593-3604.
12) Voorhoeve PM, le Sage C, Schrier M, Gillis AJ, Stoop H, Nagel R, Liu YP, van Duijse J, Drost J, Griekspoor A, Zlotorynski E, Yabuta N, De Vita G, Nojima H, Looijenga LH, Agami R. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. Cell 2006; 124: 1169-1181.
13) Tu HF, Chang KW, Cheng HW, Liu CJ. Upregulation of miR-372 and -373 associates with lymph node metastasis and poor prognosis of oral carcinomas. Laryngoscope 2015; 125: E365-370.
14) Tian YY, Jia CM, Li Y, Wang Y, Jiang L, Liu AC. Restoration of microRNA-373 suppresses growth of human T-cell lymphoma cells by repressing CCND1. Eur Rev Med Pharmacol Sci 2016; 20: 4435-4444.
15) Wei F, Wang Q, Su Q, Huang H, Luan J, Xu X, Wang J. miR-373 Inhibits Glioma Cell U251 Migration and Invasion by Down-Regulating CD44 and TGFBR2. Cell Mol Neurobiol 2016; 36: 1389-1397
16) Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 2007; 114: 97-109.
17) Lococo F, Cafarotti S, Cesario A, Dall'Armi V, Cusumano G, Lauriola L, Frederic M, Evoli A, Margaritora S, Granone P. Prognostic grading after complete resection for thymic malignancies. Eur Rev Med Pharmacol Sci 2015; 19: 2882-2891.
18) Fan X, Wang Y, Zhang C, Liu L, Yang S, Wang Y, Liu X, Qian Z, Fang S, Qiao H, Jiang T. ADAM9 expression is associate with glioma tumor grade and histological type, and acts as a prognostic factor in lower-grade gliomas. Int J Mol Sci 2016; 17: E1276.
19) Sibin MK, Bhat DI, Narasingarao KV, Lavanya Ch, Chetan GK. CDKN2A (p16) mRNA decreased expression is a marker of poor prognosis in malignant high-grade glioma. Tumour Biol 2015; 36: 7607-7614.
20) Chen D, Dang BL, Huang JZ, Chen M, Wu D, Xu ML, Li R, Yan GR. MiR-373 drives the epithelial-to-mesenchymal transition and metastasis via the miR-373-TXNIP-HIF1α-TWIST signaling axis in breast cancer. Oncotarget 2015; 6: 32701-32712.
21) Wang LQ, Zhang Y, Yan H, Liu KJ, Zhang S. MicroRNA-373 functions as an oncogene and targets YOD1 gene in cervical cancer. Biochem Biophys Res Commun 2015; 459: 515-520.
22) Eyking A, Reis H, Frank M, Gerken G, Schmid KW, Cario E. MiR-205 and miR-373 are associated with aggressive human mucinous colorectal cancer. PLoS One 2016; 11: e0156871.
23) Adi Harel S, Bossel Ben-Moshe N, Aylon Y, Bublik DR, Moskovits N, Toperoff G, Azaiza D, Biagoni F, Fuchs G, Wilder S, Hellman A, Blandino G, Domany E, Oren M. Reactivation of epigenetically silenced miR-512 and miR-373 sensitizes lung cancer cells to cisplatin and restricts tumor growth. Cell Death Differ 2015; 22: 1328-1340.
24) Liu P, Wilson MJ. miR-520c and miR-373 upregulate MMP9 expression by targeting mTOR and SIRT1, and activate the Ras/Raf/MEK/Erk signaling pathway and NF-κB factor in human fibrosarcoma cells. J Cell Physiol 2012; 227: 867-876.