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Association between downexpression of miR-1301 and poor prognosis in patients with glioma

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Abstract. – OBJECTIVE: MiR-1301 has been shown to be frequently down-regulated in various tumors. However, the clinical significance of miR-1301 in human glioma is still unclear. The aim of this study was to evaluate the prognostic impact of the expressions of miR-1301 in patients with glioma.

PATIENTS AND METHODS: Quantitative Real-time PCR was used to determine the expression miR-1301 in glioma tissues and pair-matched adjacent normal tissues. The relationships between miR-1301 expression and clinicopathological parameters were examined by X2 test. Kaplan-Meier curves and multivariate Cox proportional models were used to study the impact on clinical outcome.

RESULTS: We observed that miR-1301 expression was significantly lower in glioma tissues compared with adjacent non-cancerous tissues ($p<0.01$). Also, low expressions of miR-1301 were significantly associated with high WHO grade ($p<0.006$), low Karnofsky performance score (KPS) ($p=0.001$), and large tumor size ($p=0.004$). Furthermore, the data of Kaplan-Meier survival analysis showed that low miR-1301 expression significantly associated with a worse overall survival ($p=0.003$) and disease-specific survival ($p=0.001$). Finally, univariate and multivariate analysis showed that the miR-1301 expression was an independent predictor for both overall survival and disease-specific survival in glioma.

CONCLUSIONS: Our findings suggested lower miR-1301 expression resulted in poorer survival in patients with glioma, which may provide important indicators for further research.

Key Words:
miR-1301, Glioma, Overall survival, Disease-specific survival.

Introduction

Glioma is the most common and type of malignant primary brain tumors in human, which incidence is about 7.2 per 100,000 population[1]. The World Health Organization (WHO) classifies human gliomas into grade I, grade II, grade III, and grade IV[2]. Despite the use of aggressive surgery in combination with chemotherapy, biological therapy and radiotherapy, the unfavorable prognosis of patients with gliomas has not improved significantly[3,4]. For instance, median overall survival of primary glioblastoma patients remains around 12-15 months after diagnosis, and the 5-year survival rate is only 9.8%[5]. Up to now, there is still no effective strategy for glioma treatment. Therefore, there is an urgent need to develop novel targets for the diagnosis, accurate prognosis prediction, and novel therapeutic target. MicroRNAs (miRNAs) are small non-coding RNA molecules that are generated within cells and act as negative regulators of gene expression[6]. Accumulating evidence prove that miRNAs get involved in various biological processes, such as differentiation, apoptosis, morphogenesis and tumorigenesis[7,8]. Aberrant expression of miRNAs plays a critical role in tumor development and progression through modulating oncogenic and tumor suppressor pathways[9,10]. Although the role of abnormal regulation of miRNAs on tumor has not been fully studied, miRNAs have been suggested novel targets for tumor therapies and as molecular diagnostic or prognostic biomarker[11,12]. MiR-1301, which is located at chromosome 2p23.3, is a newly identified miRNA. Previous studies have reported that expression levels of miR-1301 were significantly down-regulated in prostate cancer[13], breast cancer[14] and glioma[15]. However, the detailed role of miR-1301 in glioma remains poorly understood. In the present work, to our best knowledge, we firstly reported whether miR-1301 could be considered novel biomarkers for the prediction of clinical outcomes in patients with glioma.

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Patients and Methods

Study Cohort and Samples

The study was approved by the Ethics Committees, and we obtained patient's permission before surgery. A total of 184 patients was analyzed in this study and underwent resection of their primary gliomas at the Department of Neurosurgery, Cangzhou Central Hospital between 2008 and 2011. All cancerous and tissues and paired normal tissues were histologically confirmed by a pathologist samples were instantly frozen in liquid nitrogen and stored at -80°C until the extraction of total RNA. None of the patients had received chemotherapy or radiotherapy preoperatively. Every 3 months, participants have been sent follow-up scheme or telephone visit to update the information. The survival status of all of the patients was confirmed in January 2017. The clinicopathological characteristics of these patients were collected from the medical records and are summarized in Table I.

