# CREB1 MODULATES GLUT1 EXPRESSION THROUGH PCREB1 AND AFFECTS GLUCOSE TRANSPORT IN GLIOMA CELLS

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***ABSTRACT***

***Objective****: This study investigates how cAMP-responsive element binding protein 1 (CREB1) regulates the glucose transporter protein (GLUT1) expression through pCREB1 and affects glucose transport in glioma cells.*

***Methods:*** *Human glioma cell line was cultured in vitro and the expression of CREB1 and GLUT1 in the human glioma cell line was detected using the immunohistochemical method. To analyse the expression of GLUT1 and glucose transport in glioma cells, the cells were divided into a normal cell group (without any treatment), negative transfer group (negative transfection control group) and siCREB1 group (down-regulated CREB1 expression). The expression of GLUT1 mRNA and protein and glucose transport in the three groups was detected through the real-time quantitative PCR assay and western blot method. Human glioma cell lines were treated with pCREB1 inhibitors or activators and divided into an inhibitor group and an activator group. Normal cells were used as a control and real-time quantitative PCR and western blot assays were used to detect GLUT1 mRNA and protein expression levels and glucose transporters in three groups of cells.*

***Results:*** *Compared with normal cell lines, both CREB1 and GLUT1 showed high expression in human glioma cell lines. Compared with normal cells and the negative transfection group, the expression of GLUT1 mRNA and protein in the siCREB1 group significantly decreased ($P<0.05$), as did the glucose absorption of the siCREB1 group ($P<0.05$). Compared with the normal cell group, expression of GLUT1 mRNA and protein in the inhibitor group markedly decreased ($P<0.05$), whereas in the activator group, it increased ($P<0.05$). Finally, compared with the normal cell group, the glucose absorption of the inhibitor group significantly decreased and that of the activator group remarkably increased ($P<0.05$).*

***Conclusion:*** *Both CREB1 and GLUT1 are highly expressed in human glioma cells. Phosphorylation of CREB1 can affect GLUT1 expression and glucose transport in cells. This suggests that the effect of CREB1 on glucose transport in glioma cells is related to the regulation of GLUT1 expression through pCREB1.*

***Keywords:*** *CREB1, pCREB1, GLUT1, glioma cells, glucose transport.*

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## Introduction

Glioma is a primary intracranial tumour of common origin in the neuroepithelium that accounts for up to 90% of brain tumours, with an annual incidence of 3 to 8 persons per 100,000 persons. Clinical manifestations include headaches, nausea and vomiting, and seizures. In addition, its effect on local brain tissue function can lead to visual loss, limb pain, and motor and sensory disorders(1). According to the malignant degree of tumour cells, it can be divided into low-grade glioma and high-grade glioma. With low-grade glioma, the prognosis of patients is relatively positive. High-grade glioma, however, is malignant and characterized by the excessive proliferation of tumour tissue and invasion of surrounding tissue. High-grade glioma features infiltrating growth, which is difficult to resect and insensitive to radiotherapy and chemotherapy. The survival rate is low, with most patients lasting only 9 to 12 months(2). Also, with the increase of carcinogenic factors, the incidence of glioma is increasing each

year. Currently, surgical resection supplemented by radiotherapy and chemotherapy is often used in the clinical treatment of glioma. However, because the tumour is metastasized at the time of diagnosis of glioma patients, the glioma cannot be completely removed by surgery, the side effects of radiotherapy and chemotherapy are relatively great, and clinical treatment is limited[3]. Therefore, it has become the focus of clinical scholars to find a safe and effective anti-gelatinoma method. cAMP-responsive element binding protein 1 (CREB1) belongs to the ATF/CREB family and regulates gene transcription[4].

Some scholars have found that CREB1 is closely related to tumour cell growth, proliferation and apoptosis, which may be due to its effort to obtain more glucose to meet its level of increase, thereby showing high expression in a variety of tumours[5]. Therefore, the purpose of this study is to analyse how CREB1 affects glucose transport in glioma cells.

## Materials and methods

### *Experimental reagents and instruments*

Human glioma cell lines were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. Dulbecco's modified Eagle's medium (DMEM) and foetal bovine serum were purchased from Gibco. Isopropanol, chloroform and agarose were purchased from China National Pharmaceutical Group. TRIzol reagent was purchased from TaKaRa, Japan. The PCR kit was purchased from TransGen Biotech Co., Ltd. The CREB1 and GLUT1 monoclonal antibodies were purchased from Abcam. The BCA protein concentration kit and RIPA lysate were purchased from Beyotime Biotechnology.

