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Expression of TAG1/APP signaling pathway in the proliferation and differentiation of glioma stem cells

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Abstract. The aim of the present study was to examine the role of the expression of transient axonal glycoprotein-1 (TAG1)/precursor protein (APP) signaling pathway in the proliferation and differentiation of glioma stem cells. A glioma cell line (U373) was used as well as fluorescence quantitative PCR, western blot analysis, and enzyme-linked immunosorbent assay (ELISA), to examine the role of the expression of TAG1/APP signaling pathway in the proliferation and differentiation of glioma stem cells after five generations of *in vitro* culture. The results showed that compared to the normal glioma cells, the expression of TAG1 and APP was significantly increased in the proliferation of glioma stem cells. The results of ELISA and western blot analysis also confirmed a significant elevation in the protein expression of TAG1 in glioma stem cells compared to normal human glioma cells. When glioma stem cells were cultured in differentiation medium, as revealed by RT-PCR, the expression of TAG1 and APP in glioma stem cells initially increased and then decreased. In addition, the protein expression of TAG1 and APP was consistent with the RT-PCR results. Compared with undifferentiated glioma stem cells, the expression of TAG1 and APP decreased gradually with the extension of differentiation time. In conclusion, the expression of TAG1/APP signaling pathway in glioma cells was abnormal. Thus, this pathway is involved in the proliferation and differentiation of glioma cells and promotes the proliferation of glioma cells to inhibit the differentiation of glioma cells.

Introduction

Glioma has been confirmed as a lethal malignant tumor due to high mortalities caused by gliomas in recent years (1). Modrek *et al* showed that gliomas account for 29% of primary tumors of system diseases, which constitutes 80% of malignant tumors (2). The incidence of gliomas is approximately 52,400/100,000 individuals (3). Previous findings have shown that approximately 0.602% of Chinese individuals exhibit varying degrees of increased primary system diseases; thus, there is a high incidence of patients with gliomas in China (4,5). Gliomas are caused by the interaction between human genetic material and the external environment; however, the related genes causing brain glioma have yet to be identified. Therefore, glioma pathogenesis remains to be determined (6).

In recent years, advances in the research on glial stem cells, and the study of the pathogenesis of brain tumors by glial stem cells, have become imperative in the study of gliomas. Robinson *et al* showed that transient axonal glycoprotein-1 (TAG1) is important in the development of the central nervous system in the human body (7). Previous findings showed that the physiological function of TAG1 is mainly expressed in the human body as a cell adhesion molecule to guide the nerve cells in the adhesion, migration, and increase of axon growth (8). Huang *et al* found a correlation between TAG1 and glioma (9). Previous results have also shown that amyloid β precursor protein (APP) is an important, widely distributed protein in brain, and plays important roles in the promotion of nerve growth, regulation of neuronal migration and differentiation (10). In the present study, we investigated the role of the expression of TAG1/APP signaling pathway in the proliferation and differentiation of glioma stem cells to provide a reference for the study of the genetic mechanism and treatment of brain glioma.

Materials and methods

Chemicals, cell lines and reagents. In this study, U373 glioma cell lines with hepatocellular function were purchased from the American Type Culture Collection (Manassas, VA, USA). The main components of the serum-free medium included DMEM/F12 + Bfgf 20 ng/ml, EGF 20 ng/ml, and B27 0.2%. Cells were cultured at 37˚C with 5% CO_2. Differentiation medium comprised DMEM/F12 culture medium containing 10% fetal bovine serum, and cells were cultured at 37˚C with 5% CO_2. The TAG1/APP primary antibodies were purchased from Roche (Mannheim, Germany).

