Downregulation of serum microRNA-205 as a potential diagnostic and prognostic biomarker for human glioma

*Xiao Yue, MS,¹ FengMing Lan, MS,² Man Hu, MS,³ Qiang Pan, MS,⁴ Qiong Wang, PhD,¹ and JinHuan Wang, PhD¹

¹Tianjin Huanhu Hospital, Tianjin Neurosurgery Institute; ²Department of Radiation Oncology, Tianjin Hospital, Tianjin;

³Department of Radiation Oncology, Shandong Cancer Hospital, Shandong Academy of Medical Sciences, Jinan; and

⁴Department of Neurosurgery, Laiwu City People's Hospital (Affiliated Hospital of Taishan Medical College), Laiwu, Shandong Province, People's Republic of China

OBJECTIVE Circulating microRNAs (miRNAs) are a new class of highly promising cancer biomarkers. Malignant glioma is one of the most devastating and lethal forms of intrinsic CNS tumor. Here, the authors evaluated serum miRNA 205 (miR-205) levels in patients with glioma.

METHODS Sixty-four patients in whom glioma was diagnosed and 45 healthy controls were recruited between October 2011 and March 2012 and randomly assigned to the screening cohort or the validation cohort. Cohorts of patients with other brain tumors, including meningioma (n = 8), primary diffuse large B-cell lymphoma of the CNS (n = 6), and pituitary adenoma (n = 5), were investigated and compared. miR-205 extraction from serum was detected by real-time quantitative reverse-transcription polymerase chain reaction. The Kaplan-Meier method was applied to perform survival analysis, the risk factors were analyzed by using a Cox regression model, and the receiver operating characteristic working curve was used to analyze the value of miR-205 in the prognostic evaluation of the patients.

RESULTS The authors first demonstrated that serum miR-205 expression was significantly lower in patients with glioma than in healthy controls (p < 0.001). It is important to note that serum miR-205 expression demonstrated a stepwise decrease with ascending pathological grades. The serum miR-205 biomarker had high sensitivity, specificity, and accuracy in patients with glioma. Serum levels of miR-205 were identified as an individual diagnostic marker and were significantly lower in the glioma cohort than in the other brain tumor cohorts. Serum miR-205 levels were significantly increased in postoperative samples over those in the preoperative samples and were reduced again during glioblastoma recurrences. Statistical analysis revealed a significant correlation between low serum miR-205 expression and both ascending pathological grades (p = 0.002) and low Karnofsky Performance Scale scores (p = 0.01). Patients with glioma at an advanced pathological grade (Grade III or IV) and a higher miR-205 serum level showed longer overall survival than those with a lower miR-205 serum concentration (p < 0.01). Furthermore, Cox regression analysis revealed that miR-205 serum levels were independently associated with overall survival.

CONCLUSIONS These data indicate that serum miR-205 expression is a novel and valuable biomarker for the diagnosis of glioma and a prognostic factor for those with a tumor at an advanced pathological grade.

<http://thejns.org/doi/abs/10.3171/2015.1.JNS141577>

KEY WORDS glioma miR-205 serum biomarker; oncology; diagnosis; progression

GLIOMA is the most common primary intracranial tumor in adults, accounting for more than 40% of primary CNS neoplasms. In accordance with WHO classification, which is based on histomorphologi-

cal criteria, gliomas are categorized as well-differentiated low-grade astrocytoma (WHO Grade I or II), anaplastic astrocytoma (WHO Grade III), or glioblastoma (GBM; WHO Grade IV).¹⁵ The most frequent and malignant glioma

ABBREVIATIONS GBM = glioblastoma; KPS = Karnofsky Performance Scale; miR-205 = microRNA 205; miRNA = microRNA; OS = overall survival; PCNSL = primary diffuse large B-cell lymphoma of the CNS; qRT-PCR = quantitative reverse-transcription polymerase chain reaction.