RNA Isolation and Quantitative Real-time-PCR

Total RNA was extracted from the collected fresh-frozen tissue specimens using the standard Trizol method. RNA concentration was measured using NanoDrop2000c (Thermo Scientific, Waltham, MA, USA). MiRNA levels were determined by qPCR using triplicate TaqMan microRNA assays (Applied Biosystems, Foster City, CA, USA). qPCR was performed with the following protocols: 93°C for 15 min, followed by 15 s at 95°C and another 1 min at 63°C for 50 cycles. GAPDH was used as an internal reference for relative gene expression quantitation. Results are expressed as means±SD of three independent experiments. The relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers for miR-1301 were purchased from Applied Biosystems (Foster City, CA, USA).

Statistical Analysis

All statistical analyses were performed using the SPSS 17.0 software package (SPSS, Chicago, IL, USA). The differences between two groups were analyzed by Student's *t*-test. The association between miR-1301 and clinicopathological features was tested using the X^2-test. Kaplan-Meier and the log-rank test were used to calculate the survival curves. Data of survival were evaluated by univariate and multivariate Cox proportional hazards models. A *p*-value of less than 0.05 was considered statistically significant.

Table I. Relationship of miR-1301 expression with clinicopathological characteristics in glioma patients.

Parameters	Number	miR-1301 expression		*p*
		High	Low	
Age				0.910
<50	74	31	43	
≥50	110	47	63	
Gender				0.786
Man	123	53	70	
Woman	61	25	36	
Tumor location				0.828
Supratentorial	89	37	52	
Infratentorial	95	41	54	
Family history of cancer				0.225
Yes	50	19	31	
No	144	69	75	
KPS				0.001
≥70	71	41	30	
<70	113	37	75	
Size				0.004
<5 cm	61	35	26	
≥5 cm	123	43	80	
WHO grade				0.006
Low	73	40	33	
High	111	38	73	

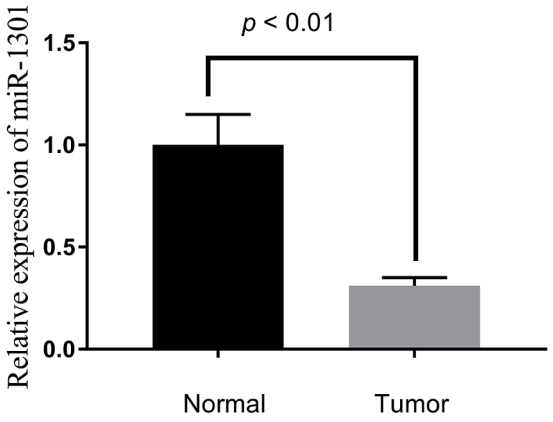

Figure 1. Comparison in miR-1301 expression level between glioma tissues and matched normal tissues.

Results

Expression of miR-1301 is Down-Regulated in Glioma Tissues

We firstly detected miR-1301 expression levels in 184 glioma tissues and matched normal brain tissues using qRT-PCR. As shown in Figure 1, miR-1301 expression was significantly up-regulated in glioma tissues compared with normal brain tissues (p<0.01). These observations informed that miR-1301 may play a negative role in progression of glioma.

Correlation of miR-1301 Expression with Clinicopathological Features

Next, we explored the correlation of miR-1301 expression level with the clinicopathological factors in glioma patients. The median expression level of miR-1301 was used as a cutoff point to divide all 184 patients into two groups: the high expression group (n = 78) and the low expression group (n = 106). The association between clinicopathological characteristics and miR-1301 expression in patients with glioma was shown in Table I. We found that low expressions of miR-1301 were significantly association with high WHO grade (p<0.006), low KPS (p=0.001), and large tumor size (p=0.004). However, we did not find a significant association of miR-1301 expression levels with other factors such as age, gender, tumor location and family history of cancer (p>0.05, respectively).

