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Evaluation of miR-302c-3p as prognostic biomarkers in glioma patients

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Abstract. – OBJECTIVE: This study aimed to explore the clinicopathological and prognostic significance of miR-302c-3p in glioma.

MATERIALS AND METHODS: The expression of miR-302c-3p in glioma tissues and normal brain tissues was measured using real-time PCR. Clinicopathological associations of miR-302c-3p expression in glioma were analyzed. The Kaplan-Meier method was used to determine the survival curves. Univariate and multivariate analysis were performed using the Cox proportional hazard analysis.

RESULTS: The relative expression of miR-302c-3p in glioma tissues was significantly lower than that in non-neoplastic brain tissues ($p < 0.001$). The decreased expression of miR-302c-3p in glioma was positively associated with WHO grade ($p < 0.001$) and Karnofsky performance status (KPS) score ($p = 0.016$). We found patients with low miR-302c-3p expression had significantly poorer OS ($p = 0.0057$) and PFS ($p = 0.0092$) by Kaplan-Meier method. Furthermore, multivariate regression analysis identified low miR-302c-3p expression as an independent predictor of poor survival.

CONCLUSIONS: Our results revealed that miR-302c-3p may serve as a tumor suppressor of malignant glioma and be used as a novel biomarker to predict the clinical prognostic of patients with gliomas.

Key Words:
Glioma, miR-302c-3p, qPCR, Progression-free survival, Overall survival.

Introduction

Glioma, arising from glial cells, accounts for the most common and primary brain tumor[1,2]. Approximately 20,000 new cases are diagnosed with glioma in the United States every year[3]. Gliomas are separated into four grades according to WHO classification, pilocytic astrocytoma (WHO grade I), diffuse astrocytoma (WHO grade II), anaplastic astrocytoma (WHO grade III), and glioblastoma (WHO grade IV)[4]. Although treating with combinations of surgery, radiotherapy and chemotherapy.

The prognosis in glioma patients has not been significantly improved. The 5-year survival rate of primary GBM patients is 30-70%, and the 5-year survival rate is only 9.8% at present[5,6]. So, new approaches to improve the efficacy of anti-glioma treatments are urgently needed. Thus, finding new biomarkers and targeted therapies for glioma are of paramount importance.

MicroRNAs (miRNAs) are small non-protein coding genes of about 19-23 nucleotides which have been proved as important regulators of malignant transformation[7-9]. MiRNAs have been proved to play diverse roles in tumorigenesis and cancer progression[10-11], more and more evidences showed that miRNAs function as oncogene or anti-oncogene in various tumors through pairing with the corresponding mRNAs[12,13]. Recently, some studies[14,15] informed that miRNAs may be useful for evaluating prognosis. Therefore, exploring novel miRNAs which is helpful to predict the prognosis of patients with glioma is necessary.

Recently, Wang et al[16] showed that overexpression of miR-302c-3p significantly inhibited invasion and proliferation of glioma cells by targeting metadherin expression. However, so far, there are no studies about the prognostic value of miR-302c-3p in patients with gliomas. Therefore, we investigated the expression levels of miR-302c-3p in human glioma tissues and the feasibility of miR-302c-3p as a novel prognostic biomarker for glioma.

Patients and Methods

Patients and Tissue Samples

This study was approved by the Research Ethics Committee Of the Linyi People's Hospital. Written informed consent was obtained from all the patients. Patient data and samples were treated according to the ethical and legal standards. A to-

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tal of 235 pairs of human gliomas and matched non-neoplastic brain tissues were collected from Department of Neurosurgery, the Linyi People's Hospital between 2007 and 2014. All human glioma tissues during the operation directly divided into two parts. One part was snap-frozen in liquid nitrogen for RNA extraction and the other part was made into paraffin sections for histopathologic diagnosis by two pathologists according to the 2007 WHO classification. The patients were 124 male and 111 female, and the median age was 44 years (range, 7-71 years) 125 of the 235 gliomas were classified as low-grade (60 WHO I, 65 WHO II), and 110 gliomas were classified as high-grade (50 WHO III, 60 WHO IV) gliomas. None of the patients received chemotherapy and radiotherapy before surgery. The clinicopathological features which included the Karnofsky performance scale (KPS) score were summarized in Table I. Overall survival (OS) time was calculated from the date of the primary surgery to death. Progression-free survival (PFS) time was calculated from the date of the initial surgery until the first evidence of local, regional, or distant tumor progression of the disease.

Quantitative reverse transcriptase PCR (qRT-PCR)

MiR-302c-3p expression in human gliomas tissues was qualified by TaqMan qRTPCR. Total RNA was extracted from the tissues TRIzol reagent (Invitrogen; Carlsbad, CA, USA) according to the manufacturer's protocol. TaqMan miRNA assays (Applied Biosystems, Foster City, CA, USA) were used to quantify the expression of MiR-302c-3p, and U6 small nuclear RNA was used as an internal control. The expression of miRNA was defined based on the threshold cycle (Ct), and the $2^{-\Delta CT}$ method was used to calculate the results.

