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Original Article

Reduced expression of miR-506 in glioma is associated with advanced tumor progression and unfavorable prognosis

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Abstract: Background: MicroRNAs regulate gene expression at the post-transcriptional level and play important roles in cancer progression. The aim of this study was to investigate the expression level of miR-506 as well as its association with glioma progression and prognosis. Methods: miR-506 expression in glioma tissues and normal brain tissues was measured by quantitative real-time PCR (qRT-PCR). Survival curves were plotted using the Kaplan-Meier method and differences in survival rates were analyzed using the log-rank test. Results: Our data showed that miR-506 expression was down-regulated in glioma tissues compared with normal brain tissues ($P<0.05$). The low miR-506 expression was found to be correlated with advanced WHO grade and low Karnofsky performance score (KPS) of glioma patients ($P<0.05$). In addition, the overall survival of patients with low miR-506 expression was shorter than those with high miR-506 expression ($P<0.05$). Furthermore, multivariate analysis suggested that miR-506 expression was an independent prognostic factor for glioma patients. Conclusion: These results indicated that miR-506 expression was decreased in human glioma and associated with tumor progression and prognosis of patients, indicating that miR-506 might be involved in glioma carcinogenesis and become a potential prognostic biomarkers for glioma.

Keywords: miR-506, glioma, prognosis

Introduction

Human glioma represents the most common malignancy in central nervous system for both children and adults [1]. According to the World Health Organization (WHO) classification, gliomas are divided into well-differentiated low grade astrocytomas (WHO grade I~II), anaplastic astrocytomas (WHO grade III) and glioblastoma multiforme (GBM, WHO grade IV) [2]. Despite recent therapeutic advances, the survival of patient with glioma is still poor with an average patient life expectancy of only 15 months after diagnosis for GBM patients [3]. Therefore, it is urgent to investigate the molecular mechanisms involved in glioma carcinogenesis, and to identify potential prognostic and therapeutic markers for human gliomas treatment.

MicroRNAs (miRNAs) are a class of small, evolutionarily conserved, short non-coding endogenous RNA molecules that regulate gene expression at the post-transcriptional level [4]. MiRNAs have been demonstrated to play normal physiologic roles in cell proliferation, migration, differentiation, epithelial-mesenchymal transition, and metabolism [5, 6]. In addition, increasing studies indicated that miRNAs play important roles in tumor progression, acting as tumor suppressors or oncogenes depending on their target genes [7]. For example, Wang et al reported that miR-451 functioned as a tumor suppressor in human non-small cell lung cancer by targeting RAB14 [8]. Tsukamoto et al showed miR-375 acted as a tumor suppressor by directly targeting PDK1 and 14-3-3 zeta in human gastric cancer [9]. Yu et al indicated that miR-96 exerted tumor oncogenic functions in prostate

Table 1. Association of miR-506 expression with clinicopathological features of glioma patients

Clinicopathological features	Group	Total	miR-506 expression		*P* value
			Low	High	
Gender	Male	47	25	22	0.597
	Female	40	19	21	
Age (years)	<50	36	16	20	0.337
	≥50	51	28	23	
Tumor size (cm)	<5 cm	56	26	30	0.299
	≥5 cm	31	18	13	
WHO grade	I	11	3	8	0.009
	II	9	2	7	
	III	29	18	11	
	IV	38	21	17	
KPS	<80	61	37	24	0.004
	≥80	26	7	19	

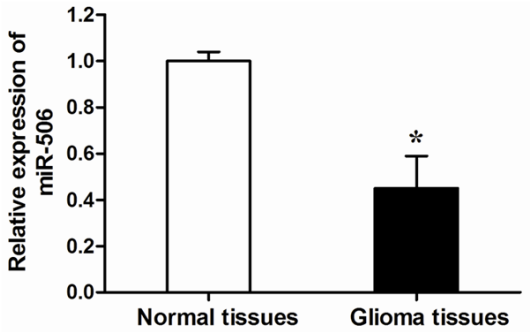

Figure 1. Expression levels of miR-506 in glioma tissues and normal brain tissues. miR-506 expression was significantly decreased in glioma tissues when compared with normal brain tissues. *P<0.05.

cancer by down-regulating of FOXO1 expression [10]. Hou et al demonstrated that miR-196a acted as a oncogene in cervical cancer through regulating FOXO1 and p27Kip1 expression [11]. These studies suggested that miRNA play critical roles in human tumor progression.

