Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Effect of ERBB2 expression on invasiveness of glioma TJ905 cells

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ARTICLE INFO

Article history:
Received 10 September 2013
Received in revised form 15 October 2013
Accepted 15 November 2013
Available online 20 December 2013

Keywords:
Glioma
Stem cells
Invasiveness

ABSTRACT

Objective: To investigate the influence and possible mechanism of ERBB2 expression on the invasiveness of glioma cells. **Methods:** Glioma TJ905 cells were separated and cultured. ERBB2 shRNA and overexpressing vectors were constructed, which were then transfected. The ERBB2 expression was up-regulated or down-regulated. Changes of invasiveness of TJ905 cells were detected by Transwell assay, and the expressions of matrix metalloprotease (MMP)-2 and MMP-9 were measured by Western blot. **Results:** ERBB2 shRNA transfection vector could effectively inhibit expression of ERBB2; while ERBB2 overexpressing vector transfection could significantly improve the expression of ERBB2 in TJ905 cells. Transwell assay showed that when ERBB2 expression was down-regulated, the invasiveness of TJ905 cells was notably decreased; when ERBB2 expression was up-regulated, the invasiveness of TJ905 cells was markedly increased. Meanwhile, Western blot indicated that down-regulating ERBB2 inhibited the expression of MMP-2 and MMP-9, while up-regulating ERBB2 enhanced their expressions. **Conclusions:** ERBB2 expression is closely related to the invasiveness of glioma TJ905 cells.

1. Introduction

Glioma is a multi-gene abnormality featured with over-proliferation of cancer cells which is caused by the activation and overexpression of cancer genes or the mutation and deficiency of cancer suppressor genes[1,2]. Recent researches have revealed that RBB2 is over-expressed in many cancers and closely related to the proliferation and invasion of cancer[3-5]. Our previous research also indicated that ERBB2 is over-expressed and is positively related to the grade of glioma. However, there is no report about the function of ERBB2 in glioma home and abroad. In this study, we will explore the mechanism by up- and down- regulating the ERBB2 expression.

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Foundation project: It is supported by project of Ministry of Health of Shaanxi Province (No.: 2012D20).

2. Materials and methods

2.1. Materials

Glioma TJ905 cell lines were purchased from the Cell Bank of Xi'an Jiaotong University. The transfection reagent Fugene HD was from Roche. MMP-2 (Catalog: sc-13595) first antibody and MMP-9 (Catalog: sc-58389) first antibody were from Santa Cruz Biotechnology. Transwell chamber was from Corning Costar. ERBB2 shRNA and ERBB2 overexpresion vectors were from ABI.

2.2. Cell culture and experimental groups

Glioma TJ905 cells were incubated in 5% CO_2 at 37 ℃, and cells grown at logarithmic growth period were separated and divided into different groups: ERBB2 blank control group, ERBB2 shRNA group and ERBB2 over-expression group.

2.3. Cell transfection

Glioma TJ905 cells (1×10^5/mL) were cultured in 12-hole culture plate. When cell density reached 70%-80%, the cells were mixed with vectors at the ratio of 3:1 using Fugene HD6. After incubatION for 15 min, the cells were put in the culture plate. Six hours later, 2/3 of the culture medium was changed and the cells were cultured for 24 h.

2.4. Detection of ERBB2 expression BY Western blot

After glioma TJ905 cells were transfected with ERBB2 and expression vectors were expressed for 24 h, the total protein was extracted; sample buffer was added in SDS-PAGE gel electrophoresis at 100 V for 1.5 h, which were then confined for 1 h with confining liquid. After washing, ERBB2 antibody (1:300) was added and it was incubated at 37 ℃ for 1.5 h. Then the mixture was washed for 3 times, each time for 5 min. The second antibody was incubated for 1.5 h and washed, then developed with hypersensitive chemiluminescent solution, and observed under gel transilluminator. GAPDH served as internal control.

2.5. Changes of cell invasiveness in Transwell assay

After glioma TJ905 cells were transfected with ERBB2 and expression vectors were expressed for 24 h, the cells were washed 3 times with serum-free medium, digested and suspended in medium containing 1% calf bovine serum. Then 1×10^4/mL cells were taken out and put in the upper chamber of the Transwell chamber paved with Matrigel gel. Medium containing 20% calf bovine serum was added the lower chamber. After 24 h incubation, the Transwell chamber was taken out, washed, fixed and dyed. When cells on the upper surface were wiped off with cotton balls, the chamber was observed under microscope (×200). The cell transit rate was calculated as follows:

Cell transit rate = number of transited cells/(number of transited cells + number of un-transited cells) × 100.