SUBMITTED July 9, 2014. **ACCEPTED** January 13, 2015.

INCLUDE WHEN CITING Published online July 31, 2015; DOI: 10.3171/2015.1.JNS141577.

DISCLOSURE The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper. The following grants funded this study: National Natural Scientific Foundation of China (Grant 81201973), College Foundation of Laiwu Hospital Affiliated to Taishan Medical College (Grant YYLX2013019), Tianjin Scientific and Technological Support Program (Grant 12ZCDZSY17700), and Tianjin Application Foundation and Cutting-edge Technology Research Program (Grant 14JCZDJC35600).

* Mr. Yue and Ms. Lan contributed equally to this work.

ma is GBM, a tumor associated with a dismal prognosis.¹⁶ Despite great progress in therapeutic technologies, such as surgery, radiotherapy, and chemotherapy, the median survival time remains a meager 15 months, with a 5-year survival rate for patients with GBM that is lower than 5%.²³ This poor prognosis for patients with GBM underscores the difficulties and challenges in effectively detecting and treating this lethal cancer.

Currently, histopathology of tumor specimens obtained during resection or stereotactic biopsy represents the gold standard for the typing and grading of glioma and relies largely on particular architectural similarities between tumor cells and nonneoplastic glial cells.¹⁹ Neuroimaging, in particular MRI, is instrumental for cancer staging and for follow-up after glioma therapy. Blood biomarkers are firmly established for screening and early diagnosis, monitoring therapeutic effects, and predicting recurrence in patients with cancer.²⁰ To date, no biomarkers in serum or plasma have been established for the routine clinical management of patients with glioma. Therefore, it is crucial to identify objective markers, particularly biomarkers that can be found noninvasively in serum or plasma, for glioma diagnosis.

MicroRNAs (miRNAs) are small, single-stranded, noncoding RNA molecules that modulate the expression of targeting genes at the posttranscriptional level, which leads to mRNA degradation or inhibition of translation.²⁴ miRNAs are involved in the regulation of various cell processes including development, apoptosis, proliferation, and differentiation.¹ Further investigations have confirmed that miRNAs can function as tumor suppressors or oncogenes and play essential roles in a wide variety of physiological and pathological processes in various cancers, including glioma.⁷ Accumulating findings have been revealing that miRNAs are notably stable in blood, and circulating miRNAs have the potential to be used as biomarkers for several malignancies.^{5,22} Based on our previous observations, microRNA 205 (miR-205) is significantly downregulated in glioma cell lines and tissue specimens.²⁶ Here, we investigated the diagnostic and prognostic potential of miR-205 in the serum of patients with glioma.

Methods

Patients and Clinical Samples

This study was approved by the Tianjin Huanhu Hospital, Tianjin Hospital, Shandong Cancer Hospital, and Laiwu City People's Hospital ethics committees. All participants signed an informed consent form for the use of their blood samples before recruitment. All specimens were handled and made anonymous according to ethical and legal standards.

Preoperative serum samples were collected from 64 patients with histologically proven glioma. Serum samples of patients with glioma ($n = 10$) were obtained before surgery and 2 weeks after surgery. Serum from 6 patients with recurrent GBM was collected and miR-205 expression levels were measured after each surgery and recurrence. As a control, serum was also collected from healthy volunteers ($n = 45$). No other type of cancer had been diagnosed in the volunteers previously, and they were matched to the patients by age, sex, and ethnicity. Cohorts of pa-

tients with other brain tumors, including meningioma ($n = 8$), primary diffuse large B-cell lymphoma of the CNS (PCNSL) ($n = 6$), and pituitary adenoma ($n = 5$), were investigated and compared. After being collected, samples were centrifuged at 1500g for 20 minutes at 4°C, and the supernatant fluids were then transferred into fresh collection tubes and stored at -80°C until further processing.

