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Borneol promotes apoptosis of human glioma cells by regulating mTORC1 / eIF4E / HIF-1a pathway

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Abstract

The main reason for the failure of malignant glioma treatment is local tumor recurrence. Tumor cells in hypoxic microenvironment activate HIF-1 α transcription, and thus promoting tumor invasion and metastasis is one of the important reasons. In our previous study, we clearly established that borneol opens the blood-brain tumor barrier and its related mechanism. However, the effects of borneol on glioma treatment have not yet been elucidated. Therefore, in this study, we evaluated the effects of borneol on cell proliferation and the expression of HIF-1a, mTORC1, eIF4E were by constructing in vivo SD rat brain glioma model and in vitro human primary cultured glioma cell model. Further, we measured the apoptosis effect and mechanism induced by borneol in human primary cultured glioma cells. We found that borneol could induce primary glioma cells apoptosis by suppressing the mTORC1 / eIF4E / HIF-1 α signaling pathway. Further, we also found that borneol could downregulate the expression of Bcl-2 and upregulation the expression of Bax and caspase-3,suggesting that borneol could further regulate the expression of apoptosis-related factors and induce the apoptosis of glioma cells. In conclusion, the findings of the present study suggest that HIF-1α may be a key factor in apoptosis of glioma cells, and mTORC1 / eIF4E / HIF-1a pathway is involved in the apoptosis induced by borneol in malignant glioma. Our results not only reveal the target and molecular mechanism and action of borneol leading to promote apoptosis in glioma cells, but also provide experimental basis and theoretical support for the clinical application of borneol.

Background

Glioma is the most common intracranial malignant tumor. It not only compresses normal brain tissues, increasing intracranial pressure and causing nervous system dysfunction, but also seriously threatens the health and life of patients [1]. High-grade gliomas (WHO III and IV grade gliomas) have a high incidence, approximately accounting from 35–45% of primary intracranial malignancies worldwide [2, 3]. Currently, the main clinical treatment of malignant gliomas is surgical, combined with radiotherapy and chemotherapy used to increase its efficacy, which is generally low.

The main reason for the failure of the treatment of malignant glioma is local tumor recurrence, which is closely related to the hypoxic microenvironment of malignant glioma. Tumor cells in a hypoxic microenvironment activated the transcription of hypoxia inducible factor-1α (HIF-1α), which was used as transcription factors promote tumor invasion, metastasis and resistance to radiotherapy and chemotherapy. In previous studies, the expression level of HIF-1α was found to be positively correlated with the pathological grade of glioma[4]. The higher pathological grade of glioma was associated with faster tumor growth and infiltration, further promoting the transcription and translation of genes related to angiogenesis. The increased neovascularization provides more oxygen and other nutrients needed for peripheral tumor growth, thus promoting continuous tumor growth and invasion [5]. Moreover, apoptosis impairment is also a hallmark of glioblastoma multiforme (GBM) [6] and is associated with tumor malignancy and poor prognosis [7, 8].The role of HIF-1α in apoptosis process is complex. HIF-1α appears

to have cell type-dependent pro- and antiapoptotic functions. Evidence has been highlighted that HIF-1α may inhibit apoptosis in GBM [9].

Studies at home and abroad have shown that the expression of HIF-1 α is regulated by many signaling pathways, among which the activation of mammalian rapamycin target (mTOR) related signaling pathway can increase the expression of HIF-1 α, maintain the acidic microenvironment of tumors, improve the invasion and metastasis of tumor cells, and lead to poor prognosis of solid tumors [10, 11]. mTOR is a well conserved tyrosine kinase with a key role in the regulation of cell proliferation, growth, survival, division, movement, and angiogenesis [12]. mTOR is subdivided into two complex subtypes, namely mTOR C1 and mTOR C2, where mTOR C1 is regulated by growth factors and nutrients to perform its function of governing cell growth and proliferation [13, 14]. Eukaryotic translation initiation factor 4E (eIF4E) is considered an important surviving protein involved in the cell cycle, cell transformation, and other vital processes; of note, mTORC1 accelerated the synthesis of some oncogene proteins by activating the function of eIF4E [15]. A previous study established that the increased expression of eIF4E increased the activity of cyclin D1, HIF-1α, and vascular endothelial growth factor; moreover, it enhanced mRNA translation and regulated cell proliferation and oxygen metabolism [16].