MiR-506 is a recently identified microRNA, which was reported to inhibit epithelial mesenchymal transition (EMT) in ovarian cancer and breast cancer [12, 13]. It was also reported that miR-506 can significantly restrain the proliferation of tumor cells in gastric cancer, oral squamous cell carcinoma, and hepatocellular cancer [14-16]. These results suggested that miR-506 might be a novel miRNA that is important in human malignancies. However, the role of miR-506 in glioma is still unclear. In the present study, the expression level of miR-506 in glioma tissues and normal brain tissues were examined by quantitative real-time PCR (qRT-PCR). Furthermore, the correlation between miR-506 expression and clinicopathological features and prognosis were analyzed.

Materials and methods

Patients and specimens

87 glioma tissue samples for qRT-PCR were obtained from patients who underwent surgery between January 2008 and December 2010 in Xinxiang Central Hospital. None of patients received radiotherapy or chemotherapy prior to surgery. Normal brain tissue specimens were taken from 18 patients who underwent surgery for reasons other than malignancy. The histomorphology of all tissue specimens had been pathologically confirmed. Tissues were snap frozen in liquid nitrogen after surgical resection until use. The clinicopathological features of all patients were showed in **Table 1**. The present study was approved by the Ethics Committee of the Xinxiang Central Hospital, informed consent was obtained from all of the patients. All specimens were handled and made anonymous according to the ethical and legal standards.

Quantitative real-time PCR

Total RNA was extracted from tissue samples using TRIzol (Invitrogen) according to the manufacturer's protocol. For miRNA qPCR, reverse transcription was performed using the QuantMir RT Kit (System Biosciences). The cDNA then serves as the template for SYBR real-time PCR using Power SYBR Green PCR Master Mix (Applied Biosystems). All reactions were run in triplicate on ABI PRISM 7000 Fluorescent Quantitative PCR System (Applied Biosystems) using miR-506 specific primers. Data was collected and analyzed by SDS2.3 Software (Applied Biosystems). The expression level of miR-506 was normalized internally by using the CT of U6. The relative quantitative value was

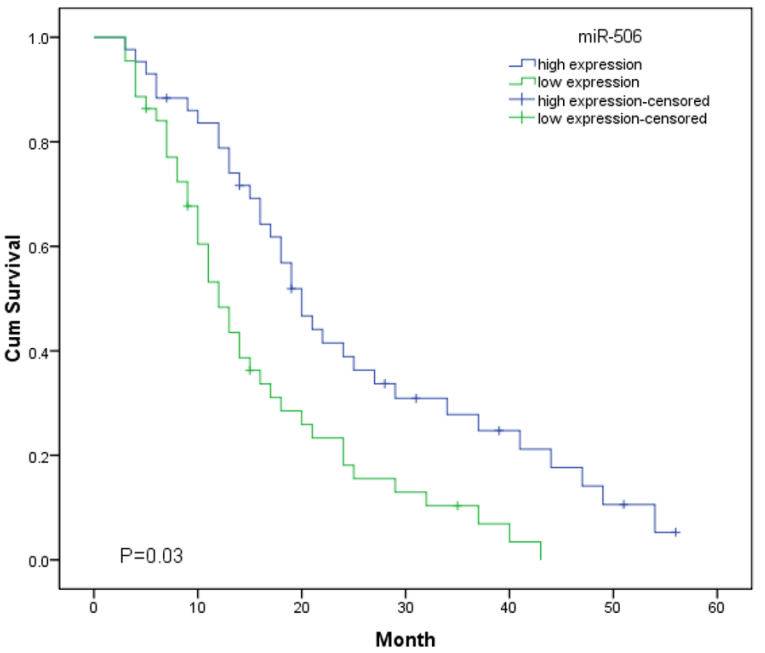

Figure 2. Survival analysis of 87 glioma patients by Kaplan-Meier method. Overall survival rate in patients with low miR-506 expression was significantly lower than that in patients with high miR-506 expression (log-rank test, P<0.05).

expressed by the $2^{-\Delta\Delta Ct}$ method. Each experiment was performed in triplicates and repeated three times.

Statistics analyses

All computations were carried out using the software of SPSS version 18.0 for Windows. Data were expressed as means ± standard deviation (SD). The correlation between the expression level of miR-506 and clinicopathological features was assessed with Chi-square test. Survival curves were plotted using the Kaplan-Meier method, and differences between survival curves were tested using the log-rank test. Multivariate analysis of the prognostic factors was performed with Cox's proportional hazards model. P<0.05 was considered statistically significant.