All the patients were followed up at regular intervals for up to 2 years. Survival time was calculated from the date of diagnosis to the date of death or last date of follow-up. Patients who died as a result of disease not directly related to glioma or as a result of unexpected events were excluded from this study.

RNA Isolation

Extraction of miRNA from serum was performed with miRNeasy RNA isolation kits (Qiagen) according to the manufacturer's protocol. The concentration and purity of all RNA samples were evaluated by their absorbance ratio at 260/280 nm using a NanoDrop 2000 spectrometer (Thermo Scientific). The isolated RNA was reverse transcribed and amplified using the TaqMan miRNA reverse-transcription kit (Applied Biosystems). The cycle parameters were defined as follows: 16°C for 30 minutes, 42°C for 30 minutes, and 85°C for 15 minutes and then stored at 4°C.

Real-Time Quantitative Reverse-Transcription Polymerase Chain Reaction

Real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was performed using TaqMan microRNA assays (Applied Biosystems) in an ABI PRISM 7900 real-time PCR system. The primers of human (has)-miR-205 and has-miR-16, used as an endogenous control,¹¹ were purchased from Applied Biosystems. Reaction mixtures were incubated at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. Signals were detected at the end of each cycle. The cycle threshold (Ct) values were calculated with SDS 2.4 software (Applied Biosystems). The relative level of miR-205 was calculated by using the $2^{-\Delta\Delta C_t}$ method. Each sample was run in triplicate for analysis.

Statistical Analysis

All computations were carried out using the software of SPSS version 13.0 for Windows (SPSS). Data are expressed as mean $\pm$ SD. ANOVA was used to determine the statistical differences among groups. Overall survival (OS) time was calculated from the date of the initial surgery to the time of death. Survival curves were made by using the Kaplan-Meier method, and the log-rank test was used to assess survival differences between the groups. Cox regression analysis was used to assess predictors related to survival. Any difference with a p value less than 0.05 was considered statistically significant.

Results

Clinical Characteristics of Patients

In total, 64 patients with glioma and 45 healthy volunteers were recruited into the present study between

October 2011 and March 2012. A total of 32 male and 32 female (1:1) patients with glioma were enrolled in this study. The median age at glioma diagnosis was 45 years (range 30–72 years). The median Karnofsky Performance Scale (KPS) score was 80 (range 70–100). The diagnosis in each patient was confirmed pathologically according to the 2007 WHO classification criteria; 7 had pilocytic astrocytoma (WHO Grade I), 9 had diffuse astrocytoma (WHO Grade II), 21 had anaplastic astrocytoma (WHO Grade III), and 27 had primary GBM (WHO Grade IV). None of the patients had received chemotherapy or radiotherapy before surgery. After surgery, 54 patients received radiotherapy combined with chemotherapy. Patients who received adjuvant therapies after surgery had similar treatment regimes. As summarized in Table 1, there was no significant difference between the patients with glioma and the controls in terms of sex, smoking status, alcohol consumption, and family history of brain tumors. The median follow-up time was 19.5 months (range 8.0–30.5 months). At the last follow-up, 34 of the 64 patients were dead, including 2 patients with a WHO Grade I tumor, 3 patients with a WHO Grade II tumor, 10 patients with a WHO Grade III tumor, and 19 patients with a WHO Grade IV tumor. The 1- and 2-year OS rates were 88.4% and 47.9%, respectively.

Serum miR-205 Expression Level as a Potential Diagnostic Biological Marker

Based on our previous observations that miR-205 was significantly downregulated in glioma cell lines and tissue specimens, we sought to test whether serum miR-205 expression can serve as a potential biomarker for glioma. First, the serum miR-205 expression levels in 12 patients with glioma and 12 healthy controls were analyzed by real-time qRT-PCR in the screening phase of the study. After normalization to miR-16 expression levels,¹¹ serum miR-205 expression levels in the patients with glioma

were significantly lower than those in the healthy volunteers ($p < 0.01$; Fig. 1).