Statistical Analysis

All data were carried out using SPSS 17.0 software package (version 17.0, SPSS Inc, Chicago, IL, USA). The chi-square test was preformed to explore the correlation of miR-302c-3p expression with clinical parameters. Patients survival and their differences were determined by the Kaplan-Meier method and log-rank test. The Cox multivariate regression analysis was used to determine the influence of each variable on survival. $p < 0.05$ was used to indicate a statistically significant difference.

Results

Expression of miR-302c-3p is Downregulated in Human Glioma Tissues

We compared the endogenous expression of miR-302c-3p in 235 glioma tissues and matched normal brain tissues by using qRT-PCR. As shown in Figure 1, we found that miR-302c-3p expression in gliomas was lower than that in the control ($p < 0.001$).

Table I. Clinicopathological features and MiR-302c-3p expression in glioma.

Variables	Cases (n = 235)	MiR-302c-3p expression level		p-value
		Low expression	High expression	
Age (years)				0.911
< 55	123	65	58	
≥ 55	112	60	52	
Gender				0.435
Man	139	71	68	
Woman	96	54	42	
WHO grade				< 0.001
I–II	113	42	71	
III–IV	122	83	39	
KPS score				0.016
< 80	112	51	61	
≥ 80	123	74	49	
Tumor size (cm)				0.068
< 5	137	66	71	
≥ 5	98	59	39	
Extent of resection				0.227
< 98%	97	46	51	
≥ 98%	138	79	59	

Relationships Between miR-302c–3p Expression and Clinicopathological

Features in Glioma

To explore the relationship between miR-302c-3p expression and clinicopathological characteristics in human gliomas, clinical follow-up was collected for all patients with glioma (Table I). The statistical analysis informed that the correlation between decreased miR-302c-3p level and WHO grade ($p < 0.001$), KPS score ($p = 0.016$)

was significant. But no significant correlations between miR-302c-3p expression and other clinicopathological variables, such as age, gender, extent of resection and tumor size were observed (all $p > 0.05$, shown in Table I).

Prognostic Value of miR-302c-3p in Glioma Patients

To explore whether the expression of miR-302c-3p was a tumor prognostic biomarker, the association between miR-302c-3p expression and prognosis of glioma patients was determined by Kaplan-Meier analysis and log-rank test. The 5-year PFS rate of glioma patients with low-miR-302c-3p expression was significantly lower than that of patients with high-miR-302c-3p expression ($p = 0.0092$, Figure 2). Furthermore, the 5-year OS rate of the patients with low-miR-302c-3p expression was significantly lower than that of the patients with high-miR-302c-3p expression ($p = 0.0057$, Figure 3). Multivariate Cox proportional hazards model suggested that down-expression of miR-302c-3p (OS $p = 0.015$, PFS $p = 0.003$) and advanced histologic grade (OS $p = 0.004$, PFS $p = 0.002$) were independent prognostic markers indicating poor prognosis for glioma patients (Tables II and III).

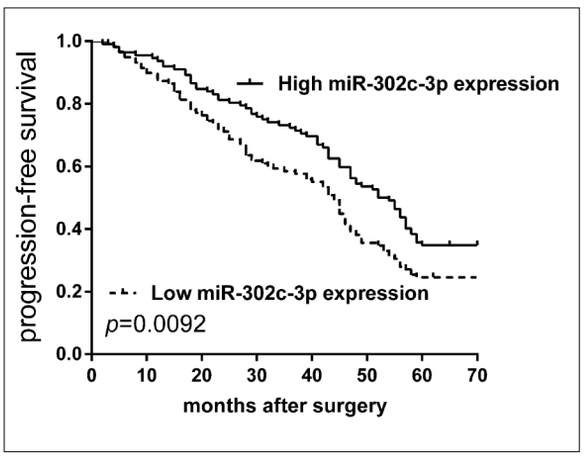

Figure 2. Kaplan-Meier curves of the progression-free survival of 235 glioma patients. Progression-free survival rate in patients with low miR-302c-3p expression was significantly lower than that in patients with high miR-302c-3p expression.

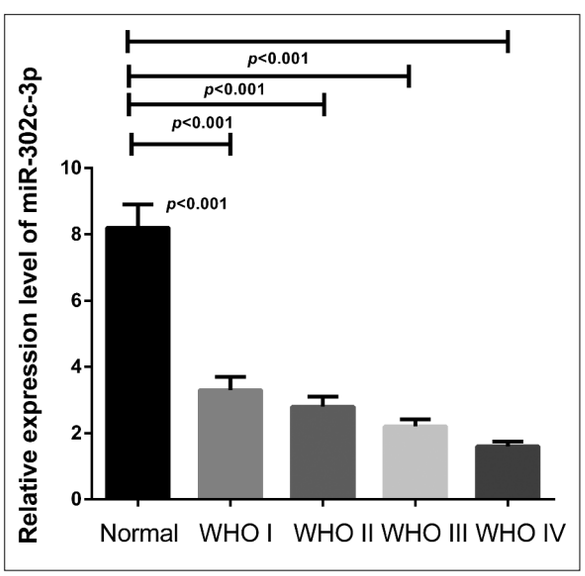

Figure 1. Real-time quantitative RT-PCR (qRT-PCR) analysis of miR-302c-3p expression in glioma tissues from different grades and adjacent normal brain tissues. U6 was used as loading control.