2.6. Expressions of MMP-2 and MMP-9 in Western blot

After glioma TJ905 cells were transfected with ERBB2 and expression vectors were expressed for 24 h, the total protein was extracted, sample buffer was added in SDS-PAGE gel electrophoresis at 100 V for 1.5 h, transferred at 300 mA for 1 h, and confined for 1 h. After the mixture was washed, MMP-2 and MMP-9 antibodies were added and incubated at 37 ℃ for 1.5 h, then the mixture was washed for 3 times, each time for 5 min. The second antibody was incubated for 1.5 h and washed, then developed with hypersensitive chemiluminescent solution, and observed with gel transilluminator. GAPDH served as internal control.

2.7. Statistical method

All results were analyzed with SPSS 11.0 software, and variance analysis was adopted. $P<0.05$ was regarded as statistically significant.

3. Results

3.1. ERBB2 expression before and after transfection

As shown in Figure 1, Western blot revealed that after ERBB2 over-expressing vectors were transfected, ERBB2 expression in TJ905 cells were significantly increased. On the other hand, After ERBB2 shRNA vectors were transfected, ERBB2 expression in TJ905 cells were notably decreased.

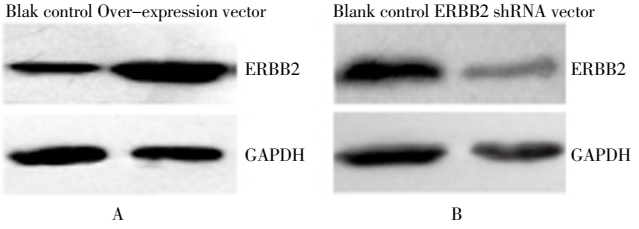

Figure 1. ERBB2 expression.

(A) ERBB2 expression before ERBB2 over-expressing vectors were transfected; (B) ERBB2 expression after ERBB2 shRNA vectors were transfected.

3.2. Effect of increased ERBB2 expression on invasiveness of glioma TJ905 cells

As indicated in Figure 2, when the ERBB2 over-expressing vectors were transfected for 48 h, the cell transit rate of TJ905 cells were notably increased compared to that of the blank control ($P<0.01$).

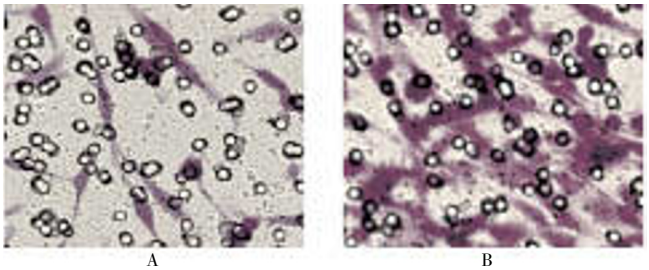

Figure 2. Influence of ERBB2 over-expressing vectors on the invasiveness of TJ905 cells.

(A) Blank control; (B) Over-expression vector.

3.3. Effect of decreased ERBB2 expression on the invasiveness of glioma TJ905 cells

After ERBB2 shRNA vectors were transfected for 48 h, the cell transit rate of TJ905 cells was remarkably reduced. As demonstrated in Figure 3, the cell transit rate of the transfected group was approximately 1/3 of that of the blank control, with statistical significance ($P<0.01$) (Figure 3).

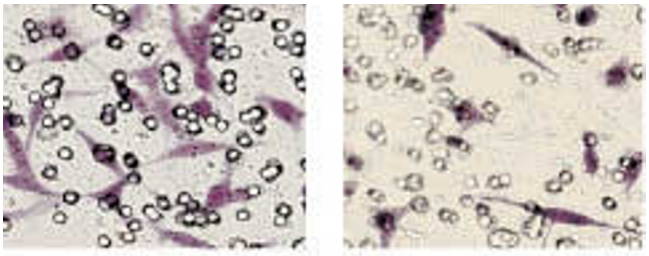

Figure 3. Influence of ERBB2 shRNA vector on the invasiveness of TJ905 cells.
(A) Blank control; (B) ERBB2 shRNA vector vector.

3.4. Effect of increased ERBB2 on MMP-2 and MMP-9 expressions

Western Blot showed that upregulated ERBB2 expression could distinctly increase the MMP-2 and MMP-9 expressions in TJ905 cells. Figure 4 showed after the ERBB2 over-expressing vectors were transfected for 48 h, the MMP-2 and MMP-9 expressions were significantly increased compared with those of the blank control ($P<0.01$).