To further evaluate the diagnostic potential of miR-205, we next examined serum miR-205 expression in 52 patients with glioma and in 33 healthy volunteers in the validation phase. We noted that serum miR-205 exhibited a relatively high expression level in healthy controls, whereas its expression was significantly downregulated in patients with glioma ($p < 0.01$). It is important to note that serum miR-205 expression demonstrated a stepwise decrease with ascending pathological grades ($p < 0.01$; Fig. 2). Serum miR-205 expression levels in high-grade (Grade III–IV) and low-grade (Grade I–II) gliomas were both significantly lower than that in healthy controls. There was also a significant difference in serum miR-205 expression between high-grade and low-grade glioma specimens ($p < 0.01$; Fig. 2).

Subsequently, we generated receiver operating characteristic (ROC) curves to assess the potential usefulness of serum miR-205 as a biomarker for the diagnosis of glioma that can be measured noninvasively. Our ROC analyses revealed that serum miR-205 levels were robust in discriminating patients with glioma from control subjects (area under the curve 0.935). Using a cutoff value of 0.16, the sensitivity, specificity, and positive and negative predictive values were 86.3%, 92.2%, 96.4%, and 65.8%, respectively, for identifying a patient with glioma. To evaluate the levels of serum miR-205 that are specifically correlated with glioma, other brain tumors were investigated and compared. We selected a healthy cohort ($n = 20$) and cohorts of patients with meningioma ($n = 8$), PCNSL ($n = 6$), pituitary adenoma ($n = 5$), and glioma ($n = 30$) to identify whether serum miR-205 can be used as an individual diagnostic marker. As shown in Fig. 3, serum miR-205 expression levels were significantly lower in the patients with glioma than those in the other cohorts.

Finally, we evaluated whether serum miR-25 levels re-

TABLE 1. Demographic and clinical features of the patients and healthy volunteers*

Demographic & Clinical Features	Healthy/No Glioma (n = 45)	WHO Grade I (n = 7)	WHO Grade II (n = 9)	WHO Grade III (n = 21)	WHO Grade IV (n = 27)	p Value
Age	45.2 ± 10.3	44.7 ± 12.6	46.8 ± 9.8	47.6 ± 11.9	47.4 ± 12.4	0.645
≤45 yrs	25	4	4	9	11	0.726
>45 yrs	20	3	5	12	16	
Sex						
Male	24	3	6	13	10	0.853
Female	21	4	3	8	17	
Smoking status						
Ever and current	23	2	4	10	12	0.647
Never	22	5	5	11	15	
Alcohol consumption						
Ever and current	20	4	4	11	13	0.786
Never	25	3	5	10	14	
Family history of brain tumor						
Yes	0	1	0	1	1	0.923
No	45	6	9	20	26	

* Values represent the mean ± SD or the number of patients.

FIG. 1. Serum miR-205 expression in patients with glioma ($n = 12$) and in healthy volunteers (HVs; $n = 12$) was analyzed by real-time qRT-PCR. The mean ($\pm$ SD) serum miR-205 expression level in patients with glioma (0.82 ± 0.13) was significantly ($p < 0.01$) lower than that in the healthy volunteers (0.22 ± 0.11). $**p < 0.01$.

flect tumor dynamics in the treatment course of patients with glioma. Serum miR-205 expression levels in a small set of 10 patients with glioma who underwent curative resection were measured before and after surgery. It was interesting to observe that serum miR-205 expression levels statistically significantly increased after surgery in the same subset of patients ($p < 0.01$; Fig. 4 upper). In addition, serum miR-205 expression levels in 6 patients with GBM were measured after surgery and after recurrence (Fig. 4 lower); a significant decrease in the serum miR-205 levels was found at recurrence in the patients with GBM. Therefore, serum miR-25 levels might be a reflection of tumor dynamics in the treatment course of patients with glioma.

