EXPRESSION OF MIR-200A IN MENINGIOMA AND ITS RELATIONSHIP WITH ANGIOGENESIS

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ABSTRACT

Objective: *To investigate the expression of microrna-miR-200a (miR-200a) in meningioma and its relationship with angiogenesis.*

Methods: *55 cases of grade III malignant meningiomas diagnosed in our hospital from February 2017 to April 201 were selected for primary cell culture, and the vasculogenic mimicry (VM) model of meningioma cells was constructed. After successful modeling, the miR-200a low expression group (miR-200a - group) and miR-200a high expression group (miR-200a + group) were established by lentivirus transfection, and the negative control group miR-200a + was set up respectively, that is, with an NC group and a miR-200a + NC group. The expression levels of EMT related proteins (twist, E-cadherin), ve cadherin, Wnt and β-catenin, the number of penetrating cells, the ability of angiogenesis and the migration distance at 0 h, 6 h, 12 h and 24 h were compared.*

Results: *The expression levels of twist and VE-cadherin protein in the miR-200a - group were significantly higher than those measured in the miR-200a + group, and the expression level of E-cadherin was significantly lower than that measured in the miR-200a + group (P<0.05). There was no significant difference in the expression of twist, VE-cadherin and E-cadherin between the miR-200a - group and the miR-200a + group (P>0.05). At 12 h and 24 h, the migration distance of the miR-200a - group was significantly higher than that of the miR-200a + group. At 12 h and 24 h, the migration distance of the miR-200a + group was significantly lower than that of the miR-200a + NC group. There was no significant difference between the miR-200a + NC group and the miR-200a-nc group (P>0.05). The number of transmembrane cells in the miR-200a - group was significantly higher than that measured in the miR-200a + group. There was no significant difference between the miR-200a + group and the miR-200a + NC group (P>0.05). The ability of angiogenesis in the miR-200a - group was significantly stronger than that measured in the miR-200a + group. The expression of Wnt and β-Catenin protein in the miR-200a - group was significantly higher than that in the miR-200a + group (P<0.05). The expression levels of Wnt and β-catenin in miR-200a + group were significantly lower than those measured in the miR-200a + NC group (P<0.05). There was no significant difference in the expression of Wnt and β-catenin between the miR-200a + NC group and the miR-200a-nc group (P>0.05).*

Conclusion: *The decreased expression of miR-200a in meningiomas can promote the migration, invasion and angiogenesis of meningioma cells, which may be achieved by activating the Wnt / β-catenin signaling pathway.*

Keywords: *Mir-200a, meningioma, angiogenesis, VE-cadherin.*

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Introduction

Malignant meningioma has the characteristics of some benign tumors, can be repeated, can require repeated surgical resection, can lead to tumor gradually malignant change and can eventually progress to meningiosarcoma. The clinical treatment of malignant meningioma is mainly surgery accompanied by radiation therapy, but the prognosis of patients is still poor after normal clinical comprehensive treatment[1]. Some scholars have found that the median survival time of patients with malignant meningioma is 1.5 years, with a 5-year survival rate of over 65 percent. Others have found that the average survival time of patients with malignant meningioma is 1.48 years[2]. The main causes of death of patients with malignant meningioma are tumor recurrence and poor sensitivity to treatment.

Therefore, it is particularly important to explore a new treatment method for malignant meningioma[3]. The cerebro-pia vascular blood supply is one of the primary ways for meningiomas to obtain blood supply, and about 60% of meningiomas exist in it[4].

It has been reported that vascular endothelial growth factor (VEGF) is closely related to angiogenesis and peritumor edema, and it can be expressed in various levels of meningioma tissues[5]. Studies related to malignant meningioma have shown that the expression level of VEGF in tumor tissues is significantly higher than that observed in atypical meningioma. Clinical reports have shown that the classical Wnt signaling pathway is involved in the initiation and progression of glioma, colon cancer and many other tumors, and β-catenin plays a key role in this signaling pathway[6].

Studies have been conducted to explore the role of the classical Wnt signaling pathway activation in malignant tumors; however, no targeted therapy for the meningioma Wnt signaling pathway has been found yet. MicroRNA-200a (miR-200a), a member of the miR-200a family, is expressed at reduced levels in many tumors. Epithelial mesenchymal transformation (EMT) can be induced by epithelial mesenchymal transformation[7].

At present, no reported studies on the relationship between miR-200a and meningioma have been found. Hence, this study aims to explore the expression of miR-200a in meningioma tissues and the relationship between miR-200a and angiogenesis.