RT-PCR and RNA extraction. RNA extraction was operated in accordance with AXYGEN kit instructions (10). Briefly,

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500 ng RNA was collected and added to 2.0 ml 5X g DNA eraser Buffer, 1.0 μg DNA eraser, and RNase-free ddH_2O to supplement the whole system to 10 ml and the DNA was eliminated from RNA, followed by the addition of 5 ml of the above reaction liquid, 0.5 ml PrimeScript RT Enzyme mix, 2.0 ml 5X Prime Script Buffer and 2.0 ml RT Primer mix. RNase-free ddH_2O was added to supplement the whole system to 10.0 ml. Fluorescent quantitative PCR reaction system was as follows: 5.0 ml SYBR Premix Ex Taq TMII (2X), 0.3 ml PCR forward primer (10 mmol/l), 0.3 ml PCR reverse primer (10 mmol/l), 1.0 ml cDNA, and H_2O to supplement the whole system to 20 ml. The system was detected as previously described (11).

Fluorescent quantitative PCR. The fluorescence quantitative PCR kit used in the present study was purchased from Takara Biotechnology Co., Ltd. (Dalian, China). The experiment was carried out in triplicate. Specific steps were conducted with reference to the specification, resulting in disease amelioration (Table I).

Western blot analysis. Roche's animal cell protein extraction kit was used to extract the total protein in the sample (specific operation according to the specification) and the operation was optimized (12). Rabbit monoclonal TAG1 antibody (dilution, 1:500; cat. no. ab133498) and rabbit monoclonal APP antibody (dilution, 1:500; cat. no. ab180140) were purchased from Abcam (Cambridge, MA, USA).

ELISA detection. The double antibody sandwich method was used to detect the expression of *TAG1/APP* gene as previously described (11). Briefly, pH 9.0 PBS buffer was used to dilute the antibody protein, at a concentration of approximately 1-10 μg/ml. Then, 0.1 ml of the sample was added in the 96-well plate and the sample was treated at 4˚C overnight. The following day, the liquid in the well was discarded and the plate was washed with PBS five times for 2 min. Subsequently, 0.1 ml of the treated serum sample was added into the 96-well plate and incubated at 37˚C for 1 h. The plate was washed five times with PBS for 2 min. Of note, the blank well was used for the negative and positive controls. After washing, 0.1 ml of the secondary antibody was added to the 96-well plate and incubated at 37˚C for 0.5-1.2 h. After staining with red, the plate was washed five times with PBS for 2 min. After washing, 0.1 ml new configured chromogenic substrate, TMB, was added to the 96-well plate and incubated at 37˚C for 30 min, followed by the addition of 0.005 ml of 0.2 M sulfuric acid stop solution.

For qualitative detection, the 96-well plate above was placed on blank paper. By reading the color depth, a qualitative observation was conducted, i.e., a deeper color indicated a higher positive degree, suggesting higher TAG1/APP protein content. The negative control hole was colorless. The 96-well plate was arranged on the enzyme standard instrument for quantitative detection with 450 nm as the wavelength. The blank well was adjusted to zero. If the OD value was >1.2-fold of the negative control value, a positive state was confirmed (12).

Statistical analysis. SPSS 20.2 statistical software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis in the experiment. Measurement data were presented as mean ± standard deviation. Countable data were tested using the Chi-square test. $P<0.05$ was considered statistically significant.

Table I. Fluorescence quantitative PCR primer.

Gene	Primer sequence
TAG1	F: 5'-AGTCACACCTGTCCTCTAG-3'
	R: 5'-ATCTGCCTATGCCTTGGTTG-3'
APP	F: 5'-GTGGCTGAGGAGATTCAAG-3'
	R: 5'-AAAGAAGGCATGAGAGCATC-3'
GAPDH	F: 5'-TCATGGGTGTGAACCATGAGAA-3'
	R: 5'-GGCAGGACTGTGGTCATGAG-3'

Results

Gene expression of TAG1/APP signaling pathway in glioma stem cell proliferation. To explore the gene expression of TAG1/APP signaling pathway in glioma stem cell proliferation, ordinary glioma and glioma stem cells were collected to extract the RNA. *TAG1/APP* gene expression status was detected (Fig. 1). The expression of TAG1/APP increased significantly in glioma stem cells. Differences were of statistical significance (t1=-3.427, P=0.018; t2=-4.201, P=0.032).