Collectively, the data demonstrate that serum miR-205

FIG. 2. Validation of miR-205 expression levels in serum samples (52 patients with glioma and 33 healthy volunteers). Serum miR-205 expression levels were still significantly lower in patients with glioma than in healthy volunteers ($p < 0.01$), and they decreased stepwise with ascending pathological grades (WHO Grades I–II vs III–IV; $p < 0.01$). $**p < 0.01$.

FIG. 3. Serum miR-205 expression levels in patients with glioma were lower than those in healthy volunteers and in the meningioma, PCNSL, and pituitary adenoma cohorts. $**p < 0.01$.

expression maybe used as a diagnostic biomarker for patients with glioma.

Association of Serum miR-205 Downregulation With Clinicopathological Characteristics of Human Glioma

We then investigated the association of serum miR-205 expression with various clinicopathological characteristics of human glioma. Glioma serum specimens that expressed miR-205 at a level less than the median expression level (0.26) were assigned to the low-expression group (mean expression value 0.21; $n = 38$), and those samples with an expression level above the median value were assigned to the high-expression group (mean expression value 0.36; $n = 26$). A statistically significant correlation was observed between low serum miR-205 expression and advanced pathological grade ($p = 0.002$) and low KPS score ($p = 0.01$; Table 2). Patients with a glioma specimen in which serum miR-205 expression was decreased tended to have a higher pathological grade and lower KPS score. There was no significant association between serum miR-205 expression and other clinicopathological parameters, including sex or age at diagnosis ($p > 0.05$; Table 2).

Serum miR-205 Is a Prognostic Marker in Patients With Malignant Glioma

Furthermore, to investigate the prognostic value of serum miR-205 expression in the OS of patients with advanced-grade glioma, the detailed clinical information of patients with malignant glioma was reviewed. Forty-eight patients were categorized into 1 of 2 groups according to their serum miR-205 level (median relative miR-205 concentration 0.17). As determined by the log-rank test, the survival rate of the patients with low serum miR-205 expression was significantly lower than that of the patients with high serum miR-205 expression ($p < 0.01$; Fig. 5).

The Cox regression analyses revealed a significant as-

FIG. 4. Evaluation of whether serum miR-205 levels can reflect tumor dynamics. **Upper:** Serum miR-205 expression levels changed before and after surgery. miR-205 levels were significantly higher in postoperative samples than in preoperative samples ($p < 0.01$). **Lower:** Comparison of serum miR-205 levels in samples obtained postoperatively and those obtained after recurrence; a significant reduction in serum miR-205 levels was found at recurrence. ** $p < 0.01$.

sociation between the miR-205 serum level and OS in patients with glioma ($p < 0.001$; hazard ratio 3.2; 95% confidence interval [CI] 1.8–7.1; Table 3). With all data considered together, serum miR-205 expression was an independent prognostic indicator for the patients with malignant glioma.

Discussion

GBM, which is characterized mainly by aggressive and rapid infiltration against neighboring normal brain tissues, is among the most feared types of cancer. Its considerable danger to patients includes not only the risk of fatality but also direct repercussions on quality of life and cognitive function.²¹ It is unfortunate that most patients with newly diagnosed GBM present with locally advanced tumors, and common imaging techniques such as MRI, radiography, CT, and PET cannot detect the exact tumor boundaries; therefore, treatment cannot be performed effectively,

TABLE 2. Serum miR-205 expression in patients with glioma with different clinicopathological features

Clinicopathological Features	No. of Cases	miR-205 Expression Level		p Value
		High (no. [%])	Low (no. [%])	
WHO grade				
I	7	7 (100.0)	0 (0)	0.002
II	9	7 (77.8)	2 (22.2)	
III	21	8 (38.1)	13 (61.9)	
IV	27	4 (14.8)	23 (85.1)	
Age				
≤45 yrs	28	15 (53.6)	13 (46.4)	NS
>45 yrs	36	17 (47.2)	19 (52.8)	
Sex				
Male	32	16 (50.0)	16 (50.0)	NS
Female	32	17 (53.1)	15 (46.9)	
KPS score				
≤80	40	10 (25.0)	30 (75.0)	0.01
>80	24	16 (66.7)	8 (33.3)	

NS = not significant.

and the clinical outcome of patients remains poor.² Nowadays, biomarker screening is an emerging field for the diagnosis of various cancers, and the role is more important in patients with glioma, because quite a few of them can not obtain a pathologic diagnosis.

