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Original Article

Expression of MicroRNA-326 in brain tissues and correlation with prognosis in patients with glioma

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Abstract: Objective: To investigate the expression of MicroRNA-326 in brain tissues and correlation with prognosis in patients with glioma. Method: We conducted analysis on the clinical characteristics and follow-up data of 106 patients with glioma enrolled in Neurosurgery Department of our hospital from February 2007 to February 2011, detected MicroRNA-326 expression level in brain tissues of patients using RT-qPCR, and investigated the factors influencing the prognosis of patients with glioma using multivariate Cox proportional hazard regression models. Results: The MicroRNA-326 expression levels in brain pathological tissues of 106 patients with glioma were the median level was 3.61 and the mean was (3.83 ± 1.72). The MicroRNA-326 expression level in brain tissues was closely correlated with pathological grades of glioma and KPS scores ($P < 0.05$). The median survival time of patients in the MicroRNA-326 high expression group was 43 months, and the 1-year, 3-year and 5-year survival rates were 92.5%, 71.7% and 26.4%, respectively; while in MicroRNA-326 low expression group, the median survival time of patients was 21 months and the 1-year, 3-year and 5-year survival rates were 77.4%, 32.1% and 3.8% respectively; there was significant difference in survival rate between the two groups ($\chi^2 = 15.019$, $P < 0.001$). Univariate analysis showed that there were statistically significant differences ($P < 0.05$) in survival of patients with glioma in different ages, pathological grades, and tumor sizes, extents of tumor resection, KPS scores and MicroRNA-326 expression levels. Multivariate Cox regression analysis showed that the pathological grades III~IV (HR = 2.51, 95% CI: 1.20~5.25) and low expression levels of MicroRNA-326 (HR = 2.37, 95% CI: 1.18~4.77) were independent risk factors for the prognosis of patients with glioma, and total resection (HR = 0.55, 95% CI: 0.357~0.86) and preoperative KPS score ≥ 70 points (HR = 0.63, 95% CI: 0.42~0.95) were independent protective factors for the prognosis of patients with glioma ($P < 0.05$). Conclusion: MicroRNA-326 expression level in brain tissues of patients with glioma is low, and the patients with low MicroRNA-326 expression level have a poor prognosis. MicroRNA-326 level may be used as a biomarker for predicting the occurrence, progression and prognosis of glioma.

Keywords: Glioma, MicroRNA-326, gene expression, prognosis

Introduction

MicroRNAs are a class of endogenous non-coding regulatory sRNA with a length of about 19 to 25 nucleotides, which can bind with 3'-UTR of target mRNA to degrade the target mRNA or inhibit the translation of proteins, and thus regulate the expression of the target genes [1]. Previous studies have shown that mRNAs are not only involved in the cell proliferation, metabolism, differentiation and apoptosis and other important biological process, but also play an important role similar to oncogene/anti-oncogene during the occurrence and progression of tumors [2, 3]. Glioma is a tumor originating from neuroglial cells of brain, and it is the most common primary intracranial tumor (about 45%~60% of the primary intracranial tumors). In recent years, the incidence of glioma has been rising [4, 5]. With advances in the diagnosis and treatment techniques, the survival time of patients with glioma is prolonged, but the overall prognosis is still poor (especially for patients with malignant glioma). Glioma remains refractory due to lack of effective diagnostic and therapeutic targets [6]. Recent studies have revealed that multiple microRNAs play an important role in occurrence and progression of the disease, and MicroRNA-326 is the most important one. Previous studies were

mainly focused on the effect of microRNAs on the proliferation and migration of glioma cells, but rarely reported their correlations with prognosis of patients [7, 8]. In this study, by investigating the clinical characteristics and follow-up data of 106 patients with glioma enrolled in Neurosurgery Department of our hospital from February 2007 to February 2011 and detecting the MicroRNA-326 expression level in brain tissues, we studied the correlation between MicroRNA-326 expression level and survival time of patients, to provide references for identifying biomarkers that can be used to predict prognosis of patients with glioma.

Materials and methods

Subjects

Patients with glioma enrolled in Neurosurgery Department of our hospital from February 2007 to February 2011 were selected as subjects in this study. The inclusion criteria: (1) pathologically diagnosed as glioma; (2) with complete clinical and follow-up (5 years after operation) data. Exclusion criteria: (1) combined with tumors of other sites; (2) severe dysfunctions of vital organs such as the heart, liver, spleen, lungs and kidneys, etc. This study was approved by the Hospital Ethics Committee. A total of 106 patients with glioma that met the criteria were included in this study, including 66 males (62.3%) and 40 females (37.7%); they were aged 21 to 80 years, with median age of 44 years and mean age of 42.6 ± 11.3 years.

