

Circulating miR-199a-3p in plasma and its potential diagnostic and prognostic value in glioma

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Abstract. – **OBJECTIVE:** The present study aimed to examine the possibility of using plasma miR-199a-3p as a biomarker for glioma.

PATIENTS AND METHODS: Plasma miR-199a-3p expression glioma patients and normal healthy controls were quantified by Quantitative reverse transcription PCR. Then, the associations of serum miR-199a-3p level with clinicopathological factors or survival of glioma patients were further evaluated. Receiver operating characteristics (ROC) and area under the ROC curve (AUC) were used to validate the diagnostic value of miR-199a-3p. Univariate and multivariate Cox regression analyses were finally performed to analyze the independent factors for overall survival.

RESULTS: The qRT-PCR results showed that the miR-199a-3p expression was significantly downregulated in glioma tissues compared with the adjacent non-tumor tissues ($p < 0.01$). Furthermore, plasma miR-199a-3p level was significantly lower in glioma patients when compared with healthy controls ($p < 0.01$). ROC curve analysis showed that plasma miR-199a-3p was a useful marker for discriminating cases from healthy controls, with an area under the ROC curve (AUC) of 0.8466 (95% confidence interval (CI) 0.772 to 0.9211, $p < 0.001$). Moreover, miR-199a-3p expression was associated with various clinicopathological parameters, including WHO grade ($p = 0.001$) and KPS score ($p = 0.008$). We found that glioma patients with low miR-199a-3p expression level had distinctly shorter overall survival than patients with high miR-199a-3p expression level ($p = 0.0067$). Univariate and multivariate analysis suggested that miR-199a-3p expression was an independent predictor of poor prognosis.

CONCLUSIONS: These findings indicated that the circulating miR-199a-3p could be used as a promising novel biomarker for the diagnosis and prognosis of glioma.

Key Words:

miR-199a-3p, Diagnosis, Prognosis, Biomarkers, Glioma.

Introduction

Glioma, the most prevalent type of adult primary brain tumor, is categorized into grades I, II, III, and IV in WHO classification by cytologic feature and degree of malignancy^{1,2}. Despite progress in surgical, radio-and chemotherapeutic approaches for the treatment of glioma, its prognosis remains poor^{3,4}. This occurs mainly due to the characteristic rapidly growth, diffuse invasion and unclear pathogenesis of this disease⁵. Therefore, the development of novel molecular markers is required to improve the prediction of the prognosis of patients and targeted therapeutic treatment strategies.

MicroRNAs (miRNAs) are a class of 18-25nt small non-coding RNA molecules, which play important roles in the post-regulation of transcription^{6,7}. MiRNAs regulated biological processes included cell proliferation, apoptosis, invasion, and differentiation^{8,9}. Accumulating evidence has demonstrated that miRNAs can either serve as a tumor suppressor or as an oncogene, depending on the genes they target^{10,11}. For instance, Li et al¹² found that over-expression of MiR-195 inhibited the proliferation of human cervical cancer cells by directly targeting cyclin D1. Liu et al¹³ showed that miR-146b-5p served as a tumor suppressor by targeting TRAF6 and the patients with higher level of miR-146b-5p had longer disease-free survival and overall survival. These experimental

data revealed the important roles of miRNAs in carcinogenesis.

Aberrant expression of miR-199a-3p has been found in many malignancies^{14,15}. However, very little is known about the expression and clinical significance of miR-199a-3p in human glioma till now. Therefore, in the current study, we focused on the diagnostic and prognostic value of plasma miR-199a-3p.

Patients and Methods

Patients and Clinical Samples

55 pairs of glioma tissues and adjacent normal tissues were collected from routine therapeutic surgery in Henan Provincial People's Hospital between 2009 and 2011. Serum specimens were from 166 glioma and 75 healthy controls at our department. None of the patients received chemotherapy and radiotherapy before initial surgical treatment. Plasma were collected and stored at -80°C freezer until RNA purification. Tumor staging was performed according to the TNM classification of malignant tumors. The clinical and pathological data of the glioma patients listed

in the study are displayed in Table I. The Ethics Committee of Henan Provincial People's Hospital approved this study. Written informed consent was obtained from all of the patients. All experiments were performed following relevant guidelines and regulations.