Biomarkers are related to tumor biology and provide a more accurate assessment in the prognosis of disease.¹⁸ Currently, only several markers for specific tumors have been identified, because discovering and testing tumor biomarkers are difficult. However, blood biomarkers can be measured noninvasively and would be easily testable because of the simplicity in obtaining samples, so they are strongly preferred, particularly in a substantial portion of patients for whom tumor biopsy is not available.⁸ More recently, miRNAs were identified as cancer biomarkers circulating in cell-free serum that can distinguish diseased individuals from healthy controls. Serum miRNAs are well protected from RNases and remain stable after being subjected to harsh conditions.²⁷ Many studies have demonstrated that miRNAs are released into the circulation and exist in markedly stable form.^{13,14} The first serum miRNA biomarker discovered was miR-21, which was found in high serum levels associated with increased relapse-free survival in patients with diffuse large B-cell lymphoma.¹² Accumulating representative reports are suggesting that circulating miRNAs in the blood of patients can act as novel biomarkers that can be obtained noninvasively for the detection or monitoring of various cancers and putative therapeutic targets.^{3,6} Indeed, their sensitivity and specificity in diseases and noninvasive collection from serum have encouraged the pursuit of research on miRNA biomarkers. Considerable study has been performed to detect circulating miRNAs; however, the circulating miRNAs in patients with glioma remain largely unknown. In our study, we obtained data indicating that the miR-205 level in serum is a diagnostic, prognostic, and predictive biomarker for glioma.

FIG. 5. Kaplan-Meier survival curves for patients with malignant glioma and high or low serum miR-205 expression.

miR-205 has been identified in many studies and is abnormally expressed in many cancers; however, it plays a converse role in different cancers.⁴ The function of miR-205 depends on the specific tumor context and targeted genes downstream. miR-205 acts as a tumor suppressor and is downregulated in glioma and breast and esophageal cancer via inhibiting proliferation and invasion, whereas it is identified as an upregulated oncogene in lung cancer, bladder cancer, ovarian cancer, and head and neck cancer cell lines through facilitating tumor initiation and proliferation.^{10,17,25,28} In previous observations, we found that miR-205 was significantly downregulated in glioma cell lines and tissue specimens and that ectopic expression of

miR-205 induces apoptosis and cell-cycle arrest and impairs cell viability, clonability, and invasive properties of glioma cells by directly targeting vascular endothelial growth factor A.²⁶ In addition, another group found that downregulation of miR-205 in tissues was associated with glioma progression.⁹ As shown in the studies discussed above, miR-205 is associated with the occurrence and development of cancers. However, the diagnostic function of miR-205 has not been reported. Here, we initially sought to test whether circulating miR-205 can serve as a biomarker for glioma.

In the present study, we found that serum miR-205 was more downregulated in human patients with glioma than in healthy controls. We also found that decreased serum miR-205 expression was significantly correlated with ascending pathological grades. It is important to note that serum miR-205 expression was associated with advanced tumor progression and lower KPS scores. In addition, Kaplan-Meier analysis revealed that patients with glioma with a low miR-205 level tended to have poorer OS. Finally, both univariate and multivariate Cox regression analyses revealed that miR-205 serum levels were independently associated with OS. Our data suggest that the level of serum miR-205 is not only a potential diagnostic biomarker but also a predictor of OS in human glioma.

