

Original Article

Serum microRNA-451a expression and its diagnostic value in glioma

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Abstract: Background: MicroRNAs (miRNA) have been confirmed to act as important diagnostic or prognostic markers in various cancers. The purpose of this study was to investigate the role of miR-451a in the diagnosis of glioma. Methods: The expression of miR-451a was detected by quantitative real-time polymerase chain reaction (qRT-PCR) analysis. And its relationship with clinicopathologic characteristics was also estimated. Besides, Receiver Operating Characteristic curve (ROC) was designed to evaluate the diagnostic value of *miR-451a* in glioma. Results: miR-451a was down-regulated in the serum of patients with glioma compared with healthy controls. And its expression was influenced by tumor size, WHO grade, KPS and lesionectomy. ROC curve proved miR-451a could be an independent diagnostic marker with an AUC of 0.816 combining with a sensitivity 81.4% of and specificity of 79.7%, respectively. Conclusion: miR-451a was decreased in patients with glioma and it might a potential diagnostic indicator in this cancer.

Keywords: Glioma, miR-451a, diagnosis

Introduction

Glioma is one of the malignant central nervous system neoplasms (CNS) which derives from astrocytic glial cells with a tendency to invade the surrounding brain tissue [1, 2]. Due to the high profile and infiltrative growth, glioma has a high recurrence and mortality rate which makes it an aggressive and malignant phenotypes [3]. However, it is also difficult to be found [4]. Therefore, it is of great importance to identify some accurate molecular markers for the early detection of glioma.

miRNAs are a class of small endogenous RNA molecule with 22-25 nucleotides long [5]. They are involved in many cell processes such as cell reprogramming, development, proliferation, differentiation, apoptosis, metabolism, cell-cycle control [6, 7]. Over 1900 human miRNAs have been determined and regulate more than 60% of the genes in mammals so far [8]. In the processes of gliomagenesis, tumor growth, proliferation, apoptosis and posttranscriptional regulation of anti-oncogenes, miRNA expression

profiling has been reported [9, 10]. miR-451a is located on human chromosome 17 and has been confirmed to be aberrant express in several cancers including lung cancer, esophageal cancer, nasopharyngeal cancer, melanoma, and hypopharyngeal squamous cell carcinoma [11-16]. Moreover, it was also reported to be down-regulated in glioma in previous studies [17, 18]. However, to our knowledge, the studies about the diagnostic value of miR-451a in glioma were never reported.

In our study, we measured the expression of miR-451a in the serum of glioma patients and healthy controls. And we further investigated the impact of miR-451a on the diagnosis of this cancer and expected to provide a new method for the early detection of glioma.

Materials and methods

Patients and serum samples

There were 118 patients diagnosed with glioma were collected from the First Affiliated Hospital

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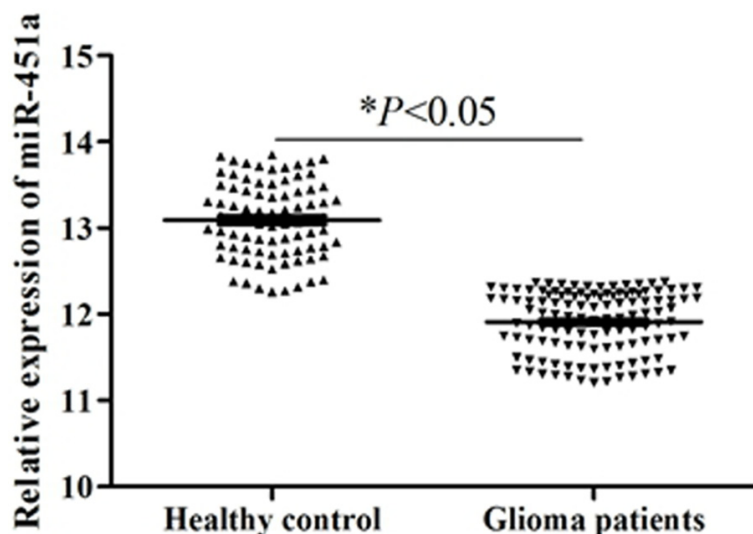


Figure 1. Expression of miR-451a in glioma patients and healthy controls. It was significantly higher in glioma patients than in healthy controls ($P<0.05$).

