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Expression of microRNA-184 in glioma

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Abstract. The aim of the present study was to examine the expression of microRNA (miRNA)-184 in gliomas with different pathological grades, and its effect on survival prognosis. For the present study, 40 participants were selected with different pathological grades of glioma tissues with grade I (n=10), grade II (n=8), grade III (n=16), and grade IV (n=6). In addition, 10 cases of normal brain tissue (obtained by decompression because of traumatic brain injury) were selected. RT-PCR and immunohistochemical techniques were used to detect the expression level and intensity of miRNA-184 in different grades of glioma tissues. The length of survival of miRNA-184-positive patients was analyzed. miRNA-184 mRNA expression was found in normal tissues and tumor tissues, and the expression in tumor tissues was significant (P<0.05). Statistically significant differences of miRNA-184 expression were observed among different grades (P<0.05). miRNA-184 expression increased with the increase of grade level. The differences in expression across grade levels was statistically significant (P<0.05). A positive expression was not related to the pathological types of glioma cells. The median survival time of patients with miRNA-184-positive expression was significantly shorter than that of the negative expression group (P<0.05). miRNA-184 is highly expressed in gliomas, which is positively correlated with pathological grade, and is not correlated with pathological type, and negatively correlated with survival time. Thus, miRNA-184 is a potentially important molecular marker for glioma.

Introduction

Glioma originates from the neural epithelium, which is the most common primary malignant tumor in the brain, responsible for 40-65% of these tumors (1). The incidence of glioma is on the increase among youth in China (2). Pathological types of gliomas can be divided into 4 grades; the higher the grade, the higher the degree of malignancy (3). Glioma develops faster, according to the location, structure and tumor size. Additionally, it presents different clinical symptoms such as elevated intracranial pressure (headache, vomiting, papilloedema and consciousness disturbance) and focal symptoms and signs (movement disorders, sensory impairment and epilepsy) (4).

Surgery remains the main method of treatment in combination with various chemotherapeutic regimens. These treatment modalities extend survival time to some extent, but the quality of life remains unsatisfactory (5). The high postoperative recurrence rate is the main cause of death in patients (6). Aggressive growth of tumors constitutes the underlying causes of poor prognosis (7). Cellular and molecular biological characteristics have shown that abnormal gene expression, which regulates tumor growth, proliferation, migration, differentiation and apoptosis, is an important factor in glioma (8). MicroRNA (miRNA) is involved in 90% of gene transcription and translation process, affecting the expression of protein, and activation of the cell signaling pathway (9). Previous findings showed that miRNA is an important tumor control factor (10). There are 18 types of miRNA expression upregulation in gliomas, such as miRNA-9-2, miRNA-21, 13 types of miRNA expression downregulation, such as miRNA-128-1, and miRNA-181 (11). miRNA-184 is a newly identified miRNA abnormally expressed in many malignant tumors, such as liver cancer, lung cancer, and nasopharyngeal carcinoma (12). It appears to regulate the c-Myc and BCL2 signaling pathways, act as a cancer gene regulatory factor, or upregulate SND1 signal to promote tumor occurrence (13-15). Based on these prior finings, the present study analyzed the expression of miRNA-184 in different pathological grades of glioma, and the relationship with survival prognosis, to provide a reference for clinical diagnosis and treatment.

Materials and methods

Object data. Forty patients diagnosed as having glioma for the first time were selected from January 2013 to January 2016, of which 26 cases were male and 14 were female. The participants were 42-76 years of age, with an average age of

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(56.8±14.6) years. Grading of the gliomas as per WHO revealed 10 grade I cases, all of which were hair cell astrocytoma; 8 grade II cases, of which 2 cases of astrocytoma were of the original type, 4 cases were ependymoma, and 2 cases were diffuse astrocytoma; 16 grade III cases, of which 4 cases were oligodendroglial tumors, 4 cases were central neurocytoma, 2 cases were anaplastic cell tumor, 4 cases were anaplastic astrocytoma, 2 cases were anaplastic room tube membrane tumor; and 6 grade IV cases, including 4 cases of glioblastoma, and 2 cases of medulloblastoma. Ten cases of normal brain tissue were selected (obtained by decompression because of traumatic brain injury), including 6 males and 4 females aged 40-78 years with an average age of 55.7±13.5 years. Sex and age of the two groups were comparable. Exclusion criteria included a history of chemotherapy, brain trauma, meningitis, with underlying diseases such as serious heart, liver, lung, kidney and other organ dysfunctions, autoimmune diseases, infection diseases and other types of benign and malignant tumors.