MicroRNA-326 detection

The pathological paraffin blocks of glioma tissue from patients in the Pathology Department were used to cut into specimens of 10 μm thick for testing. (1) Extraction of total RNA: the total RNA in glioma paraffin specimens was extracted by Trizol method, and then the RNA purity and concentration were determined by UV spectrophotometer (identification of RNA purity by maintaining OD260/280 at 1.8~2.1); (2) Reverse transcription: cDNA was obtained by reverse transcription using the TaqMan MicroRNA reverse transcription kit (purchased from TIANGEN Biotech (Beijing) Co., Ltd.); (3) qRT-PCR: PCR amplification using TaqMan Universal Master Mix II with the reaction systems of TaqMan Universal Master Mix II 10.00 μl, Nucleare free water 7.67 μl, TaqMan Assay (20×) 1.00 μl and cDNA 5.00 μl, and U6 as an internal reference. All experiments were performed at least three times.

Treatment

Eighty-two patients (77.4%) received total resection/near total resection (tumor resection rate ≥ 90% according to the visual/microscopic observation during the surgery and the brain MRI scan results). 24 patients received partial resection (tumor resection rate < 90% according to the visual/microscopic observation during the surgery and the brain MRI scan results).

Follow-up approach

All patients were followed up after surgery by telephone or outpatient reexamination every 3 months in average. The patients were followed up until March 1, 2016 or terminated due to death and the follow-up rate was 100%. Survival time was defined as the period from treatment to the end of follow up.

Study method

By reviewing the materials such as hospitalization records, various auxiliary test reports and follow-up records, the clinical and follow-up data were collected for patients with glioma, and relevant information was extracted using a uniform questionnaire, mainly including sex, age, pathological type and grade of brain glioma [according to WHO pathological classification for tumors in the central nervous system (2007 version) [9], tumor size, pre-operative KPS score, treatment method, last follow-up time and survival time etc.

Statistical analysis

Statistical analysis was performed with the SPSS 21.0 software, measurement data were described in mean ± standard deviation ($\bar{x} \pm S$), and counting data were expressed in relative numbers such as rate and constituent ratio. χ^2 test was used to analyze the relationship between MicroRNA-326 expression and clinical pathological characteristics. The Kaplan-Meier method was used to estimate the survival rate of patients with glioma with different characteristrics and the log-rank test was adopted for comparison. Finally, the multiple Cox proportional hazard regression model

Table 1. Correlation between MicroRNA-326 expression and clinical pathological characteristics in patients with glioma (n = 106)

Clinical characteristics		MicroRNA-326 expression		χ^2	P
		High expression	Low expression		
Age (year)	≤ 45	30	25	0.945	0.331
	> 45	23	28		
Sex	M	35	31	0.642	0.423
	F	18	22		
Number of lesions	Single	28	34	1.399	0.237
	Multiple	25	19		
Tissue type	Astrocytoma	37	43	1.835	0.176
	Non-astrocytoma	16	10		
Pathological grade[&]	I	13	2	23.598	< 0.001*
	II	18	5		
	III	18	30		
	IV	4	16		
Tumor diameter	≤ 5 cm	20	16	0.673	0.412
	> 5 cm	33	37		
KPS SCORE	< 70	6	29	22.655	< 0.001*
	≥ 70	47	24		
Pre-operative epilepsy	Yes	12	16	0.777	0.378
	No	41	37		

Note: [&]indicated χ^2 trend test, *P < 0.05.