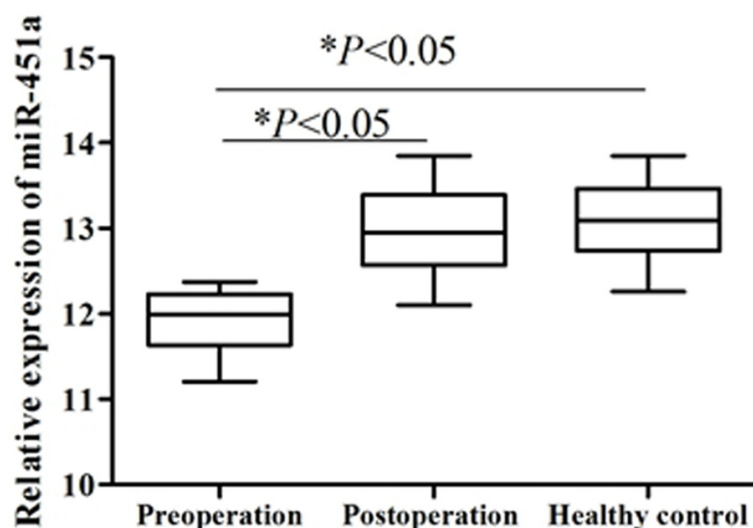


Figure 2. Comparison of miR-451a expression in preoperation and postoperation serum as well as in healthy control. MiR-451a expression was increased in postoperation serum compared that in preoperation serum of patients with glioma while it was lower than that in healthy controls ($P<0.05$).

of Xinxiang Medical University and the study was permitted by the Ethnic Committee of the hospital. 84 healthy people were selected as healthy controls. All patients had never received any chemical and physical therapies before sampling. Written informed consents were signed in advance.

Preoperative serum samples from 118 patients and serum from 84 healthy people were

obtained and put into blood collection tube of EDTA, respectively. Then the samples were stored at -80°C for RNA extraction. The expression of miR-451a was detected by quantitative real-time polymerase chain reaction (qRT-PCR). The clinicopathologic characteristics including age, gender, tumor size, WHO grade, KPS, lesionectomy and therapy methods were recorded in a databases in advance.

RNA extraction and QRT-PCR analysis

Total RNA was isolated from the serum of glioma patients and healthy controls using mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA). The first chain of cDNA was synthesized by reverse transcription with TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). RT-PCR reaction was performed in the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). RUN44 was taken as internal controls. The relative quantification of *miR-451a* expression was evaluated via the comparative cycle threshold (CT) method. Each sample was in triplicate.

Statistical analysis

All data were stated as Mean \pm SD. SPSS 13.0 software and GraphPad primes 5 were used to make statistical analysis and design relative figures. The differences between two groups were analyzed by Students' t test. The relationship between miR-451a expression and clinical features was evaluated through chi-square test. ROC curve was established to estimate the diagnostic value of miR-451a for distinguishing the glioma patients from healthy controls. The difference was considered to be significant when $P<0.05$.

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Table 1. Relationship between expression of miR-451a and clinicopathological characteristics

Clinicopathological characteristics	n	miR-451a expression		χ^2	P
		High	Low		
Age				2.758	0.097
≤50	60	26	34		
>50	58	34	24		
Sex				1.737	0.188
Female	47	20	27		
Male	71	40	31		
Tumor size				15.935	0.000
≤5 cm	53	16	37		
>5 cm	65	44	21		
WHO grade				26.434	0.000
I	27	7	20		
II	33	10	23		
III	33	22	11		
IV	25	21	4		
KPS				9.969	0.002
≤90	58	38	20		
>90	60	22	38		
Lesionectomy				9.978	0.002
Partial removal	49	34	15		
Total removal	69	26	43		
Therapy methods				2.281	0.131
Operation	57	34	23		
Conservative treatment	61	26	35		

Results

miR-451a was down-regulated in the serum of patients with glioma

QRT-PCR was used to measure the expression of miR-451a. As shown in **Figure 1**, miR-451a expression was lower in patients with glioma than that in healthy controls ($P<0.05$). This revealed that miR-451a was related to glioma and it might be act as a tumor suppressor.

miR-451a was increased in glioma patients after surgery

The expression level of miR-451a in the serum of preoperation and postoperation (7 days after tumor resection) in 72 patients detected with qRT-PCR analysis, too. The outcome revealed that serum *miR-451a* expression was significantly increased in postoperation serum compared with that in preoperation (**Figure 2**).

However, comparing with healthy controls, serum miR-451a expression level of post-operation was still lower (**Figure 2**).

The relationship between miR-451a expression and clinicopathologic characteristics

To explore whether miR-451a was related to the progress and development of glioma, we analyzed the relationship between it and clinicopathologic characteristics. Tumor size ($P<0.001$), WHO grade ($P<0.001$), KPS ($P=0.002$) and lesionectomy ($P=0.001$) were proved to be influential factors for the expression of miR-451a (**Table 1**). This outcome manifested that miR-451a was involved in the development of glioma.