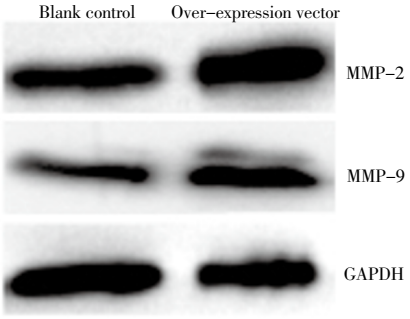

Figure 4. Influence of ERBB2 over-expressing vectors on the expressions of MMP-2 and MMP-9.

3.5. Effect of reduced ERBB2 expression on MMP-2 and MMP-9 expressions

As shown in Figure 5, after ERBB2 shRNA vectors were transfected for 48 h, Western blot indicted that MMP-2 and MMP-9 expressions were significantly reduced compared with those of the blank control ($P<0.01$).

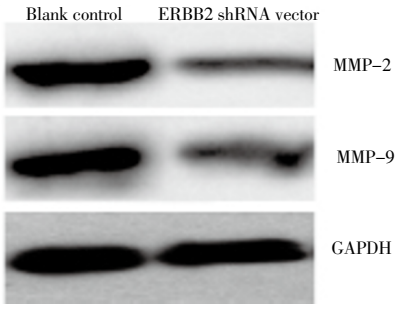

Figure 5. Influence of ERBB2 shRNA vectors on MMP-2 and MMP-9 expressions.

4. Discussion

Although distant metastasis seldom occurs in glioma cells, they have strong invasiveness. Moreover, the higher the malignancy of the glioma is, the stronger the invasion will be, which directly leads to the low rate of radical surgery, high rate of recurrence and poor prognosis of glioma[6-8]. Therefore, it's meaningful to target the invasion in treatment of glioma[9-12]. Previous researches have found that ERBB2, as an epidermal growth factor receptor, overexpresses in many malignant cancer tissues[13,14]. Latest findings show that ERBB2 is highly expressed in glioma. However, there is few reports about the influence of ERBB2 on the invasiveness of glioma home and abroad[15-18]. In this study, we explored the influence of ERBB2 on glioma TJ905 cells by upregulating and downregulating the ERBB2 expression, and investigated the correlation between the invasiveness of glioma TJ905 cells and MMP-2 and MMP-9.

Firstly we constructed upregulation and downregulation vectors of ERBB2, transfected glioma TJ905 cells, and observed the effect of ERBB2 expression on the invasiveness of TJ905 cells with Transwell blot. Our findings showed that, when ERBB2 expression was upregulated, the cell transit rate of TJ905 cells was significantly increased, indicating the cell invasiveness was enhanced; when ERBB2 expression was downregulated, the cell transit rate of TJ905 cells was remarkably decreased, showing the cell invasiveness was inhibited. This also verified that ERBB2 expression in TJ905 cells was closely related to the invasiveness of glioma.

It has been reported the mechanism of ERBB2 is that the proliferation and invasion of cancer cells is related to FAK signal pathway[19-21]. Activated ERBB2 could activate downstream FAK protein phosphorylation, whose Tyr-397 once combined with Src kinase, would further activate downstream MAPK pathway and boost cell proliferation and invasion. Our research showed that the invasiveness of glioma TJ905 cells regulated with ERBB2 was closely correlated to MMP-2 and MMP-9, which are principal members of matrix metalloproteinases (MMPs), and can mediate extracellular matrix degradation and facilitatie cancer cell invasion[22-24]. Highly-expressed MMP-2 and

MMP-9 in glioma could markedly destroy the intactness of basement membrane, furthering the invasion and metastasis of glioma, which led to the invasion of cancer[25]. Our research indicated that ERBB2 overexpression could remarkably upregulate the MMP-2 and MMP-9 expressions, while downregulating ERBB2 expression by ERBB2 shRNA vectors could notably inhibit MMP-2 and MMP-9 expressions. It indicates that invasiveness of TJ905 cells regulated by ERBB2 expression is correlated to the MMP-2 and MMP-9 expressions.

To conclude, we believe that ERBB2 expression is closely related to the invasiveness of glioma TJ905 cells, which may provide evidence for the gene therapy of glioma targeted at ERBB2.

Conflict of interest statement

We declare that we have no conflict of interest.

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