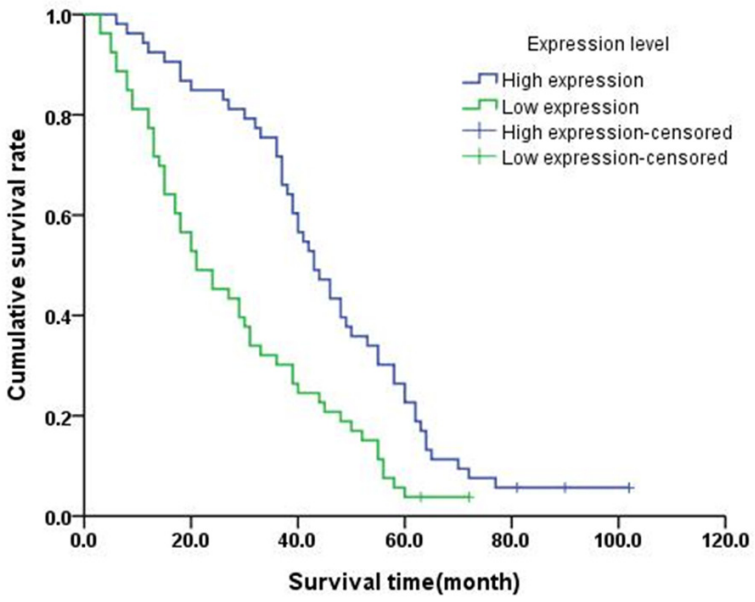

Figure 1. The survival curves of patients with different MicroRNA-326 expression levels. (Cumulative survival rate; Survival time (month); High expression; Low expression).

(?) was used to explore the relevant factors having an impact on prognosis of the patients. The test level $\alpha = 0.05$, and P < 0.05 indicated statistically significant differences.

Results

MicroRNA-326 expression level and its correlation with clinical characteristics

The MicroRNA-326 expression levels in pathological tissues of brain in 106 cases were 0.25~7.13, and the median expression level was 3.61, the mean was 3.83 ± 1.72. Using the median expression level as boundary, the 106 patients with glioma were divided into the MicroRNA-326 high expression group and MicroRNA-326 low expression group, 53 cases in each group.

Analysis on correlation between the MicroRNA-326 expression level and clinical characteristics showed that the MicroRNA-326 expression level was not associated with patient age, gender, number of lesions, glioma tissue type, tumor diameter and epilepsy before operation (P > 0.05), but closely associated with the pathological grades of glioma and KPS scores (P < 0.05) (**Table 1**).

Survival length of patients with different MicroRNA-326 expression levels

The follow-up survival time of 106 patients with glioma was from 3 to 102 months, with a median of 38.0 months, 1-year survival rate of 84.9% (90/106), 3-year survival rate of 51.9% (55/106), and 5-year survival rate of 15.1% (16/106). Survival of patients with different

Table 2. Univariate analysis of prognosis for patients with glioma (n = 106)

Factors	Number of cases [n (%)]	Median survival time (month)	χ^2	*P*
Age (year)				
≤ 45	55 (51.9)	43	9.342	0.002*
> 45	51 (48.1)	33		
Sex				
M	66 (62.3)	37	0.091	0.764
F	40 (37.7)	40		
Number of lesions				
Single	62 (58.5)	36	1.056	0.304
Multiple	44 (41.5)	40		
Tissue type				
Astrocytoma	80 (75.5)	37	0.165	0.921
Non astrocytoma	26 (24.5)	40		
Pathological grade				
I~II	38 (35.8)	50	23.571	< 0.001*
III~IV	68 (64.2)	28		
Tumor diameter				
≤ 5 cm	36 (34.0)	44	5.102	0.024*
> 5 cm	70 (66.0)	33		
Degree of tumor resection				
Total/subtotal resection	76 (71.7)	43	16.953	< 0.001*
Partial resection	30 (28.3)	28		
KPS SCORE				
< 70	35 (30.0)	30	8.452	0.015*
≥ 70	71 (70.0)	42		
Pre-operative epilepsy				
Yes	28 (26.4)	35	1.385	0.500
No	78 (73.1)	39		
MicroRNA-326				
High expression	53 (50.0)	43	15.019	< 0.001*
Low expression	53 (50.0)	21		

Note: *the survive in first value compare with that in second value in each variable, P < 0.05.

MicroRNA-326 expression levels: (1) among 53 cases with high MicroRNA-326 expression, the follow-up survival time was 6~102 months, with a median of 43 months; 1-year, 3-year and 5-year survival rates were 92.5%, 71.7% and 26.4%, respectively; (2) among 53 cases with low MicroRNA-326 expression, the follow-up survival time was 3~72 months, with a median survival time of 21 months; 1-year, 3-year and 5-year survival rates were 77.4%, 32.1% and 3.8%, respectively; there was statistically significant difference in survival rate between the two groups (χ^2 = 15.019, P < 0.001) (**Figure 1**).

Univariate analysis of prognosis for patients with glioma

Univariate analysis with log-rank test showed that there was no significant difference in survival of the patients in sex, number of lesions, tissue type and preoperative epilepsy (P > 0.05), but there was significant difference in survival of patients with different age, histological grade, tumor size, extent of tumor resection, KPS score and MicroRNA-326 expression level (P < 0.05) (**Table 2**).