Conclusions

We found that serum miR-205 is a novel and valuable biomarker for the diagnosis of glioma and an independent prognostic parameter for OS in advanced-grade cancers. This study also shows that detecting miRNA levels in serum is a promising area for identifying biomarkers and has great potential for clinical use in cancers. Certainly, further supporting evidence from larger independent studies is needed before clinical application.

References

1. Ameres SL, Zamore PD: Diversifying microRNA sequence and function. *Nat Rev Mol Cell Biol* **14**:475–488, 2013
2. Castro MG, Baker GJM, Lowenstein PR: Blocking immunosuppressive checkpoints for glioma therapy: the more the merrier! *Clin Cancer Res* **20**:5147–5149, 2014
3. Challagundla KB, Fanini F, Vannini I, Wise P, Murtadha M, Malinconico L, et al: microRNAs in the tumor microenvironment: solving the riddle for a better diagnostics. *Expert Rev Mol Diagn* **14**:565–574, 2014
4. Chao CH, Chang CC, Wu MJ, Ko HW, Wang D, Hung MC, et al: MicroRNA-205 signaling regulates mammary stem cell fate and tumorigenesis. *J Clin Invest* **124**:3093–3106, 2014
5. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al: Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* **18**:997–1006, 2008
6. Ellinger J, Muller SC: MicroRNAs: a novel non-invasive biomarker for patients with urological malignancies. *Curr Pharm Biotechnol* **15**:486–491, 2014
7. Esteller M: Non-coding RNAs in human disease. *Nat Rev Genet* **12**:861–874, 2011
8. Gruessner C, Gruessner A, Glaser K, AbuShahin N, Zhou Y, Laughren C, et al: Flutamide and biomarkers of risk for ovarian cancer: pre-clinical and clinical study. *Cancer Prev Res (Phila)* [epub ahead of print], 2014
9. Hou SX, Ding BJ, Li HZ, Wang L, Xia F, Du F, et al: Identifying

TABLE 3. Univariate and multivariate analyses of prognostic parameters in patients with glioma

Parameter	Univariate Analysis		Multivariate Analysis	
	p Value	HR (95% CI)	p Value	HR (95% CI)
Age (≥ 45 vs < 45 yrs)	0.85	2.3 (1.5–4.2)		
Sex (F vs M)	0.74	1.6 (0.5–3.6)		
KPS score (≥ 80 vs < 80)	0.11	7.6 (1.8–15.7)		
WHO grade (I–II vs III–IV)	< 0.001	8.7 (2.5–21.3)	< 0.001	4.4 (1.6–9.4)
Radiotherapy (yes vs no)	0.25	1.8 (0.7–4.2)		
Chemotherapy (yes vs no)	0.02	5.4 (2.8–11.3)	0.21	2.6 (1.3–5.8)
Serum miR-205 expression (high vs low)	< 0.001	6.7 (2.0–16.2)	< 0.001	3.2 (1.8–7.1)

HR = hazard ratio.