Materials and methods

Experimental materials

Fifty-five cases of WHO grade III malignant meningioma diagnosed in our hospital from February 2017 to April 201 were selected for primary cell culture, and an angiogenic mimicry (VM) model of meningioma cells was established.

Main reagents and instruments

Reagents

Fetal bovine serum was purchased from Zhejiang Tianhang Biological Technology Co., Ltd. DMEM high sugar medium was purchased from Zhejiang Lianshuo Biotechnology Co. Ltd. 0.5% trypsin was purchased from Emmett Technology Co., Ltd. DEPC water was purchased from Nanjing Saihongrui Biological Technology Co., Ltd. Monoclonal antibodies against Twist, VE-Ca, E-Ca and β-catenin were purchased from Icogene (Wuhan) Biotechnology Co., Ltd. Protein lysate was purchased from Sangon Bioengineering (Shanghai) Co., Ltd. GAPDH monoclonal antibody was purchased from Wuhan Bafel Biotechnology Service Co., Ltd. HRP-labeled anti-rabbit was purchased from Wuhan Aijibaike Biological Technology Co., Ltd. Protein pre-dyeing marker was purchased from China Keritai (Beijing) Biotechnology Co., Ltd. A protein immunoassay kit was purchased from Nanjing Senbeijia Biological Technology Co., Ltd.

Instruments

Low temperature refrigerators of -20°C and -80°C were purchased from Senxi Technology Co., Ltd. A low temperature liquid nitrogen tank was purchased from Guangzhou Beamate Instrument Co., Ltd. A super clean workbench was purchased from Jinan Bohang Scientific Instrument Co., Ltd. An enzyme label instrument was purchased from Beijing Keyue Huacheng Technology Co., Ltd.

An SDS vertical electrophoresis tank was purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. An electric thermostatic water bath box was purchased from Guangzhou Juneng Nano Biotechnology Co., Ltd. A horizontal shaker was purchased from Wuxi Naisi Biological Technology Co., Ltd. A desktop high speed centrifuge was purchased from Wuxi Leifsi Biological Experimental Equipment Co., Ltd. An inverted microscope was purchased from Dongguan Pibiao Experimental Equipment Technology Co., Ltd. An electronic analytical balance was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. A digital display, constant temperature magnetic stirrer was purchased from Suzhou Weidu Biotechnology Co., Ltd.

Grouping and methods

• After the successful modeling of angiogenesis mimicry (VM) model of meningioma cells, the low expression group of miR-200a (miR-200a- group) and the high expression group of miR-200a (miR-200a+ group) were established by lentivirus transfection. The miR-200a - NC group and miR-200a + NC groups were respectively set as negative control groups.

• EMT-related proteins (Twist, E-cadherin) and vascular endothelial cadherin in each group were detected by Western blotting. VE-cadherin, Wnt and β-catenin protein expression levels were measured.

• The cell migration distance of each group was detected via a scratch test at the 0 h, 6 h, 12 h and 24 h time points, respectively.

• Transwell chamber and immunohistochemistry methods were used to detect cell invasion and angiogenesis in each group.

Statistical methods

The levels of EMT-related protein and VE-cadherin protein expression were expressed by ($\bar{x}\pm s$). A t-test was used between the two groups as well as a multifactor analysis of variance. This institute analyzed its data using the SPSS23.0 software package, and a P-value of less than 0.05 is regarded as indicative of a statistically significant result.

Results

Comparison of the expression levels of EMT-related proteins and VE-cadherin in each group

The protein expression levels of Twist and VE-cadherina in the miR-200a-group were significantly higher than those in the miR-200a + group, and the expression level of E-cadherina was significantly lower than those measured in the miR-200a + group (P<0.05). The expression levels of Twist, VE-cadherina and E-cadherina in miR-200a- group were not significantly different from those in the miR-200a+ group (P>0.05). See Table 1 and Figure 1.

Group	Twist	VE-cadherina	E-cadherina
miR-200a -	13.99±1.55[a]	9.02±1.98[a]	1.99±0.05[a]
miR-200a - NC	10.05±1.01	10.55±1.56	9.97±1.55
miR-200a +	8.43±1.25	6.23±1.55	8.47±2.18
miR-200a + NC	10.36±1.02	10.69±1.66	10.15±1.20

Table 1: Comparison of the expression levels of EMT-related proteins and VE-cadherin in each group ($\bar{x}\pm s$).
Note: Compared with miR-200a+ group, [a]P<0.05.