The present study was approved by the Ethics Committee of Shandong Provincial Hospital (Shandong, China). Written and signed informed consent was obtained from the patients and their families.

Methods. RT-PCR and immunohistochemical methods were used to detect the expression level and intensity of miRNA-184 in different grades of glioma tissues. The length of survival of miRNA-184-positive expressed patients was followed up and analyzed.

RT-PCR. Conventional TRIzol reagent and miRNA extraction kit were used to extract total RNA as per manufacturer instructions. Determination of miRNA concentration was performed using a spectrophotometer (Hitachi, Tokyo, Japan) and completion was detected by agarose gel electrophoresis. The reverse transcriptase kit was used to produce cDNA, and design primers. The primers used were miR-184: forward, 5'-GCATGCCTAAATGTTGACAGCC-3' and reverse, 5'-CAC AUGUAUGAAUUGACAGCC-3'; reference U6: forward, 5'-GCGCGTCGTGAAGCGTTC-3' and reverse, 5'-GTGCAG GGTCCGAGGT-3'. The reaction system was cDNA 4 μl + SYBR Master Mix 10 μl + upstream and downstream primers each 0.2 μl, with 20 μl of water. The reaction conditions were 95˚C for 5 min, 95˚C for 15 sec, 60˚C for l min, a total of 35 cycles, stored at 4˚C. A standard curve and melting curve were constructed. The results were expressed by $2^{-\Delta\Delta Cq}$ method of cycle number.

Immunohistochemical method. Conventional paraffin sections were prepared. Xylene dewaxing and gradient ethanol hydration were performed. Antigen repair and close endogenous peroxidase were used. The section were incubated at room temperature for 20 min in 3% H_2O_2, and PBS was used for washing for 5 min x 3 times with oscillation. Closed non-specific binding was performed and normal goat serum was then added to the sections, in the wet box at room temperature, followed by incubation for 30 min. Primary antibody was added as follows: goat serum on the surface of the sections was discarded, the mouse anti-human miR-184 monoclonal antibody (cat. no. SY786520; Wuhan Sanying Co., Wuhan, China) was added at a dilution of 1:200. The sections were then incubated in the wet box at 4˚C overnight, and normal mice IgG replaced the primary antibody as the negative control. Then the goat anti-rabbit secondary antibody (dilution, 1:2,000; cat. no. ab6721; Abcam, Cambridge, MA, USA) was added as follows: the primary antibody on the surface of the section was discarded, PBS was used for washing for 5 min x 3 times with oscillation, the biotin-labeled secondary anti working liquid in the SP immunohistochemistry kit was added to the section, and they were incubated in the wet box at room temperature for 20 min. HRP streptavidin solution was added by discarding the secondary antibody on the surface of the section, and washing with PBS for 5 min x 3 times with oscillation, adding HRP streptavidin solution to the section, followed by incubation in the wet box at room temperature for 20 min, and washing with PBS for 5 min x 3 times with oscillation. DAB color rendering was then performed, and the sections were counterstained with hematoxylin, hydrochloric acid alcohol differentiation was conducted, ammonia back to blue, gradient ethanol dehydration, xylene transparent, and neutral gum mounting were applied. The sections were then dried at room temperature.

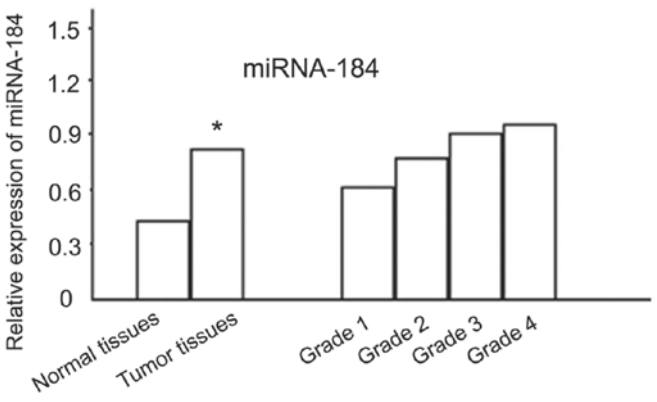

Figure 1. RT-PCR was used to detect the expression level of miRNA-184 mRNA. *P<0.05.