Borneolum Syntheticum is an antidote commonly used against fainting and convulsions [17]. In addition, borneol was found to easily cross the blood-brain barrier, and some anti-tumor drugs, such as arsenic trioxide, nimustine, methotrexate, and cisplatin, promoted its penetration through the blood-brain tumor barrier [18–20]. Furthermore, borneol has been used as a medicine since ancient times and is now also prescribed for brain disorders. It was shown to improve the energy metabolism in the area of the disorder and to protect the brain tissue from ischemic damage [21, 22].

Our research team has previously established intracranial transplantation tumor models of glioma C6, RG2, and 9L rats. We comprehensively studied the role and mechanism of action of borneol in opening the blood-brain tumor barrier[25]. Nevertheless, the effect of borneol on HIF-1a expression and glioblastoma growth are still unclear. Therefore, the purpose of this study was to investigate the effect of apoptosis induced by borneol in human malignant glioma primary cells, and the effect of borneol on regulation of the mTORC1/eIF4E/HIF-1a signaling pathway. Better understanding the involvement of borneol in the target and molecular mechanism of apoptosis will promote its clinical application for the treatment of gliomas.

Methods

Construction of SD rat brain C6 glioma transplantation model

SPF male SD rats weighing 250–300 g were provided by Shanghai sipur bikai experimental animal Co., Ltd. Before the operation, the animals were tested according to the routine. After confirming that there was no abnormal rotation behavior, they were anesthetized by intraperitoneal injection of 3% Pentobarbital Sodium 50 mg / kg. Then the rats were fixed on the brain stereotaxic instrument, the hair was removed from the head and disinfected. Under aseptic condition, the skin of the cranial crest was cut

along the median line, the periosteum was peeled off, and the anterior fontanelle was exposed. According to the atlas of brain stereotaxic localization wrote by Bao Xinmin et al, the right caudate nucleus was determined: 1 mm behind the anterior fontanelle, 3.0 mm to the right of the median line and 5.0 mm under the dura. The skull was drilled carefully in the required area of the operation, and 15 ul cell suspension was injected into the right caudate nucleus of the rat by microinjector. The injection time is 10 minutes, keep the needle for 5 minutes, and then exit the microinjector slowly. After the operation, the skull hole was filled with bone wax, the incision skin was sutured. After the animals were awake, they were put back into the feeding cage.

Animal grouping

After the establishment of the model, the rats were kept in SPF environment for 2 weeks to establish a stable and reliable transplanted tumor model. The rats were divided into 4 groups. A: Model group; B: borneol low dose group (4 mg / kg); C: borneol medium dose group (8 mg / kg); D: borneol high dose group (16 mg / kg)

Rats inoculated with C6 glioma cells for 2 weeks were given borneol for 10 days. During the administration period, the general condition and survival period of each group of animals were recorded, and the animals' foraging, drinking water and limb movement were observed. The rats were killed and the glioma tissue was removed for subsequent experimental research.

The expression of HIF-1 α, mTORC1 and eIF4E protein in C6 rat model of intracranial transplantation tumor treated by borneol was observed by immunohistochemistry