A crystal working table was purchased from Shanghai Yuantuo Purification Equipment Factory. An ultra-low temperature centrifuge was purchased from Eppendorf. The paraffin slicer was purchased from Thermo Fisher Scientific Co., Ltd. The $CO_2$ incubator was purchased from Sheldon Manufacturing. The confocal microscope was purchased from ZEISS. The enzyme labelling analyser was purchased from Bio-Rad Laboratories, Inc. The PCR was purchased from Gene Co., Ltd. Finally, the fluorescence image analyser was purchased from Fujifilm Holdings Corporation.

### *Cell culture*

After centrifugation, the cells were mixed with the 5 ml cell medium and transferred into the culture dish. The cells were then cultured at 37 °C with 5% $CO_2$, and the culture medium was replaced after adhering to the wall. Next, 1 ml of 0.05% trypsin solution was added and cultured at 37 °C for 2 min in a 5% $CO_2$ incubator for cell passage.

The cells in the logarithmic growth phase were digested by trypsin. After centrifugation, an appropriate amount of cell cryopreservation fluid was added to the precipitate and put into the cryopreservation tube to be stored in a -80 °C refrigerator or liquid nitrogen for long-term storage.

### *siRNA interference*

Before transfection, the cells were cultured in the DMEM without antibiotics. When the concentration reached 60% to 70%, RNA transfection was performed using Lipofectamine 2000 Reagent.

The cells were cultured at 37 °C with 5% $CO_2$ for 24 to 72 h. the transfection results were observed and the gene silencing was analysed. The untreated human glioma cells were used as the normal cell group, negative control cells as the negative transfection group and cells with siCREB1 transfection as the siCREB1 group.

### *Test methods*

A real-time quantitative PCR was used to detect the expression of genes. After the cells were taken out, trypsin and RNAiso Plus were added to fully homogenize the cells. After standing at room temperature, the cells were centrifuged, and the supernatant was placed in a new EP tube. Chloroform was added and was mixed and shook for 5 minutes, the cells were again centrifuged, and the supernatant was placed in a new EP tube. Then, isopropanol was added and the steps were repeated. Next, ethanol was added and, after centrifugation, the supernatant was discarded, leached and dissolved in DEPC water. The total RNA concentration of the cells was measured. The RNA was purified and reverse transcribed, and the PCR primers of the corresponding genes were designed by Primer5. PCR amplification was then carried out and photographed using a gel imaging system.

### *Western blot assay for protein expression*

The cells were washed using D-Hanks solution and the mixed lysate was added. The cells in each well were scraped with the cell brush. Finally, the scraped cells were moved into the 1.5 ml centrifuge tube with a pipette and placed on ice for 30 min. After centrifugation, the supernatant was taken and placed in a new centrifuge tube for further use.

Next, 45 μl of PBS buffer and 5 μl of extracted protein was added to the centrifuge tube for dilution of the total protein. Then, 20 μl of diluted protein was placed into the corresponding well of the enzyme label plate, and the AB mixture was added into each well. After 30 min, the total protein concentration was determined by an enzyme label instrument.

Finally, a series of operations, such as protein electrophoresis, membrane transfer, sealing and antibody incubating, were carried out, and the grey values of each band were scanned by Quantity One software for analysis.

*Glucose testing*

Human glucose enzyme-linked immunoassay was used to detect glucose concentration in cells.

***Statistical methods***

The measurement data in this study were expressed as ($\bar{x}\pm s$). A repeated measurement variance analysis was used to compare multi-group data, and a t-test was used to compare data between the two groups. P<0.05 indicated having a statistical difference. All the data of this study were analysed by the SPSS21.0 software package.

## Results

***Expression of CREB1 and GLUT1 in human glioma cell lines***

Immunohistochemical results showed that compared with normal cell lines, both CREB1 and GLUT1 showed high expression in human glioma cell lines. The results are shown in Figure 1.

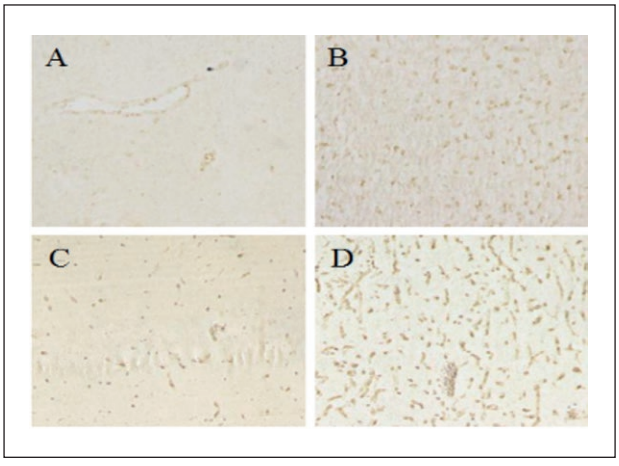


**Figure 1:** Expression of CREB1 and GLUT1 in human glioma cell lines. A: CREB1 expression in normal cell lines; B: CREB1 expression in human glioma cell line; C: GLUT1 expression in normal cell lines; D: GLUT1 expression in human glioma cell line.