The results were shown according to the staining intensity and positive cell number: 0, no staining; 1-3, light to dark brown. Positive cell rate according to random selection of 5 visual fields was: 0, positive cells; 1, 25% positive cells, 2, 25-50% positive cells and 3, >50% positive cells. The score was the product of both, indicated as: 0, negative (-); 1-2, weak positive (+); 3-4, positive (+ +); and 5-9, strong positive (+ + +).

Statistical methods. IBM SPSS 20.0 (Armonk, NY, USA) software was used for statistical analysis. Measurement data were expressed as mean ± standard deviation. The comparison of two groups was tested by independent sample t-test and the multiple group comparison was analyzed by single-factor ANOVA analysis. Pairwise comparison was tested by LSD method. Countable data were expressed by cases or percentage. Inter-group comparison was tested by (correction) χ^2 or Fisher's exact probability and ranked data were tested by rank sum. Survival time was compared by Kaplan-Meier method with log-rank χ^2 test. P<0.05 was considered to indicate a statistically significant difference.

Table I. Comparison of microRNA-184-positive expression rate [cases (%)].

Group	No. of cases	Negative	Weak positive	Positive	Strong positive	Positive rate
Normal brain tissue	10	8 (80.0)	2 (20.0)	0	0	2 (20.0)
Grade I gliomas	10	6 (60.0)	2 (20.0)	1 (10.0)	1 (10.0)	4 (40.0)
Grade II	8	3 (37.5)	2 (25.0)	2 (25.0)	1 (12.5)	5 (62.5)
Grade III	16	3 (18.8)	4 (25.0)	6 (37.5)	3 (18.8)	13 (81.3)
Grade IV	6	1 (16.7)	1 (16.7)	1 (16.7)	3 (50.0)	5 (83.3)
χ^2		26.537				13.127
P-value		0.000				0.011

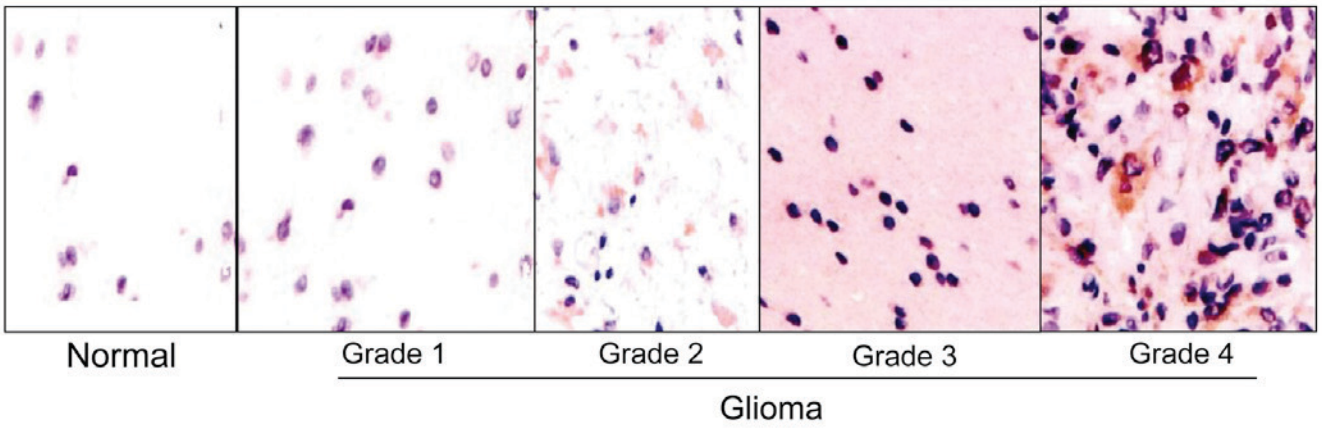

Figure 2. Immunohistochemical staining of microRNA-184-positive expression (magnification, x40, from left to right normal tissue, glioma grade I, II, III and IV).