The brain tissue of the rats was fixed with 10% neutral formaldehyde, cut along the injection site, and then embedded into slices. Paraffin sections were dried at 60 ℃ for 1 hour, dewaxed by xylene and graded alcohol (100% 3 min, 100% 3 min, 95% 3 min, 90% 3 min, 85% 3 min, 75% 3 min, distilled water 3 min, distilled water 3 min, PBS 3 min). Antigen repair: place the section in 0.1 mol/l citric acid solution with pH = 6.0, use 100 fire power of microwave oven for 3 minutes to slightly boil, use 50 fire power for 7 minutes, stop heating and then naturally cool for 20–30 minutes. Rinse with PBS for 5 min × 3 / time. Immunohybridization: incubation with 3% H2O2 for 10 minutes to eliminate endogenous peroxidase activity. PBS washing, 5 minutes × 3 times. Add 5% BSA to the closed solution, and keep it in a wet box for 30 minutes. Wipe off the sealing liquid with filter paper, do not wash. Drop the first antibody of appropriate concentration (1:50 in average) and incubate it overnight in a wet box at 4 ℃, then wash the first antibody with PBS for 5 minutes × 3 times, and wipe off PBS outside the sample with filter paper. Drop the biotinylated second antibody working solution, incubate in a wet box at room temperature for 20 minutes, wash the second antibody with PBS for 5 minutes × 3 times, and wipe off PBS outside the sample with filter paper. Drop the working solution of streptavidin labeled with horseradish enzyme, incubate it in a wet box at room temperature for 20 minutes, wash it with PBS for 5 minutes × 3 times, and wipe off PBS outside the sample with filter paper. DAB chromogenic agent and tap water should be fully rinsed. Then the hematoxylin was re dyed, dehydrated, transparent and neutral resin sealed. The tumor tissues of rats were randomly selected under 10 × 40 times microscope.

Construction of an in vitro model of primary cultured glioma cells

Fresh human glioma tissue pieces were rinsed with serum-free culture medium and cut into small pieces of lmm 3 in size. 0.25% trypsin, digested in 37℃ water bath for 30 minutes, and filtered through a 100-mesh steel mesh. Transfer to a centrifuge tube and centrifuge at 1500 to 2000 rpm for 10 minutes, then remove the supernatant. The pellet was resuspended in a culture solution containing no calf serum, centrifuged twice at the same speed and the same time, and the supernatant was removed. Cells were resuspended in 1640 medium containing 10% A and bovine serum. count. 5×10^5 cells per well were transplanted into a 96 culture plate, and all cells were cultured in a humid environment at 37 ° C and 5% CO_2. The growing cells were collected and observed under a microscope and a laser confocal microscope, and compared with the results of the original surgical pathological examination to confirm that the growing cells were human glioma cells. This study was approved by the Medical Ethical Committee of Zhejiang Cancer Hospital.

Construction of mTORC1 gene silencing and eIF4E gene silencing primary glioma cells

Design and synthesize the siRNA sequence of mTORC1. According to the product instructions, use Opti-MEM (Invitrogen) and Oligofectamine (Invitrogen) to transfect siRNA into primary glioma cells and silence the mTORC1 gene in primary glioma cells.

Design and synthesize the eIF4E siRNA sequence. Follow the product instructions to use Opti-MEM (Invitrogen) and Oligofectamine (Invitrogen) to transfect siRNA into primary glioma cells, and silence the eIF4E gene in primary glioma cells.

Drug treatment and cell proliferation test (CCK-8)

Simulate hypoxic conditions (1% O_2), take human glioma primary cell suspension in logarithmic growth phase and inoculate it into a 96-well plate, and place the culture plate in the incubator for 24 hours; according to the normal group, The solvent control group (PBS), 10 μg / ml borneol group, 20 μg / ml borneol group, 40 μg / ml borneol group, 80 μg / ml borneol group were administered in groups for experiments, and 5 Gy radiation dose X-rays were given. After 48 hours of administration, 10 μL of CCK-8 solution was added to each well and incubated in an incubator. The absorbance at 450 nm was measured with a microplate reader, and the cell survival rate was calculated. Cell activity = (A value of the experimental group-blank zero A value) / (control group A value-blank zero A value) × 100%. Three replicates of each group of cells were measured in parallel.