Prognostic Value of miR-1301 Expression in Glioma Patients

Whether miR-1301 expression has prognostic potential for the survival time of glioma patients was explored. Kaplan-Meier method was performed to evaluate the correlation between miR-1301 expression level and the overall survival/disease-specific survival of glioma patients. As shown in Figure 2, the overall survival rate of glioma patients with high-miR-1301 expression was significantly higher than that of patients with low-miR-1301 expression (p<0.001). Moreover, we also observed that decreased miR-1301 expression was associated with poor disease-specific survival of patients (p<0.001, Figure 3). Cox proportional hazards regression analysis at the univariate level indicated that KPS, size, WHO grade, and miR-1301, were significantly associated with overall survival and disease-specific survival of glioma patients (Table II). Then, multivariate analysis further confirmed that miR-1301 expression was an independent prognostic factor for the both overall survival (p=0.003) and disease-specific survival (p=0.006) of glioma patients (Table III).

Discussion

Glioma is characterized by its high growth potential and poor clinical prognosis[16]. Identifying of prognostic marker in glioma is crucial to select optimal therapeutic strategies[17]. Recently, many efforts have been devoted to understand the underlying molecular mechanisms and explore susceptible markers of glioma to predict prognosis[18,19]. In recent years, miRNAs

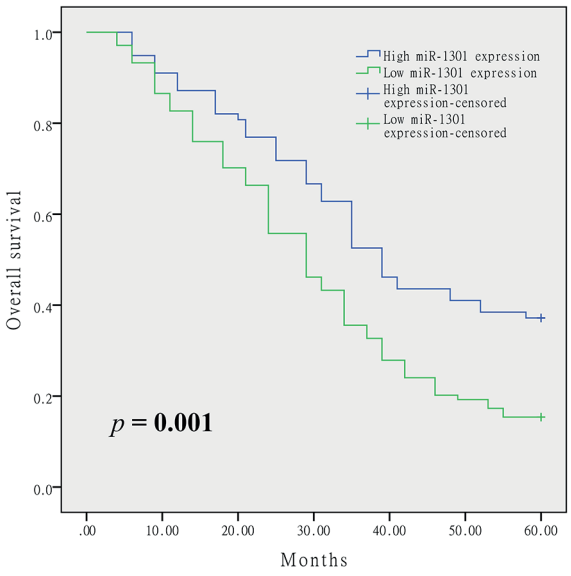

Figure 2. Kaplan-Meier plots of overall survival in patients with glioma and with low and high level of miR-1301.

Table II. Univariate analyses for overall survival and disease-specific survival by Cox regression model.

	Overall survival			Disease-specific survival		
Variable	**HR**	**95% CI**	**p**	**HR**	**95% CI**	**p**
Age						
<50	1.000			1.000		
≥50	0.823	0.473-1.428	0.471	0.793	0.542-1.321	0.523
Gender						
Man	1.000					
Woman	0.893	0.634-1.655	0.313	0.832	0.552-1.437	0.377
Tumor location						
Supratentorial	1.000			1.000		
Infratentorial	0.721	0.552-1.213	0.563	0.873	0.673-1.553	0.473
Family history of cancer						
Yes	1.000			1.000		
No	0.931	0.774-1.542	0.188	0.831	0.652-1.669	0.135
KPS						
≥70	1.000			1.000		
<70	3.753	1.321-4.321	0.004	3.341	1.134-3.341	0.007
Size						
<5 cm	1.000			1.000		
≥5 cm	2.459	1.542-3.778	0.012	2.321	1.223-3.034	0.017
WHO grade						
Low	1.000			1.000		
High	2.873	1.239-4.733	0.008	2.452	1.348-4.236	0.011
miR-1301						
High	1.000			1.000		
Low	2.931	1.652-5.663	0.001	2.569	1.583-5.291	0.001

Table III. Multivariate analyses for overall survival and disease-specific survival by Cox regression model.