- fication of microRNA-205 as a potential prognostic indicator for human glioma. **J Clin Neurosci** **20**:933–937, 2013
10. Kalogirou C, Spahn M, Krebs M, Joniau S, Lerut E, Burger M, et al: MiR-205 is progressively down-regulated in lymph node metastasis but fails as a prognostic biomarker in high-risk prostate cancer. **Int J Mol Sci** **14**:21414–21434, 2013
 11. Komatsu S, Ichikawa D, Hirajima S, Kawaguchi T, Miyamae M, Okajima W, et al: Plasma microRNA profiles: identification of miR-25 as a novel diagnostic and monitoring biomarker in oesophageal squamous cell carcinoma. **Br J Cancer** **111**:1614–1624, 2014
 12. Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, et al: Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. **Br J Haematol** **141**:672–675, 2008
 13. Lee SJ, Seo JW, Chae YS, Kim JG, Kang BW, Kim WW, et al: Genetic polymorphism of miR-196a as a prognostic biomarker for early breast cancer. **Anticancer Res** **34**:2943–2949, 2014
 14. Li X, Shi Y, Yin Z, Xue X, Zhou B: An eight-miRNA signature as a potential biomarker for predicting survival in lung adenocarcinoma. **J Transl Med** **12**:159, 2014
 15. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al: The 2007 WHO classification of tumours of the central nervous system. **Acta Neuropathol** **114**:97–109, 2007
 16. Omuro A, DeAngelis LM: Glioblastoma and other malignant gliomas: a clinical review. **JAMA** **310**:1842–1850, 2013
 17. Orang AV, Safaralizadeh R, Hosseinpour Feizi MA, Somi MH: Diagnostic and prognostic value of miR-205 in colorectal cancer. **Asian Pac J Cancer Prev** **15**:4033–4037, 2014
 18. Reimers MS, Zeestraten EC, van Alphen TC, Dekker JW, Putter H, Saadatmand S, et al: Combined analysis of biomarkers of proliferation and apoptosis in colon cancer: an immunohistochemistry-based study using tissue microarray. **Int J Colorectal Dis** **29**:1043–1052, 2014
 19. Riemenschneider MJ, Jeuken JW, Wesseling P, Reifenberger G: Molecular diagnostics of gliomas: state of the art. **Acta Neuropathol** **120**:567–584, 2010
 20. Sawyers CL: The cancer biomarker problem. **Nature** **452**:548–552, 2008
 21. Sweeney KJ, Jarzabek MA, Dicker P, O'Brien DF, Callanan JJ, Byrne AT, et al: Validation of an imageable surgical resection animal model of Glioblastoma (GBM). **J Neurosci Methods** **233**:99–104, 2014
 22. Turchinovich A, Weiz L, Langhein A, Burwinkel B: Characterization of extracellular circulating microRNA. **Nucleic Acids Res** **39**:7223–7233, 2011
 23. Weller M, Pfister SM, Wick W, Hegi ME, Reifenberger G, Stupp R: Molecular neuro-oncology in clinical practice: a new horizon. **Lancet Oncol** **14**:e370–e379, 2013
 24. Yates LA, Norbury CJ, Gilbert RJ: The long and short of microRNA. **Cell** **153**:516–519, 2013
 25. Yin WZ, Li F, Zhang L, Ren XP, Zhang N, Wen JF: Down-regulation of microRNA-205 promotes gastric cancer cell proliferation. **Eur Rev Med Pharmacol Sci** **18**:1027–1032, 2014
 26. Yue X, Wang P, Xu J, Zhu Y, Sun G, Pang Q, et al: MicroRNA-205 functions as a tumor suppressor in human glioblastoma cells by targeting VEGF-A. **Oncol Rep** **27**:1200–1206, 2012
 27. Zhang Y, Zhong Q, Chen X, Fang J, Huang Z: Diagnostic value of microRNAs in discriminating malignant thyroid nodules from benign ones on fine-needle aspiration samples. **Tumour Biol** **35**:9343–9353, 2014
 28. Zhou J, Liu H, Chen Y, Wen J, Li L, Wu X: [Expression and significance of VEGF, miR-205 and target protein Ezrin and Lamin A/C in ovarian cancer.] **Zhong Nan Da Xue Xue Bao Yi Xue Ban** **39**:142–150, 2014 (Chinese)

Author Contributions

Conception and design: Yue. Acquisition of data: Lan, Hu. Analysis and interpretation of data: Pan. Drafting the article: Q Wang. Critically revising the article: JH Wang.

Correspondence

Xiao Yue, Department of Neurosurgery, Tianjin Huanhu Hospital, Tianjin Neurosurgery Institute, 122 Qixiangtai Rd., Tianjin 300060, People's Republic of China. email: yuexiaofm@163.com.