Immunofluorescence detection

Take mTORC1 gene silencing and eIF4E gene silencing primary glioma primary cells, culture them for 48 hours under hypoxic conditions (1% O2), collect cell culture fluids, put them into sterile high-cleanness coverslips, and then take 60–70% cell density seed plate, add 1–2 mL of pre-chilled 4% paraformaldehyde (PFA) to each well after attaching the cells, fix for 10 min and aspirate, rinse 3 times in

PBS for 5 min each time; add 1 mL-2 mL 0.5 per well % Triton X-100, aspirate for about 2 min, add 1–2 mL PBS for rinsing, aspirate PBS and rinse 3 times for 5 min each time; block with appropriate blocking solution for 0.5 h (blocking solution: 3% BSA); incubate the primary antibody Rinse 3 times in PBS for 5 min each; incubate secondary antibodies: add 80 μL of secondary antibody to each cover slip (the dilution ratio of the secondary antibody is diluted with 5% goat serum according to the instructions, generally 1: 500), and incubated at room temperature for 0.5 h in the dark Remove the secondary antibody (Note: All steps after the secondary antibody step need to be protected from light), rinse PBS twice for 5 min each time; nuclear staining: add nuclear staining reagent (DAPI, generally 1–2 min can be used, depending on the specific As the case may be). After staining, remove the nucleating reagent, rinse once with PBS, and then add 1 mL of PBS to keep it wet. Preparation: First add an appropriate amount of mounting media (Mounting media, anti-fluorescence quenching blocking tablets, usually placed on -20 ° C), use a curved needle to lift the coverslip with the side of the cell facing down, shake the slide slightly to dry it, and then cover the adhered slide containing the quencher. Squeeze to the other side and suck excess mounting media away with absorbent paper. Coverslip: Capillary drops of nail polish are placed on the four corners of the slide for covertling, and protected from light and dried. Finally, ZEISS was observed and photographed under an inverted fluorescence microscope.

Real-time PCR detection

The primary glioma cells of mTORC1 gene silencing and eIF4E gene silencing were taken and cultured for 48 h under hypoxic conditions (1% O2). Cell culture fluids were collected and the cells were lysed to extract total RNA. The cells in the cell culture dish were washed twice with 1 mL of PBS, added with 1 ml of Trizol solution, pipetted and mixed, and aspirated into a 1.5 mL EP tube to fully lyse the cells, and allowed to stand at room temperature for 5 min. Place in a clean bench, incubate for 5 min, 12000 rpm, and centrifuge for 10 min. Aspirate the supernatant in a new 1.5 mL centrifuge tube, add 200 μL of chloroform, shake well, and let stand at room temperature for 2 minutes, 4 ° C, 12000 rpm, and centrifuge for 10 minutes. Aspirate the supernatant in a new 1.5 mL centrifuge tube, add 600 μL of isopropanol, mix well, leave it at room temperature for 15 min, 4 ° C, 12000 rpm, centrifuge for 15 min, and discard the supernatant. Add 1 mL of 75% absolute ethanol (750 μL of absolute ethanol and 250 μL of DEPC water) to rinse the pellet, centrifuge at 12,000 rpm at 4 ° C for 5 minutes, and discard the supernatant. Add 1 mL of absolute ethanol, rinse the pellet, centrifuge at 12,000 rpm at 4 ° C for 5 min, discard the supernatant, and dry at room temperature for 10 min. Add 40 μL of DEPC water to dissolve RNA and store in -80 ° C refrigerator for future use. The following reaction system was prepared for reverse transcription reaction. Reaction conditions: 42 ° C, 15 min; 85 ° C, 5 min. Prepare the following reaction system for real-time PCR reaction. Thoroughly mix the solution in the tube with a vortex shaker and briefly centrifuge at low speed. Reaction conditions: 95 ° C, 10 min denaturation; 95 ° C, 15 s; 60 ° C, 60 s; 40 cycles.

The primary glioma cells were taken and cultured under hypoxic conditions (1% O2). After four different concentrations (10, 20, 40, 80 μ g / ml) of borneol were used to treat cells for 24 hours, mRNA was extracted from cells of each group, and the expression of HIF-1 α, mTORC1, eIF4E, Bcl-2, Bax and caspase-3 were detected by real-time PCR.

Western blot detection

Primary human glioma cells were taken under hypoxic conditions (1% O2), after 4 different concentrations (10, 20, 40, 80 μg/ml) intervention for 24 hours, collect the cell culture solution, lyse the cells to extract the total protein, use the BCA method to measure and balance the protein concentration, isolate the protein by 10% SDS-PAGE, transfer the protein to the PVDF membrane by semi-dry electrophoresis, and block at room temperature for 2 h. The primary antibody was incubated overnight, washed, and the secondary antibody was incubated for 1 h. The ECL method was used to develop the color and the tablet was exposed. The gel imager analysis system was used to scan the western blot detection band for gray value. The protein expression of HIF-1 α, mTORC1, eIF4E, p-eIF4E, Bcl-2, Bax and caspase-3 was detected.

