Original Article
Decreased expression of miR-378 correlates with tumor invasiveness and poor prognosis of patients with glioma

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Abstract: microRNAs (miRNAs) are a class of small non-coding RNAs that play important roles in a variety of biological process. It has been reported that dysregulation of miRNA is always associated with cancer progression and development, and miR-378 aberrant expression has been found in some types of cancers. However, the association of miR-378 and glioma has not been evaluated. In this work, we measured the expression of miR-378 in glioma tissues and non-neoplastic brain tissues was measured using real-time PCR, and found that miRNA-378 expression level was significantly lower in glioma tissues compared with non-neoplastic brain tissues. Patients with lower miR-378 expression level had significantly poorer overall survival. Multivariate Cox regression analysis showed that miR-378 expression was an independent prognostic factor for 5-year overall survival. Over-expression of miR-378 inhibits glioma cell migration and invasion. In conclusion, our results indicated that miR-378 may serve as a tumor suppressor and play an important role in inhibiting tumor migration and invasion. Our work implicates the potential effect of miR-378 on the prognosis of glioma.

Keywords: miR-378, glioma, prognosis, cell migration and invasion

Introduction

Glioma is one of the most common malignant brain tumors and represents about 30% of all intrinsic neoplasms of the central nervous system. It have been reported that glioma has a poor prognosis because of the characteristic progressive overgrowth and diffuse invasion. The World Health Organization (WHO) classification divides glioma into four grades (I to IV), however, this criteria may not sufficient to estimate patient prognosis [1-3]. Therefore, investigating the pathogenesis and biological features of glioma is crucial to enhance early detection and treatment.

MicroRNAs (miRNAs) are a class of small non-coding RNAs with the size range of 21-25 nucleotides, and it plays important role at post-transcriptional regulation levels [4]. It can lead to mRNA degradation or inhibition of translation through imperfect hybridization to target 3’ untranslated regions (UTR). Many evidences suggest that miRNAs play important roles in various biological processes, including cell proliferation and differentiation [5, 6]. In addition, studies have de-monstrated that dysregulation of miRNA is al-ways associated with the tumorigenesis and progression of various types of tumors, and a number of miRNAs have been reported to regulate tumor metastasis [7, 8]. Many miRNAs may serve as markers for cancer diagnosis and prognosis.

Dysregulation of miRNA expression have been documented to play crucial role in tumorigene-sis and cancer progression [9]. Recently, the expression of miR-378 was shown to be des-regulated in several human cancers, such as colorectal cancer and oral carcinoma [10, 11]. However, the underlying molecular mechanism and clinical significance of miR-378 in glioma
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remains unclear. In this work, we explored the expression difference between glioma tissues and non-neoplastic brain tissues, and evaluated the clinical significance of miR-378 in glioma patients. Furthermore, we examined the effects of miR-378 on glioma cells migration and invasion, which implicates the potential effects of miR-378 on glioma prognosis.

**Materials and methods**

**Patients and tissue samples**

Surgical specimens of glioma tissues and adjacent non-neoplastic brain tissues were obtained from patients with a diagnosis of glioma who underwent surgery at the Wuxi Second People's Hospital (Jiangsu Province, China) between April 2006 and June 2012. All patients had complete five-year follow-up, and informed written consents were obtained from all pa-

**Table 1. Clinicopathological associations of miR-378 expression in glioma**

<table>
<thead>
<tr>
<th>miR-378 expression</th>
<th>Low (n = 50)</th>
<th>High (n = 50)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages (years)</td>
<td></td>
<td></td>
<td>0.18</td>
</tr>
<tr>
<td>&lt; 50</td>
<td>19</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>≥ 50</td>
<td>31</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.98</td>
</tr>
<tr>
<td>Male</td>
<td>28</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td>0.012</td>
</tr>
<tr>
<td>&lt; 5 cm</td>
<td>32</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>≥ 5 cm</td>
<td>18</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>KPS</td>
<td></td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td>&lt; 90</td>
<td>35</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>≥ 90</td>
<td>15</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>WHO grade</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>20</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>29</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** Relative expression levels of miR-378 in glioma tissues and cell lines. A. The relative expression of miR-378 in glioma tissues and adjacent non-neoplastic brain tissues. The bars represent the means of the relative expression of miR-378. B. The relative expression of miR-378 in glioma cell lines.