***Effect of down-regulation of CREB1 expression on GLUT1 mRNA and protein***

A real-time quantitative PCR test showed that compared with normal cells and the negative transfection group, the expression of GLUT1 mRNA in the siCREB1 group significantly decreased (P<0.05).

A western blot assay showed that the expression of GLUT1 protein in the siCREB1 group markedly decreased compared with normal cells and the negative transfection group (P<0.05). The results are shown in Figure 2 and Table 1.

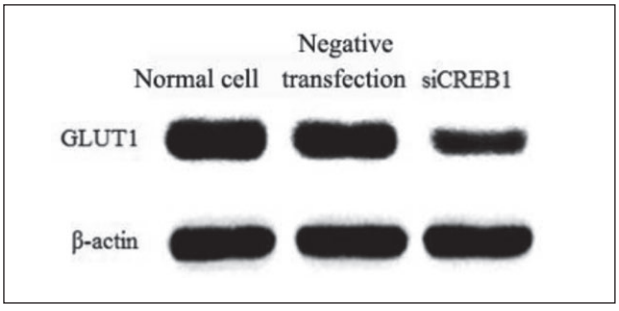


**Figure 2:** Effect of down-regulation of CREB1 expression on GLUT1 protein.

| Groups | Cases | GLUT1 mRNA | GLUT1 protein |
|---|---|---|---|
| Normal cell group | 5 | 1.01±0.01* | 1.02±0.03* |
| Negative transfection group | 5 | 0.85±0.06* | 0.82±0.05* |
| siCREB1 group | 5 | 0.42±0.03 | 0.53±0.02 |

**Table 1:** Effect of down-regulation of CREB1 expression on GLUT1 mRNA and protein.
*Notes: Compared with siCREB1 group, *P<0.05.*

***Effect of down-regulation of CREB1 expression on glucose transport in glioma cell lines***

After 48 h of transfection, the glucose absorption of the siCREB1 group decreased significantly compared with normal cells and the negative transfection group (P<0.05). The results are shown in Figure 3.

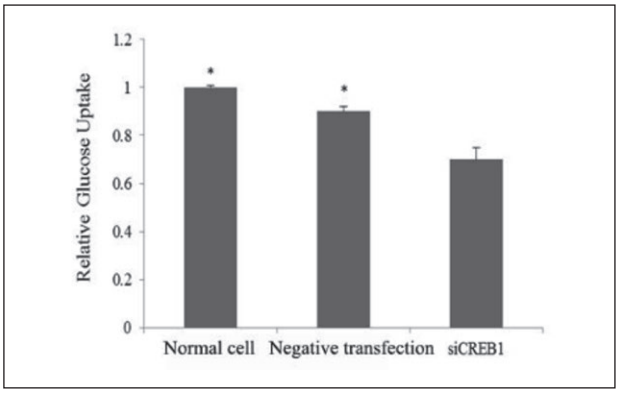


**Figure 3:** Effect of down-regulation of CREB1 expression on glucose transport in glioma cell lines.
*Notes: Compared with siCREB1 group, *P<0.05.*

***Effect of pCREB1 inhibitor or activator on GLUT1 mRNA and protein***

The results of a real-time quantitative PCR and the western blot method showed that compared with the normal cell group, expression of GLUT1 mRNA and protein in the inhibitor group markedly decreased (P<0.05), whereas in the activator group, it increased (P<0.05). The results are shown in Figure 4 and Table 2.

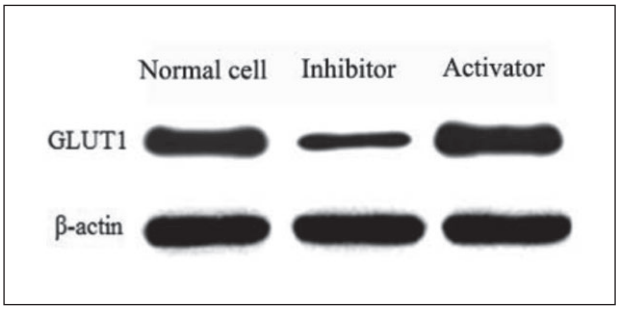


**Figure 4:** Effect of pCREB1 inhibitor or activator on GLUT1 protein.

| Groups | Cases | GLUT1 mRNA | GLUT1 protein |
|---|---|---|---|
| Normal cell group | 5 | 1.02±0.03 | 1.01±0.01 |
| Inhibitor group | 5 | 0.78±0.05* | 0.75±0.06* |
| Activator group | 5 | 1.26±0.08* | 1.20±0.09* |

**Table 2:** Effect of pCREB1 inhibitor or activator on GLUT1 mRNA and protein.
*Notes: Compared with normal cell group, *P<0.05.*

***Effect of pCREB1 inhibitor or activator on glucose transport in glioma cell lines***

Compared with the normal cell group, the glucose absorption of the inhibitor group significantly decreased, and that of the activator group remarkably increased (P<0.05). The results are shown in Figure 5.