	Overall survival			Disease-specific survival		
Variable	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age	0.732	0.562-1.341	0.399	0.656	0.762-1.452	0.442
Gender	0.852	0.672-1.553	0.328	0.723	0.822-1.347	0.495
Tumor location	0.923	0.663-1.342	0.288	0.831	0.582-1.762	0.219
Family history of cancer	1.231	0.774-1.673	0.113	1.431	0.893-1.665	0.164
KPS	3.321	1.213-3.893	0.007	2.893	1.139-3.452	0.011
Size	2.139	1.133-3.231	0.017	1.893	1.323-2.993	0.021
WHO grade	2.783	1.382-4.552	0.009	2.663	1.213-3.892	0.011
miR-1301	2.563	1.423-5.113	0.003	2.362	1.238-4.374	0.006

have become a novel ‘‘hot topic’’ in exploring prognostic biomarker field. Many miRNAs have been identified to have potential to predict prognosis of glioma[20-22]. It is necessary to explore the prognostic role of more miRNAs in glioma. Studies have shown that miR-1301 has a crucial role in cancer development. Fang et al[23] reported that overexpression of miR-1301 promoted liver cancer cell proliferation and migration by targeting tumor suppresser KLF6-FL. Bi et al[13] found that miR-1301 played an oncogenic role in prostate cancer by targeting tumor suppressor PPP2R2C. Lin et al[14] revealed that upregulation of miR-1301 enhanced the proliferation of breast cancer cells by down-regulating ICAT. All of these findings indicated that miR-1301 expression was up-regulated in these tumors. However, Zhi et al[15] found that the expression levels of miR-1301 in glioma tissues were significantly downregulated, and forced expression of miR-1301 suppressed inhibits proliferation of human glioma cells by di-

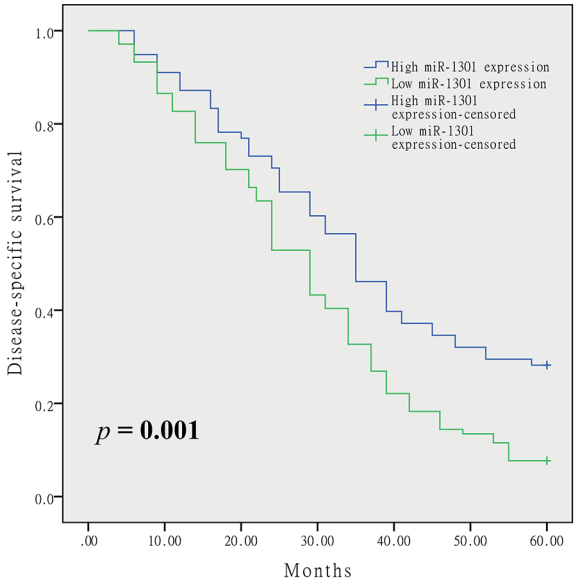

Figure 3. Kaplan-Meier plots of disease-specific survival in patients with glioma and with low and high level of miR-1301.

rectly targeting N-Ras. These data showed that miR-1301 was downregulated or upregulated in different tumor types. Although miR-1301 has been confirmed to serve as a tumor suppressor in progression of glioma, the clinical significance of miR-1301 in glioma patients remains unknown. In our investigation, we determined miR-1301 expression by qRT-PCR. We found that miR-1301 expression was significantly lower in glioma tissues compared with adjacent non-cancerous tissues. In addition to its aberrant expression, we observed that low expressions of miR-1301 were significantly association with high WHO grade, KPS, and large tumor size. Furthermore, we proved that miR-1301 low expression was associated with lower overall survival and disease-specific survival rates and could be an independent prognostic factor in patients with glioma. The above findings suggested that reduced miR-1301 expression could be a promising biomarker for predicting poor survival in glioma patients.

Conclusions

Our findings indicated that miR-1301 was downregulated in glioma tissues, and low miR-1301 expression was significantly associated with aggressive variables and poorer prognosis. Further studies are needed to validate the prognostic value of miR-1301 expression in other cohorts.

Conflict of interest

The authors declare no conflicts of interest.

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