**Figure 2.** Kaplan-Meier survival curves of patients with glioma based on miR-378 expression status. Patients with low expression group have significantly poorer prognosis than those in high expression group.
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Table 2. Univariate and multivariate analyses of prognostic factors in glioma

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Risk 95% CI P-value</td>
<td>Risk 95% CI P-value</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>1.06 0.96-1.22 0.59</td>
<td>1.01 0.92-1.22 0.55</td>
</tr>
<tr>
<td>Gender</td>
<td>1.16 0.82-1.48 0.44</td>
<td>1.09 0.88-1.44 0.43</td>
</tr>
<tr>
<td>KPS</td>
<td>1.41 0.76-2.55 0.11</td>
<td>1.21 0.74-2.11 0.09</td>
</tr>
<tr>
<td>Tumor size</td>
<td>1.08 0.96-1.24 0.12</td>
<td>1.06 0.96-1.68 0.26</td>
</tr>
<tr>
<td>WHO grade</td>
<td>2.52 1.22-4.47 &lt; 0.001</td>
<td>1.78 1.11-3.68 0.001</td>
</tr>
<tr>
<td>miR-378</td>
<td>2.26 1.16-3.05 &lt; 0.001</td>
<td>1.68 1.14-5.96 0.001</td>
</tr>
</tbody>
</table>

In vitro migration and invasion assays

The cell migration and invasion abilities were assessed using transwell inserts with 8 µm pores (BD Biosciences, San Jose, CA, USA). For transwell migration assay, 2 × 10^5 cells were added into the top chamber lined with a noncoated membrane. For invasion assay, 2 × 10^5 cells were placed to each upper compartment of the chamber pre-coated with matrigel matrix. In both assays, cells were suspended in medium without growth or serum factors, and medium containing 10% fetal bovine serum was used as the chemoattractant in the lower chamber. After the incubation for 48 hours and stained with hematoxylin for 10 minutes, the non-invaded cells were removed from the top chambers. The number of cells on the lower surface of the membrane was counted under a microscope in five random fields.

Statistical analysis

The data presented in this work have been repeated at least three independent experiments. The differences between groups were measured using the nonparametric test (Mann-Whitney U test) and categorical data were evaluated using Chi-square test. Kaplan-Meier method was used to calculate the overall survival rate. The Cox proportional hazards model was employed for the multivariate analysis. All statistical analyses were performed using SPSS version 16.0 (SPSS, Chicago, IL, USA). All of the P values were two-sided, and the differences were considered to be statistically significant at P-value < 0.05.

Results

Expression of miR-378 in glioma tissues and cell lines

At first, we explored the expression levels of miR-378 in 100 pairs of glioma tissues and adjacent non-neoplastic brain tissues from 100 glioma patients by qRT-PCR. The result showed that miR-378 expression level was significantly lower in glioma tissues (2.3 ± 0.77) compared with adjacent non-neoplastic brain tissues (4.5

RNA extraction and RT-PCR

The total RNA was extracted using the TRIzol Reagent (Invitrogen, Carlsbad, USA) according to the manufacturer’s protocol. Quantitative RT-PCR was performed using All-in-OneTM miRNA quantitative RT-PCR Detection Kit (GeneCopoeia, Rockville, MD). The primer sequences of miR-378 and U6 were purchased from Applied Biosystems (ABI, Foster City, CA, USA). RT-PCR was performed using TaKaRa SYBR Green PCR Kit (TaKaRa), and measured in a LightCycler 480 system (Roche, Basel, Switzerland). Relative quantification of miR-378 expression was calculated by using the 2^-ΔΔCT method.