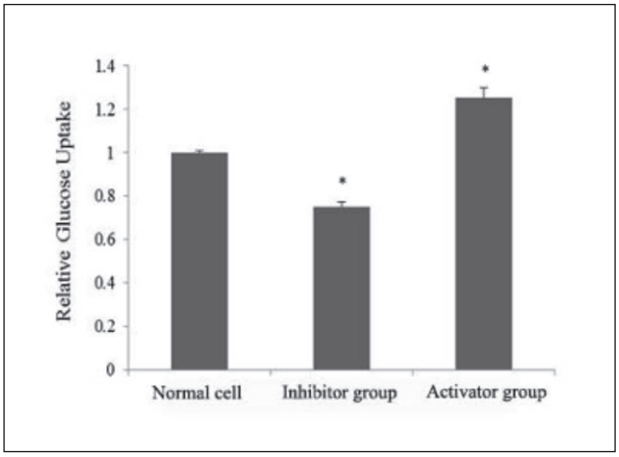


**Figure 5:** Effect of pCREB1 inhibitor or activator on glucose transport in glioma cell lines.

## Discussion

Glioma is the most common tumour in the central nervous system, has a wide range of invasiveness and can transform from low grade to high grade. Differentiating glioma is difficult and proliferation is fast. At present, these tumours cannot be completely resected in operations. The recurrence rate is high, and its causes and the factors promoting a patient's deterioration are still unclear[6]. Cancer cells usually have high metabolic activity and require more energy to grow faster than normal cells.

Because extra energy is needed during glycolysis and catabolism, the demand for glucose in tumour cells increases significantly[7]. Glucose transporter (GLUT) protein is the main vector mediating glucose uptake in cells. It has been confirmed that GLUT family proteins are closely related to the regulation of glioma growth and infiltration[8]. GLUT 1 is a protein composed of 492 amino acid residues, is widely found in eukaryotes and prokaryotes and is the main glucose transporter in the brain[9]. Related data show that over-expression of GLUT1 in bladder cancer and other malignant tumours can increase the proliferation of tumour cells and drive tumour invasion and metastasis[10]. Some researchers found that GLUT1 could regulate the EGFR/MAPK signalling pathway and inhibit proliferation, and down-regulating the expression of GLUT1 can inhibit the proliferation and glucose absorption of breast cancer cells.

The CREB1 genome is located in human chromosome 2q34 and is composed of 341 amino acid residues. It mainly adjusts the expression of genes through the cAMP-dependent cell signal transduction pathway and plays an important role in the life process[12]. CREB1 exists in resting cells in a dephosphorylation state and has no transcriptional activity. Extracellular signals such as neurotransmitters, hormones and growth factors can bind to G protein-coupled receptors on cell membranes, increase intracellular cAMP concentration, promote the activation of PKA and regulate the activity of CREB1[13]. Some foreign scholars have found that CREB1 is highly expressed in breast cancer and is closely related to its severity and the prognosis of patients[14]. It has also been reported that CREB1 regulates GLUT1 expression in mouse embryonic stem cells and has an important effect on glioma cell proliferation[15].

In this study, the results of the immunohistochemical method showed that compared with normal cell lines, both CREB1 and GLUT1 presented high expression in human glioma cell lines. This suggests

that CREB1 plays an important role in the development of glioma. To further analyse the relationship between the two proteins, the expression of CREB1 was down-regulated in this study, and it was found that compared with normal cells and the negative transfection group, the expression of GLUT1 mRNA and protein, as well as glucose absorption, in the si-CREB1 group significantly decreased ($P<0.05$). This suggests that CREB1 regulates glucose absorption in glioma cell lines and is associated with GLUT1 expression. The effects of pCREB1 inhibitors or activators on GLUT1 mRNA and protein were analysed and it was found that compared with the normal cell group, expression of GLUT1 mRNA and protein in the inhibitor group markedly decreased ($P<0.05$), whereas in the activator group, it increased ($P<0.05$). Also, the glucose absorption of the inhibitor group significantly decreased and that of the activator group remarkably increased ($P<0.05$). This suggests that the effect of CREB1 on glucose transport may be realized by regulating GLUT1.

In conclusion, both CREB1 and GLUT1 exhibit high expression in human glioma cells. CREB1 phosphorylation can affect GLUT1 expression and glucose transport in glioma cells. This suggests that the effect of CREB1 on glucose transport in glioma cells is associated with regulating GLUT1 expression through pCREB1.

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