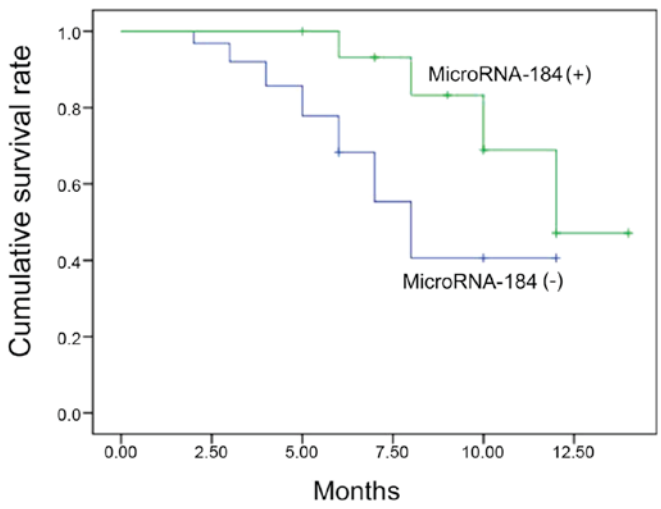

Figure 3. Kaplan-Meier analysis of survival time (green line, microRNA-184-positive expression group; purple line, microRNA-184-negative expression group).

Results

Quantitative comparison of miRNA-184 mRNA. miRNA-184 mRNA expression was found in normal tissues and tumor tissues, and the expression significantly increased in tumor tissues, and the difference was statistically significant (P<0.05). miRNA-184 mRNA expression in different grades was compared, and the difference was statistically significant (P<0.05) with a higher grade being associated with a higher expression level (Fig. 1).

Comparison of miRNA-184-positive expression rate. miRNA-184-positive expression rate increased with the increase of tumor grade; the higher the level, the higher the positive expression intensity. The differences were statistically significant (P<0.05). Positive expression was not associated with the type of glioma cells (Table I and Fig. 2).

Comparison of survival time. The median survival time of miRNA-184-positive expression was significantly shorter than that of the negative expression group [compare (8.0±0.4) with (12.0±0.1), χ^2=12.480, P<0.001] (Fig. 3).

Discussion

Although there are many miRNAs, the common molecular biological characteristics, including similar frame structure having no open reading frame can be found in all eukaryotic genomes, and code for proteins that are similar in length, approximately the size of 18-22 nT. They are small RNA molecules of a short sequence (16). miRNA is an important regulatory factor, involved in cell growth, proliferation, differentiation, metabolism and apoptosis, angiogenesis, cell invasion and metastasis, including stem cell regulation (17).

The present study demonstrates that miRNA-184 mRNA expression is expressed at a statistically significant greater level in glioma tissue than normal tissue. miRNA-184 mRNA

expression for each tumor grade also showed statistically significant differences with increasing expression as the grade increased. The present findings also show that miRNA-184 expression is upregulated in the occurrence or progression of glioma, which may promote the occurrence and development of gliomas. The miRNA-184-positive expression rate increased with the increase of the tumor grade and the higher the level of expression, the higher the positive expression intensity. Differences for each grade level were statistically significant. A positive expression is not related to the type of glioma cells. miRNA-184 does not express in neurons, and is only expressed in glial cells, and mainly expressed in plasma cells, but is not expressed in normal brain tissue. The presence of glioma indicates a positive expression of miR-184, and an increase of staining suggess a correlation with the degree of glioma pathological grades. An increase of pathological grade leads to stronger staining, which is not related to the type of glioma cells. Thus, miR-184 can be used as a marker for the pathological grade of gliomas. Furthermore, the follow up showed that the median survival time of miRNA-184-positive expression was significantly shorter than that of the negative expression group.

In conclusion, miRNA-184 is highly expressed in gliomas, which is positively correlated with pathological grade, but is not correlated with pathological type, and negatively correlated with survival time, and may be an important molecular marker. Future studies aim to examine the miRNA-184 modulation mechanisms of glioma cell proliferation, migration and apoptosis, such as cell signaling pathways and regulators and identify appropriate targets for intervention and provide clues for clinical diagnosis, treatment and prognostic evaluation.

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