The diagnostic value of miR-451a in glioma

ROC curve was established to estimate the diagnostic value of miR-451a. The result demonstrated that miR-451a could reliably distinguish patients with glioma from healthy normal people, with an AUC value of 0.816 corresponding with a sensitivity 81.4% of and specificity of 79.7%, respectively (**Figure 3**). And the optimal cut-off value was 11.977. The result indicated that the diagnostic value of miR-451a was high.

Discussion

The role of miR-451a was largely unknown in glioma. In current study, the expression of miR-451a and its role in the diagnosis of glioma were assessed. miR-451a expression was significantly lower in glioma patients' serum samples than that in the serum of healthy controls. The result suggested miR-451a could sever as a tumor suppressor and was vital for the early detection of glioma.

Glioma is divided into four grades including pilocytic astrocytoma (PA, WHO grade I), diffuse astrocytoma (DA, WHO grade II), anaplastic astrocytoma (AA, WHO grade III), and glioblastoma (GBM, WHO grade IV) by the World Health Organization (WHO) according to the increasing degree of malignancy [19, 20]. The diagnosis at

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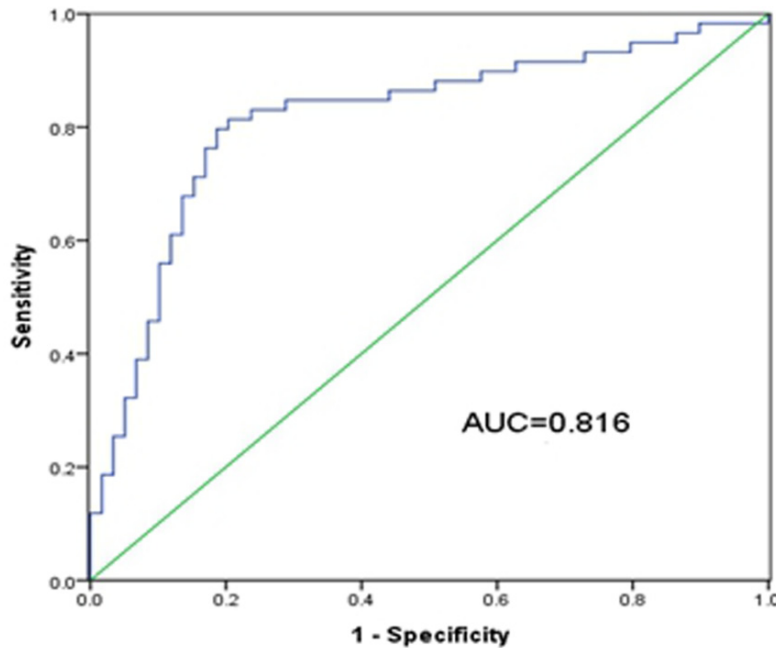


Figure 3. ROC curve for the analysis of diagnostic value of miR-451a in glioma. The AUC of 0.816, the high sensitivity and specificity revealed that miR-451a could distinguish glioma patients from healthy people.

early time is necessary for its cure when it is at low grade. Previous researches for circulating biomarkers of glioma include proteins, miRNAs and so on [21, 22]. However, the detection of proteins is complicated and expensive while miRNAs are easily to be detected with a low cost [23].

Recently, studies on miRNAs have shown significant changes of expression in malignant gliomas which make them act as biomarkers in the diagnosis or prognosis or both of them [22]. For instance, Qu et al. found the expression of miR-21 was up-regulated in serum of patients with higher grade malignant glioma compared with the control group which indicated that miR-21 might be a potential diagnostic biomarker in glioma [24]. miR-125b was confirmed to be low-expression and could be a potential diagnostic biomarker with relatively high accuracy in glioma [25]. In the study of Lai et al., the high expression of miR-210 was not only serve as a diagnostic but a potential prognostic indicator in glioma [26]. The down-regulation of miR-128 in the serum of glioma patients was proved to very sensitive and accurate in the early diagnosis of glioma according to the study of Sun et al. [27]. In this study, we found miR-451a showed a high diagnostic value with an AUC of 0.816.

However, its accuracy for predictive glioma still need further to be studied.

miRNA is considered to function as an oncogene when it is increased in cancer while it acts as a tumor suppressor due to its down-regulated. The cancer phenotype, the clinic pathological appearance and prognosis are influenced by these alterations of miRNA expression [22]. Therefore, the change of miRNAs expression could be special marker for the various processes of cancers. As a consequence, it has been used as biomarkers of various cancers. Although our study preliminary evaluate the diagnostic value of miR-451a, the detailed mechanism of this

effect and other function of miR-451a in glioma were not clear.

In conclusion, miR-451a is down-regulated in glioma and can be a potential promising diagnostic marker in the early detection of glioma. However, because the limitation of the sample scale and other unfavorable factors, further studies remain to be done in future.

Disclosure of conflict of interest

None.

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