Multivariate analysis of prognosis for patients with glioma

The variables with significance in the univariate analysis such as age, pathological grading, tumor size, extent of tumor resection, KPS score and MicroRNA-326 expression level were included in the multiple Cox stepwise regression analysis. Results showed that the pathological grades III~IV (HR = 2.51, 95% CI: 1.20~5.25) and low expression of MicroRNA-326 (HR = 2.37, 95% CI: 1.18~4.77) were independent risk factors for the prognosis of patients with glioma, total resection (HR = 0.55, 95% CI: 0.357~0.86) and preoperative KPS score ≥ 70 points (HR = 0.63, 95% CI: 0.42~0.95) were independent protective factors for the prognosis of patients with glioma (P < 0.05) (**Table 3**).

Discussion

As the most commonly seen intracranial malignant tumor, glioma is characterized by high incidence, difficulty in treatment, and poor progno-

Table 3. Multiple Cox stepwise regression analysis of patients with glioma (n = 106)

Independent variable	Regression coefficient	Standard error	Wald χ^2	P	*HR* (95% *CI*)
Pathological grade III~IV (reference group = I~II)	0.921	0.376	17.892	< 0.001*	2.51 (1.20~5.25)
KPS SCORE ≥ 70 (reference group < 70)	-0.457	0.228	5.311	0.021*	0.63 (0.42~0.95)
Total resection (reference group = partial resection)	-0.601	0.231	18.526	< 0.001*	0.55 (0.35~0.86)
Low expression of MicroRNA-326 (reference group = high expression)	0.862	0.357	16.513	< 0.001*	2.37 (1.18~4.77)

Note: *compare with each reference group of variable, P < 0.05.

sis, etc. In recent years, studies on glioma have always been the focus in the area of neurosurgery [10]. At present, there is lack of effective biomarkers for predicting the occurrence, progression and prognosis of glioma in clinical diagnosis and treatment.

Previous studies have shown that MicroRNAs can regulate the tumor differentiation, invasion and metastasis by controlling the key genes in tumor occurrence and progression processes, playing a critical role in the processes [11]. MicroRNA-326 belongs to the miRNAs family, and related studies have shown [12] that MicroRNA-326 affects apoptosis, invasion and proliferation of glioma cells in glioblastoma multiforme. Besides, some studies showed that MicroRNA-326 could inhibit activation of signaling pathways by inhibiting expression of the key gene Smo in the Hedgehog signaling pathway, and ultimately inhibited the proliferation of cerebellar neuron progenitor cells and tumor cells [13]. However, previous studies were rarely concerned on the correlation between MicroRNA-326 and prognosis of patients with glioma. In this study, we detected the MicroRNA-326 expression levels in brain tissues in 106 patients, and results showed that MicroRNA-326 expression was low in the brain tissues, and the higher the pathological grading of glioma, the lower the MicroRNA-326 expression level; the MicroRNA-326 expression level was positively correlated with the KPS scores; these results were consistent with those reported in previous studies [14]. This study also revealed that patients with low expression of MicroRNA-326 had a poor prognosis (HR = 2.37, 95% CI: 1.18~4.77). Results showed that MicroRNA-326 could be an important indicator for prognosis of patients with glioma, suggesting that it may be used as a biomarker for predicting occurrence, progression and prognosis of glioma.

This study showed that the risk of death for patients receiving total resection/subtotal resection (compared with partial resection) decreased significantly (HR = 0.55, 95% CI: 0.35~0.86), similar to the results in previous studies [15, 16]. The results revealed that total resection was preferred for patients with glioma. This study also showed that patients with high preoperative KPS score (≥ 70) had decreased risk of prognosis (HR = 0.63, 95% CI: 0.42~0.95), consistent with the previous studies [17]; it is possibly because KPS is an indictor reflecting the body's functional status, and the higher the KPS score, the better the physical condition of the patient, and the more able to withstand a variety of side effects from treatment, to accept a comprehensive treatment. In this study, we also revealed that patients with high pathological grade (grade III~IV) had significantly increased risk of prognosis (HR = 2.51, 95% CI: 1.20~5.25), consistent with the results in previous studies [18, 19], possibly because glioma grew invasively, and the higher the pathological grading, the higher the malignant degree, and the stronger the tumor invasiveness, difficult to be completely removed without damages to the brain functions, and it is prone to recurrence, affecting the prognosis of patients.

In summary, patients with glioma have low MicroRNA-326 expression level in their brain tissues, and the higher the pathological grading of glioma, the lower the MicroRNA-326 expression level. Patients with low MicroRNA-326 expression level have a poor prognosis, which can be used as a biomarker for predicting the occurrence, progression and prognosis of glioma.

Disclosure of conflict of interest

None.

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