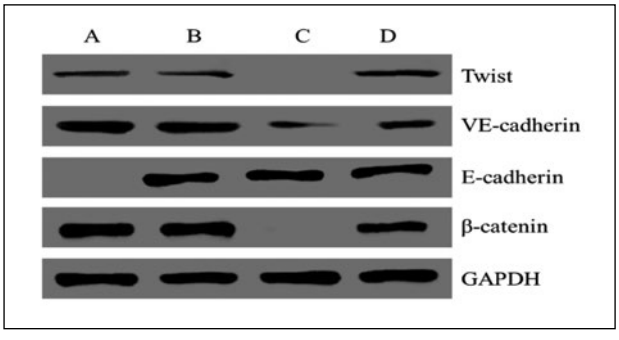

Figure 1: Comparison of the expression levels of EMT-associated proteins and VE-cadherin in each group.
Note: A: miR-200a - group; B: miR-200a - NC group; C: miR-200a + group; D: miR-200a + NC group.

Comparison of cell migration ability in each group

At 12 h and 24 h, the cell migration distance of the miR-200a - group was significantly higher than that measured in the miR-200a+ group (P<0.05). At 12 h and 24 h, the cell migration distance in the miR-200a + group was significantly lower than that measured in the miR-200a + NC group (P<0.05). There was no significant difference in migration distance between the miR-200a + NC group and the miR-200a - NC group (P>0.05). These results are shown in Table 2.

Group	0 h (mm)	6 h (mm)	12 h (mm)	24 h (mm)
miR-200a-	0.00	0.48±0.05	1.53±0.12[a]	3.37±0.28[a]
miR-200a - NC	0.00	0.21±0.01	0.85±0.05	1.28±0.11
miR-200a +	0.00	0.11±0.02	0.38±0.08[b]	0.58±0.05[b]
miR-200a + NC	0.00	0.16±0.08	0.84±0.07	1.52±0.15

Table 2: Comparison of cell migration ability in each group ($\bar{x}\pm s$).
Note: Compared with miR-200a + group, [a]P<0.05; Compared with miR-200a + NC group, [b]P<0.05.

Comparison of cell invasion ability of each group

The number of transmembrane cells in the miR-200a - group was significantly higher than that measured in the miR-200a + group (P<0.05). The number of transmembrane cells in the miR-200a + group was not statistically significantly different than that measured in the miR-200a + NC group (P>0.05). These results are given in Table 3.

Group	Number of transmembrane cells
miR-200a -	475.33±35.99[a]
miR-200a - NC	37.71±2.55
miR-200a +	45.18±4.19
miR-200a + NC	55.11±8.55

Table 3: Comparison of the number of membrane penetrating cells in each group ($\bar{x}\pm s$).
Note: Compared with miR-200a + group, [a]P<0.05

Comparison of angiogenesis ability of each group

The angiogenesis ability of the miR-200a - group was significantly higher than that of the miR-200a + group (P<0.05). There was no statistically significant difference in the angiogenesis ability between the miR-200a + group and the miR-200a + NC group (P>0.05). These results are shown in Table 4.

Group	VM (mm^2)
miR-200a -	13.28±2.29[a]
miR-200a - NC	7.33±1.55
miR-200a +	4.25±0.58
miR-200a + NC	6.67±1.23

Table 4: Comparison of angiogenic ability of each group ($\bar{x}\pm s$).
Note: Compared with miR-200a + group, [a]P<0.05.

Comparison of Wnt and β-catenin protein expression levels in each group

The protein expression levels of Wnt and β-catenin in the miR-200a - group were significantly higher than those measured in the miR-200a + group (P<0.05). The protein expression levels of Wnt and β-catenin in the miR-200a + group were significantly lower than those measured in the miR-200a + NC group (P<0.05).

The expression levels of Wnt and β-catenin in miR-200a + NC cells were not significantly different from those measured in the miR-200a - NC group (P>0.05). These results are provided in Table 5 and Figure 2.

Group	Wnt	β-catenin
miR-200a -	10.58±1.01[a]	9.53±1.62[a]
miR-200a - NC	11.22±1.89	10.05±1.55
miR-200a +	2.10±0.99[b]	1.92±0.98[b]
miR-200a + NC	10.55±1.43	10.11±1.42

Table 5: Comparison of Wnt and β-catenin protein expression levels in each group ($\bar{x}\pm s$).
Note: Compared with miR-200a+ group, [a]P<0.05; Compared with miR-200a+ NC group, [b]P<0.05

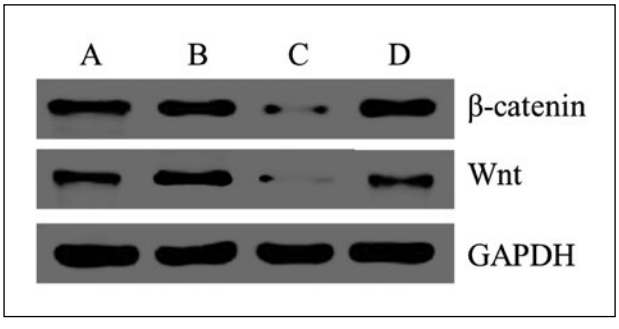

Figure 2: Comparison of Wnt and β-catenin protein expression levels in each group.
Note: A: miR-200a - group; B: miR-200a - NC group; C: miR-200a + group; D: miR-200a + NC group.