Protein expression of TAG1/APP signaling pathway in glioma stem cell proliferation. The protein expression levels of TAG1/APP protein were studied using ELISA and western blotting, and the results are shown in Fig. 2. The expression of protein of TAG1/APP in glioma stem cells was significantly higher in comparison to the ordinary cells (t3=-3.49, P=0.021; t4=-9,782, P=0.012).

Gene expression of TAG1/APP in the differentiation of glioma stem cells. To further examine the gene expression of TAG1/APP signaling pathway and the differentiation of glioma stem cells, the protein of ordinary glioma and glioma stem cells cultured in differentiation medium was extracted. RNA expression levels of TAG1/APP were determined by RT-PCR. The results revealed a significant increase in the RNA expression levels of TAG1/APP in glioma stem cells (P1=0.003, P2=0.004) (Fig. 3).

Protein expression of TAG1/APP signaling pathway in the differentiation of glioma stem cells. The protein expression of TAG1/APP signaling pathway in glioma stem cell differentiation was examined following protein extraction in ordinary glioma and glioma stem cells cultured in differentiation medium, using ELISA and western blotting. The results shown in Fig. 4 suggest that, the protein expression of TAG1/APP was significantly higher in glioma stem cells (P1=0.002, P2=0.001).

Discussion

The recent increase in the incidence of cerebral gliomas results in the deepening of the research on brain glioma (13).

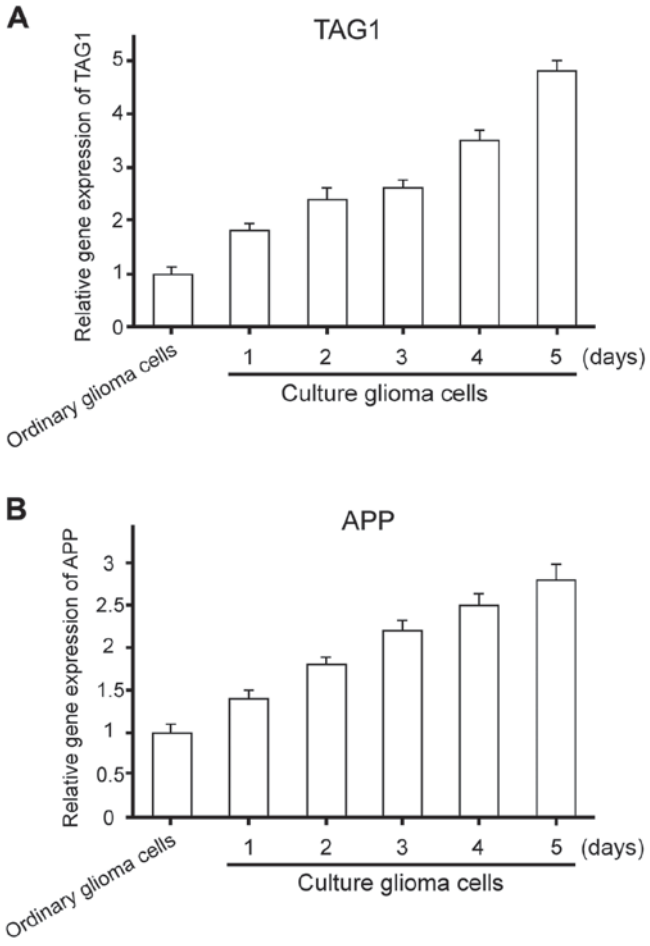

Figure 1. Gene expression of TAG1/APP signaling pathway and glioma stem cell proliferation.