qRT-PCR for miRNA

The extraction of total RNA from sera was carried out using mirVana PARIS Kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. MiRNAs were isolated using the miRNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. qRT-PCR was performed using Hairpin-it miRNAs qPCR Quantitation Kit (catalog number QPM-010, Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The reaction condition was 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/elongation step at 60°C for 30 seconds. Results were normalized to U6 snRNA using the comparative threshold cycle (Ct) method. The primers used for the expression analysis were as follows: U6-forward, 5'-CTCGCTTCGGCAGCA-CA-3'; U6 - reverse, 5'-AACGCTTCACGAATTT-

Table I. Serum miR-199a-3p expressions and clinicopathological features in 166 glioma specimens

| Variable | Number of cases | Expression of miR-199a-3p | | |
|--------------------------|-----------------|---------------------------|------|---------|
| | | Low | High | p-value |
| Age (years) | | | | 0.446 |
| <45 | 94 | 44 | 50 | |
| ≥45 | 72 | 38 | 34 | |
| Gender | | | | 0.322 |
| Male | 55 | 24 | 31 | |
| Female | 111 | 58 | 53 | |
| Family history of cancer | | | | 0.259 |
| Yes | 45 | 19 | 26 | |
| No | 121 | 63 | 58 | |
| Tumor location | | | | 0.106 |
| Supratentorial | 51 | 30 | 21 | |
| Infratentorial | 115 | 52 | 63 | |
| Tumor size | | | | 0.837 |
| <5 cm | 60 | 29 | 31 | |
| ≥5 cm | 106 | 53 | 53 | |
| WHO grade | | | | 0.001 |
| Low, I-II | 73 | 21 | 52 | |
| High, III-IV | 93 | 61 | 32 | |
| KPS score | | | | 0.008 |
| <90 | 86 | 51 | 35 | |
| ≥90 | 80 | 31 | 49 | |
| Surgery | | | | 0.660 |
| GTR | 43 | 20 | 23 | |
| PR | 123 | 62 | 61 | |

Abbreviation: NS, difference between groups was not statistically significant.

GCGT-3'. miR-199-3p-forward, 5'-GCGGCGGA-CAGTAGTCTGCAC-3': miR-199-3p-reverse, 5'-ATCCAGTGCAGGGTCCGAGG-3'.

Statistical Analysis

Statistical analyses and graphs were generated using GraphPad Prism version 5.0 (La Jolla, CA, USA). Differences between groups were analyzed by the Student t-test or the X^2 test. Receivers operating characteristic (ROC) curves were derived to detect the diagnostic accuracy of miR-199-3p. Survival curves were plotted by the Kaplan-Meier method and the log-rank test evaluated the significance. Survival data were evaluated through univariate and multivariate Cox regression analysis. For all tests, p -values of < 0.05 were interpreted as statistically significant.

Results

Down-regulation of miR-199a-3p in Glioma

To study the role of miR-199a-3p in glioma, we examined the miR-199a-3p levels in 55 cases of glioma tissues and their matched adjacent non-tumor tissues. As shown in Figure 1, the expression levels of miR-199a-3p were markedly decreased in glioma tissues compared with the matched adjacent non-tumor tissues ($p < 0.01$).

The Expression of Plasma Circulating miR-199a-3p and its Predictive value for Glioma

Next, we used a stem-loop RT-PCR assay to detect the expression of miR-199a-3p in the plasma

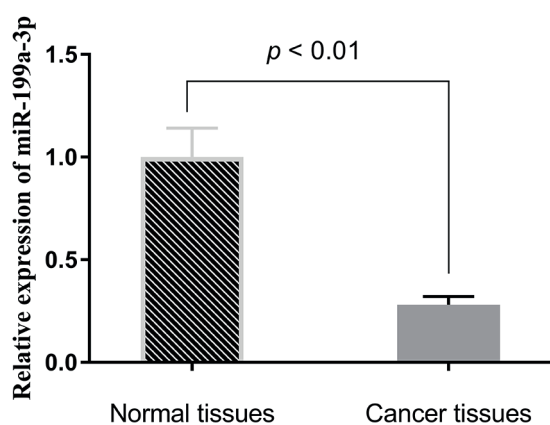


Figure 1. miR-199a-3p expression in 166 paired glioma and adjacent non-tumour tissues.