Results

miR-506 is down-regulated in human glioma tissues

Clinicopathological features of all patients with glioma recruited were showed in **Table 1**. The expression levels of miR-506 were detected in 87 glioma and 18 normal brain tissues normalized to U6. As shown in **Figure 1**, the expression level of miR-506 in glioma tissues was significantly lower than that in normal brain tissues (P<0.05). In order to facilitate further analysis, we defined gliomas with miR-506 expression less than the median expression level as the low expression group, and tumors with miR-506 expression above the median value were defined as high expression group.

Association of miR-506 expression with clinicopathological features of glioma

As miR-506 expression was found to be decreased in glioma, indicating a potential tumor suppressor role of miR-506 in this disease. We further explored the association of miR-506 expression with clinicopathological features. As shown in **Table 1**, the decreased expression of miR-506 was significantly more common in glioma tissues with advanced WHO grade than those with low WHO grade (P<0.05). In addition, a significant relationship was also found between miR-506 expression and Karnofsky performance score (KPS). The down-regulation of miR-506 more frequently occurred in tumors with low KPS than those with high KPS (P<0.05). However, there was no significant association between miR-506 expression and other clinicopathological features, including patients' gender, age, and tumor size.

Relationship of miR-506 expression with overall survival in glioma patients

To further investigate the correlation of miR-506 expression level with overall survival of glioma patients, Kaplan-Meier analysis and log-rank test were performed. We found that low expression of miR-506 in glioma was significantly associated with poor overall survival of patients (**Figure 2**, log-rank test, P<0.05). Univariate analysis indicated that KPS, WHO grade, and miR-506 expression were statistically significant risk factors affecting the overall

Table 2. Univariate and multivariate analysis of overall survival in glioma patients

Clinicopathological features	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*P*	Hazard ratio	95% CI	*P*
Gender	1.138	0.518-1.934	0.283			
Age (years)	0.917	0.436-2.306	0.385			
Tumor size	1.372	0.831-3.674	0.165			
WHO grade	2.648	1.473-6.058	0.009	2.381	1.295-5.668	0.013
KPS	1.749	1.236-4.261	0.012	1.584	1.173-3.965	0.007
MiR-506	2.276	1.303-5.774	0.005	2.107	1.225-5.391	0.003

survival of patients with glioma (**Table 2**). No significant associations were found for gender, age, and tumor size. Multivariate analysis using the Cox proportional hazard model confirmed that KPS, WHO grade, and miR-506 expression were independent prognostic factors for patients with glioma (**Table 2**, P<0.05). These results demonstrated that miR-506 could be an independent prognostic factor of overall survival for patients with glioma.

Discussion

In recent years, increasing studies showed that dysregulation expression of miRNAs contributes to the initiation and progression of cancers [17]. Moreover, the physiological and pathological roles of miRNAs have also been demonstrated in most tumor types and miRNAs may play an important role in the diagnosis and treatment of cancer [18, 19]. Therefore, the correlation between miRNAs and cancers has become a focus of cancer studies.

In this study, we explored the expression of miR-506 in glioma, we found that the expression level of miR-506 in glioma tissues was significantly lower than that in the normal brain tissues. Meanwhile, our data revealed that low miR-506 expression was associated with advanced WHO grade and low KPS. Furthermore, Kaplan-Meier analysis showed that patients with low miR-506 expression had a poorer overall survival than those with high miR-506 expression group. Univariate and multivariate analyses indicated that miR-506 down-regulation was an independent predictor for shorter overall survival of glioma patients.

Previous researches demonstrated that miR-506 was deregulated in types of cancers and play important roles in tumor progression. For example, Sakimura et al suggested that miR-506 induced epithelial mesenchymal transition was involved in poor prognosis for patients with gastric cancer [20]. Sun et al indicated that miR-506 inhibited vimentin and N-cad in the epithelial mesenchymal transition network and decreased expression of miR-506 was associated with poor prognosis in epithelial ovarian cancer [12]. Yang et al reported that miR-506 was decreased in renal cancer and associated with an advanced clinical stage and poor prognosis of patients, furthermore, they found that miR-506 could inhibit renal cancer cell growth and metastasis by directly targeting FLOT1 [21]. These data suggested that miR-506 might be novel miRNA that is important in human malignancies. Our study expanded the tumor suppressive role of miR-506 in glioma progression.

In conclusion, our study suggested that the expression of miR-506 was decreased in glioma, and associated with advanced tumor progression and unfavorable prognosis, indicating that miR-506 could serve as an efficient prognostic factor for glioma patients.

Disclosure of conflict of interest

None.

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