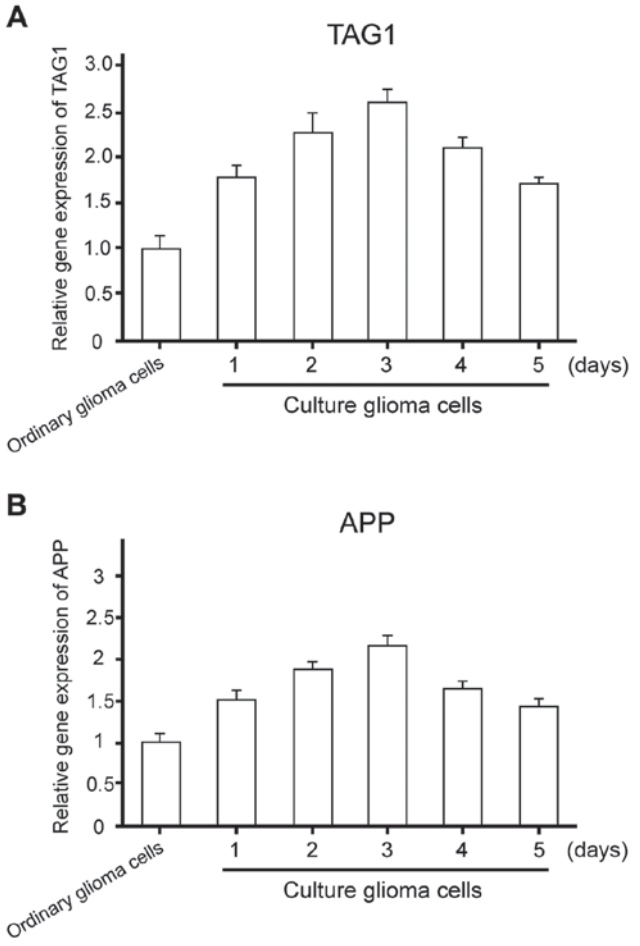

Figure 3. Gene expression of TAG1/APP in the differentiation of glioma stem cells.

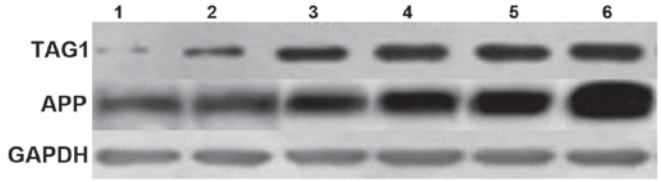

Figure 2. Protein expression of TAG1/APP signaling pathway and the glioma stem cell proliferation.

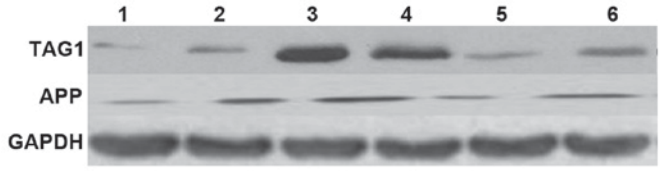

Figure 4. Expression of TAG1/APP protein in the signal and differentiation of glioma stem cells.

Researchers are focused on important genes, which are able to affect gliomas such as TAG1 and APP. However, research on the relevant signaling pathway in glioma is rarely reported (14). The present study has focused on this aspect of glioma research and has explored the expression profiles of TAG1/APP signaling pathway in the proliferation and differentiation of glioma stem cells. *TAG1* gene is crucial in the signal pathway and can promote axonal formation and remodeling (15). Furthermore, the interaction of TAG1 with glial cells plays an important role in the regulation of glial cell migration (16). We observed a significant increase in the expression profiles of TAG1 in the present study and the results are consistent with an earlier study by Liu *et al* (17).

The APP protein is another important factor that has signal transfer function (18,19). Results by Nagai *et al* (20) showed that the *APP* gene is also involved in brain and nervous system development and maturation. Chen *et al* suggested the association of this gene with many types of gliomas (21). We also observed a significant increase in its expression profiles, as observed by Mirzayans *et al* (22). Therefore, it can be inferred from the abovementioned studies and results that *TAG1/APP* gene may be involved in the development and maturation of the nervous system, to a certain extent. At present, there are few reports about the expression of TAG1/APP signal pathway that is abnormally expressed in glioma cells. In addition, the TAG1/APP signaling pathway involves glioma stem cell proliferation and differentiation, promotes glioma stem cell proliferation, and inhibits glioma differentiation, which provides certain theoretical and experimental basis to the subsequent diagnosis and treatment of glioma.

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