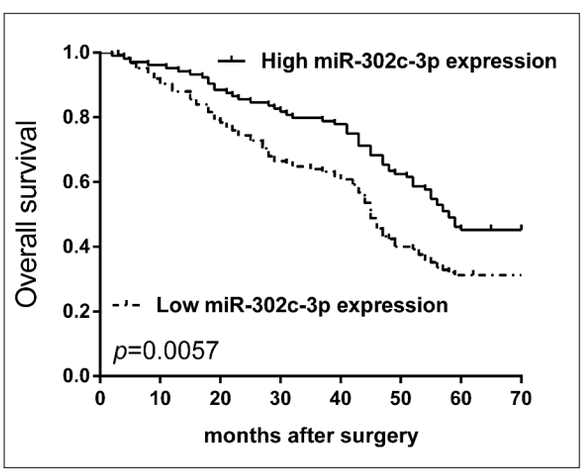

Figure 3. Kaplan-Meier curves of the overall survival of 235 glioma patients. Overall survival rate in patients with low miR-302c-3p expression was significantly lower than that in patients with high miR-302c-3p expression.

Table II. Univariate and multivariate analyses for overall survival.

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio	p	Hazard ratio	p
Age	1.32	0.45	1.21	0.32
Gender	0.77	0.92	0.81	0.73
WHO grade	3.18	0.003	3.12	0.004
KPS score	1.89	0.17	2.21	0.11
Extent of resection	1.92	0.12	1.83	0.15
Tumor size	2.31	0.09	2.42	0.08
MiR-302-3p expression level	2.26	0.02	2.54	0.015

Discussion

Glioma counts as one of the most common malignant tumors[17]. Even if the recent development in tumor diagnosis and treatment have been marked, the outcome of the patients with glioma remains poor. Especially in the patients diagnosed with high WHO tumor grades (III, IV). One of the reasons that resulted in the poor prognosis of glioma was that most cases of glioma were diagnosed at advanced clinical stages[18,19]. Many researches have been conducted to explore significant biomarkers with prognosis in glioma[20]. As a newly discovered miRNA, there is little research about the role of MiR-302c-3p in the progression of the tumor. Recently, Wang et al[16] found that MiR-302c-3p played an important role in invasion and proliferation of glioma cells. However, no association between MiR-302c-3p and clinicopathological parameters has been reported.

MiR-302c has been observed to be involved in multiple physiological and pathological processes. For example, Rosa et al[21] informed that the miR-302 family played an important role in the differentiation of human embryonic stem cells. Zhu et al[22] found that miR-302c-3p expression was significantly down-regulated in HCC tissues. Furthermore, they found that miR-302c-3p could suppress tumor growth of hepatocellular carcinoma by targeting MTDH. Similarly, another report showed that the expression level of miR-302c-3p was down-regulated and miR-302c-3p functioned as a tumor suppressor gene via targeting metadherin (MTDH) expression[16]. Moreover, Yoshimoto et al[23] revealed that miR-302c directly targeted the estrogen receptor in human breast cancer. Collectively, these results suggested that miR-302-3p played an oncogenic role in tumors.

In our present work, we measured the expression of miR-302c-3p in human glioma tissue. Quantitative PCR (qPCR) showed that miR-302c-3p was significantly up-expressed in high-grade glioma, compared with low-grade glioma and matched brain tissues. Patients with lower miR-302c-3p expression had significantly poorer OS and PFS. Furthermore, in a multivariate Cox model, we found that miR-302c-3p expression was an independent predictor of poor prognosis. All those results suggested that miR-302c-3p played an important role in prognostic of patients with glioma.

Table III. Univariate and multivariate analyses for progression-free survival.

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio	p	Hazard ratio	p
Age	1.37	0.42	1.33	0.36
Gender	0.83	0.66	0.93	0.71
WHO grade	2.79	0.007	3.31	0.002
KPS score	1.97	0.09	1.81	0.08
Extent of resection	2.11	0.11	2.23	0.09
Tumor size	1.61	0.07	2.02	0.08
MiR-302-3p expression level	2.41	0.006	2.92	0.003

Conclusions

To our knowledge, this is the first time to demonstrate that miR-302c-3p was significantly downregulated in glioma and correlated with poor prognosis, suggesting that miR-302c-3p are potential biomarkers for detection and prognosis of glioma.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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