of glioma patients and healthy controls. We found that circulating miR-199a-3p was downregulated in glioma patients compared to healthy controls (Figure 2A, $p < 0.01$). Furthermore, the specificity and sensitivity of miR-199a-3p as a glioma diagnosis biomarker were calculated by receiver operating characteristic (ROC) curves. As shown in Figure 2B, the results showed plasma levels of miR-199a-3p discriminated glioma patients from healthy controls, with an AUC of 0.8466 [95 % confidence interval (CI) 0.772 to 0.9211; $p < 0.001$]. These findings provided evidence that plasma miR-199a-3p levels can be used to distinguish early stage glioma patients from controls.

miR-199a-3p Expression and Clinicopathologic Factors in Glioma

To explore the association between miR-199a-3p and clinicopathologic factors, the expression levels of miR-199a-3p in tumor tissues were categorized as low or high about the mean value. As shown in Table I, the results showed that miR-199a-3p expression was associated with various clinicopathological parameters, including WHO grade ($p = 0.001$) and KPS score ($p = 0.008$). No significant difference was observed between miR-199a-3p expression and patients' other factors.

Correlation between miR-199a-3p Expression and Prognosis.

Using Kaplan-Meier method and log-rank test, the overall survival of glioma patients with low miR-199-3p expression was significantly shorter than those with high miR-199-3p expression (Figure 3; $p < 0.0067$). Also, univariate analysis revealed that in WHO grade ($p = 0.014$), KPS score ($p = 0.012$) and miR-199a-3p expression ($p = 0.011$) were significantly related to overall survival of the patients (Table II). Then, the three parameters were subjected to the Cox regression analysis. The results confirmed miR-199a-3p expression as an independent prognostic factor for patients with glioma, while the other factors were excluded as independent prognostic predictors (All $p > 0.05$, respectively; Table II).

Discussion

Gliomas account for approximately 30% of all brain and central nervous system tumors and 80% of all malignant brain tumors¹⁶. Lack of diagnostic biomarkers for early detection has made glioma one of the human cancers with the worst

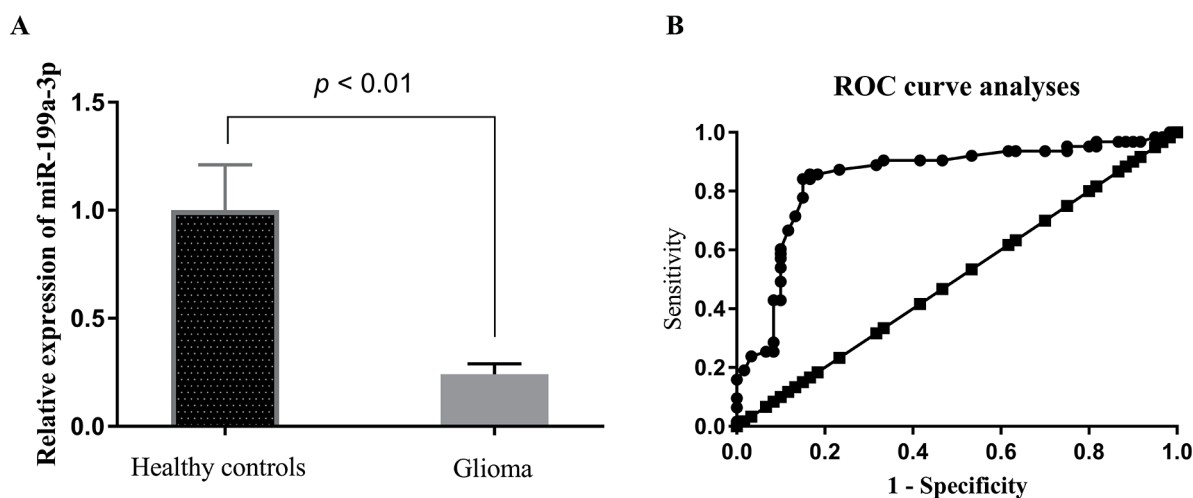


Figure 2. The expression of miR-199a-3p in the serum of glioma patients was significantly downregulated and may be a potential biomarker for glioma. A, miR-199a-3p expression levels in serum samples of healthy controls and glioma patients. B, Receiver operating characteristic (ROC) analysis was performed to determine the sensitivity and specificity of the miR-199a-3p expression level using area under the ROC curve (AUC) analysis.

prognosis. More and more articles confirmed the important role of miRNAs in the progression of tumors¹⁷. Therefore, circulating miRNAs are considered as potential diagnostic and prognostic biomarkers in the cancer detection. Recently, Zhuang et al¹⁸ found that miR-30e expression was significantly lower in HCC sera compared with sera from chronic liver disease patients, and demonstrated that miR-30e could serve as a diagnostic biomarker of hepatocellular carcinoma. Wang et al¹⁹ also provided convincing evidence for the potential application of miR-152 as a diagnostic and prognostic indicator in osteosarcoma. However, to our knowledge, the prognostic and diagnostic value of serum miR-199-3p has not been reported.