Discussion

Meningioma is a common intracranial tumor which mainly presents with symptoms such as headache, nausea and vomiting. It is characterized by rapid onset, high aggression and poor prognosis[8]. Relevant literature shows that the overall recurrence rate of patients with meningioma is about 80%, and the 5-year survival rate of patients is basically 0%[9]. Therefore, it is the focus of current research to explore new therapeutic methods to assist meningioma patients in improvings their condition.

With the continuous improvement of medical technology, molecular targeted therapy has become a new method for the treatment of malignant meningioma. Hence, more and more prognostic factors and molecular markers in the signal transduction pathway have been widely studied by clinical scholars. In particular, the function of miRNA in regulating tumor biology has received attention from clinical scholars[11]. The miR-200a expression legacy can lead to the progression of many tumors and is mainly involved in tumor cell proliferation and invasion[12]. VM is one of the primary factors in the regulation of angiogenesis of meningioma. Clinical studies have shown that it is the most widely used method in the treatment of malignant tumor patients before the release of the monoclonal antibody against VEGF-bevacizumab, and the clinical effect is relatively ideal[13].

Other reports have shown that VEGF plays an important role in inducing capillary tubular formation and endothelial cell migration in vitro and is a necessary condition for endothelioid vascular formation. Previous reports have shown that the findings of VM illustrate the drawbacks of traditional anti-tumor angiogenesis and provide a direction for the further research of clinical therapeutic drugs. In clinical practice, the application of anti-tumor methods combining VM and endothelial angiogenesis can achieve good results to a certain extent and, therefore, may become one of the primary targets for clinical drug development. The Wnt signaling pathway is involved in tumor initiation and progression. If it is not activated, β-catenin, axin, glycogen synthase kinase 3β, and colon adenomatous polyp genes in the cytoplasm bind, and then β-catenin S45 is phosphorylated by glycogen synthase kinase 3β and eventually degraded by ubiquitin. β-catenin is always at a low level in the cytoplasm[14]. When the Wnt signaling pathway is abnormally activated, the

prosynthase kinase 3β cannot degrade the β-catenin protein, which in turn triggers the aggregation of β-catenin in the cytoplasm and allows it to migrate to the nucleus, where it further binds to TCF4 to produce transcription complexes and finally initiates the transcription of downstream target genes in the pathway to regulate cell growth[15].

The results of this study showed that, first, the expression levels of EMT-related proteins and VE-cadherin in each group of cells were detected by Western blotting, and it was found that the expression levels of Twist and VE-cadherina in the miR-200a - group were significantly higher than those in the miR-200a + group. The expression level of E-cadherina in the miR-200a + group was significantly lower than that measured in the miR-200a + group ($P<0.05$). It is suggested that the down-regulation of miR-200a expression levels can affect the generation of VM. Next, we detected the cell migration distance, the number of transmembrane cells and the ability of VM formation in each group via scratch test,

Transwell chamber and immunohistochemistry methods. The results showed that at 12 h and 24 h, the cell migration distance in the miR-200a - group was significantly higher than that measured in the miR-200a + group ($P<0.05$). The number of transmembrane cells in the miR-200a - group was significantly higher than that measured in the miR-200a + group ($P<0.05$). The angiogenesis ability of the miR-200a - group was significantly higher than that measured in the miR-200a + group ($P<0.05$). These results suggest that the down-regulation of miR-200a expression can promote the migration, invasion and angiogenesis of meningioma cells. Finally, we found that the expression levels of Wnt and β-catenin in the miR-200a - group were significantly higher than those measured in the miR-200a + group ($P<0.05$). These results suggest that the down-regulation of miR-200a can activate the Wnt/β-catenin signaling pathway and lead to the EMT process.

The decreased expression of miR-200a in meningioma tissues can promote the migration, invasion and angiogenesis of meningioma cells, and the mechanism may be through the activation of the Wnt/β-catenin signaling pathway.

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