Cell culture and transfection of miRNA

Four human glioma cell lines (U87, U251, MT330 and SJ-G2) and the normal skin fibroblasts (HF) were obtained from the American Type Culture Collection and cultured in RPMI-1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum, 100 µ/ml penicillin and 100 mg/ml streptomycin, at 37°C in a humidified atmosphere containing 5% CO_2_. The pre-miR miRNA-378 (Pre-miR-378), pre-miR negative control (Pre-miR-nc) were purchased from Ribobio (China). A final concentration of 2 × 10^5 cells were seeded into each well of a 6-well plate and transfected for 48 hours using Lipofectamine 2000 Reagent (Invitrogen) according to the manufacturer’s protocol.

Patients. None of the patients had received radiotherapy before surgery excision. The tumor tissue and adjacent non-neoplastic brain tissues were divided by tissue laser microdissection. All tissue samples were immediately frozen in liquid nitrogen and stored at -80°C until further use.
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Further analysis of miR-378 expression in cell lines showed that miR-378 expressions in glioma cell lines (U87, U251, MT330 and SJ-G2) were significantly lower compared with normal skin fibroblast cell line (Figure 1B).

Clinical significance of miR-378 expression in glioma

To measure the association between miR-378 expression and clinicopathological characteristics, the 100 patients with glioma were divided into two groups according to the median value of miR-378 expression level, including high-expression group (n = 50) and low-expression group (n = 50), respectively. The correlation between miR-378 expression and pathological factors was evaluated (Table 1), and the result showed that low expression of miR-378 was significantly associated with tumor size (P = 0.012), KPS (P = 0.005) and WHO grade (P < 0.001). No significant differences about gender, age were found. In the Kaplan-Meier survival analysis, the patients with lower miR-378 expression level had a significantly poorer prognosis than those with high miR-378 expression level (P = 0.001, Figure 2). Univariate proportional hazard model showed that the WHO grade and miR-378 expression level were prognostic predictors. Those parameters which significantly associated with overall survive in the univariate analysis were further evaluated by multivariate analysis. The result showed that WHO grade (P = 0.001) and miR-378 expression level (P = 0.001, Table 2) were independent prognostic factors for overall survival.

In vitro effect of miR-378 on glioma cell migration and invasion

To investigate whether miR-378 regulates glioma cell migration and invasion, we performed in vitro glioma cell migration and invasion assays by transfecting pre-miR-378 or pre-miR-nc into U87 cell. The results showed that over-expression of miR-378 significantly inhibited glioma cell migration and invasion (Figure 3).

Discussion

Glioma is one of the most common tumors with a relative low survival rate. Prognostic factor detection in glioma is essential to predict pa...
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patients’ survival and determine optimal therapeutic strategies. Up to date, many efforts have been devoted to explore specific biological markers with prognosis in glioma [12]. As a novel biomarker, the potential rules of miRNAs in predicting prognosis has been extensively identified. Until now, nearly 1900 human miRNAs have been identified, which can regulate 60%~80% of the genes in humans [13]. Due to the crucial role of miRNA regulation at the post-transcription level, miRNAs are involved in many important biological functions, such as differentiation, proliferation. It has also been implemented in diverse pathological processes in cancers [14-16].

It has been reported that dysregulation of miR-378 performed an important function in diverse cancer types, such as oral carcinoma and colorectal cancer [10, 11, 17, 18]. However, there are still no studies on the association between miR-378 and glioma. In this work, we investigated the expression level of miR-378 in glioma and adjacent non-neoplastic brain tissues. Moreover, we determined whether miR-378 expression could predict the outcome of glioma patients. The results showed that the expression of miR-378 was extensively down-regulated in glioma tissues and cell lines. Further multivariate survival analysis showed that miR-378 might be involved in glioma and could be used as a potential prognostic biomarker for glioma. Subsequent work showed that overexpression of miR-378 could significantly inhibit cell migration and invasion, which indicates that miR-378 might function as a tumor suppressor in glioma.

In conclusion, our present work showed that miR-378 expression was decreased in glioma and is tightly associated with cancer cell migration and invasion. Our data implicated for the first time that miR-378 expression was an independent prognostic factor of glioma patients.

Disclosure of conflict of interest

None.

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References

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