Reduced miR-199a-3p expression is a frequent molecular event in human malignancies. Many studies reported the effect of miR-199a-3p in different tumors. For instance, Tian et al²⁰ showed that miR-199a-3p inhibited osteosarcoma cells' migration and invasion through downregulation of phosphorylated AKT. Kinose et al²¹ found that miR-199a-3p inhibited ovarian cancer progression through the downregulation of c-Met expression. Han et al²² reported that miR-199a-3p suppressed proliferation, migration and invasion in colorectal cancer through directly targeting 3'-UTR of NLK gene. Further clinical research indicated that low expression of miR-199a-3p correlated with a shorter overall survival rate of patients with colorectal cancer. For glioma, recently, Shen et al²³ found that miR-199a-3p function as a tumor suppressor via the AKT/mTOR signaling pathway. All these

findings indicated that miR-199a-3p had a potential for application as a therapeutic target in glioma. The aim of the present study was to explore whether miR-199a-3p could be a potential noninvasive biomarker for the diagnosis and prognosis of human glioma.

In the present study, we confirmed that miR-199a-3p was frequently downregulated in glioma tissues. We also found that the levels of circulating miR-199a-3p were significantly decreased in the plasma of patients with glioma. Next, we found that the expression of miR-199a-3p in glioma tissues was significantly associated with the TNM stage and KPS score. These results revealed that miR-199a-3p might participate in tumor formation and development of glioma. ROC curve

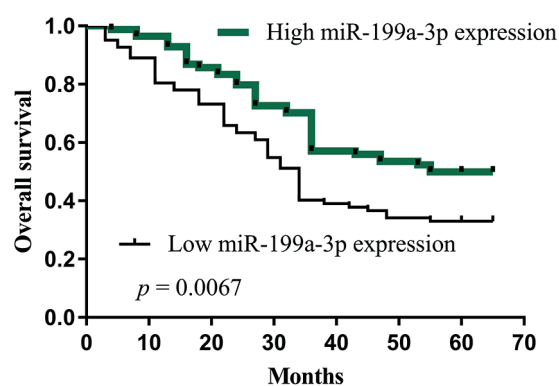


Figure 3. Kaplan-Meier analysis of the correlation between the serum miR-199a-3p expression and the overall survival of glioma patients.

Table II. Cox regression analysis of prognostic factors for overall survival in glioma patients (n = 166).

| | Univariate | | | Multivariate | | |
|--------------------------|------------|-------------|---------|--------------|---------------|---------|
| | HR | 95% CI | p-value | HR | 95% CI | p-value |
| miR-199a-3p | 4.338 | 1.562-7.793 | 0.011 | 3.552 | (1.239-6.193) | 0.005 |
| Age | 1.872 | 0.634-2.139 | 0.563 | | | |
| Gender | 1.438 | 0.603-1.945 | 0.358 | | | |
| Family history of cancer | 2.543 | 1.155-3.945 | 0.492 | | | |
| Tumor location | 3.238 | 1.437-5.332 | 0.351 | | | |
| Tumor size | 2.239 | 0.933-4.148 | 0.238 | | | |
| WHO grade | 2.437 | 1.158-4.623 | 0.014 | 2.044 | 0.893-5.573 | 0.006 |
| KPS score | 3.562 | 2.237-6.733 | 0.012 | 3.038 | 1.782-5.643 | 0.008 |
| Surgery | 2.211 | 1.041-3.893 | 0.255 | | | |

analysis showed that plasma miR-199a-3p was a useful marker for discriminating cases from healthy controls, with an area under the ROC curve (AUC) of 0.8466. Kaplan-Meier survival and log-rank test analysis showed that low expression of miR-199a-3p was associated with shorter overall survival. Moreover, multivariate Cox analysis proved that miR-199a-3p was an independent prognostic indicator for glioma patients.

Conclusions

Our work showed that miR-199a-3p may be a promising biomarker for the detection of glioma and its downregulation may be potentially associated with unfavorable prognosis of glioma. Further studies are warranting validating these results.

Conflicts of interest

The authors declare no conflicts of interest.

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