Detection Of Apoptosis Rate By Flow Cytometry

After treated with 4 different concentrations (10, 20, 40, 80 μg/ml) of borneol for 24 hours, apoptosis was detected by flow cytometry. The human glioma cells in the logarithmic growth stage were implanted in the 6-well plate with a volume of 2 ml per well, and the cell inoculation density was 1.2×10^6 cells per well. After transfection, the cells were collected and washed twice by precooling PBS, and the cell concentration was adjusted to 1×10^6 cells / ml. Add 500 μl of binding buffer, centrifugate the supernatant, add 100 μl of binding buffer and mix well, respectively add 5 μl of annexin V-FITC and 10 μl of PI, mix well, and react at room temperature without light for 15 minutes. Finally, 400 μl of the binding buffer was added and the apoptotic rate was detected by flow cytometry. The experiment was repeated three times.

Statistical Analysis

SPSS 22.0 statistical software was used for statistical analysis. All data were expressed as mean ± standard deviation ($\bar{\chi} \pm s$). Comparison between two samples was performed by t test. $P < 0.05$ was considered significant difference.

Results

Effect of borneol on the expression of HIF-1 α, mTORC1 and eIF4E in glioma tissue

The results of immunohistochemistry showed that HIF-1 α, mTORC1 and eIF4E were all expressed in the brain of each group. Compared with the model control group, the positive expression of HIF-1 α and eIF4E in each group was significantly lower ($P < 0.05$ or $P < 0.01$)(Figure 1, Table 1; Fig. 2, Table 3). Compared with the model group, the positive expression of mTORC1 protein in each group was decreased, but only the middle dose group and high dose group of borneol had significant difference ($P < 0.05$ or $P < 0.01$) (Fig. 3, Table 2).

Table 1
the effect of Borneol on the expression of HIF-1 α protein in brain glioma of C6 rats($\bar{x}$±s,n = 6)

Group	Area	IOD
Model Group	129894.0 ± 27834.9	7614.2 ± 1761.8
Low dose group of Borneol(4 mg/Kg)	89446.2 ± 21464.8[△]	4510.0 ± 1252.8[△]
Medium dose roup of Borneol(8 mg/kg)	72740.5 ± 18369.5[△△]	4043.3 ± 1132.0[△△]
High dose group of Borneol(16 mg/kg)	50668.7 ± 12418.1[△△]	2694.2 ± 753.6[△△]
Compare with model group: [△]P<0.05,[△△]P<0.01		

Table 2
Effect of Borneol on the expression of mTORC1 protein in brain glioma of C6 rats($\bar{x}$±s,n = 6)

Group	Area	IOD
Model Group	135075.3 ± 39024.2	22351.7 ± 7236.9
Low dose group of Borneol(4 mg/Kg)	105222.5 ± 41448.5	16569.1 ± 7716.7
Medium dose roup of Borneol(8 mg/kg)	89601.7 ± 20803.4[△]	13659.4 ± 3868.9[△]
High dose group of Borneol(16 mg/kg)	47369.5 ± 13712.6[△△]	6002.6 ± 2674.9[△△]
Compare with model group: [△]P<0.05,[△△]P<0.01		

Table 3
Effect of Borneol on the expression of eIF4E protein in C6 rat brain glioma model($\bar{x}$±s,n = 6)

Group	Area	IOD
Model Group	152238 ± 17344.3	13570.6 ± 1601.3
Low dose group of Borneol(4 mg/Kg)	109643.8 ± 22019.1[△△]	9636.1 ± 2035.3[△△]
Medium dose roup of Borneol(8 mg/kg)	82896.3 ± 19349.0[△△]	7264.8 ± 1772.9[△△]
High dose group of Borneol(16 mg/kg)	42909.2 ± 15126.7[△△]	3620.4 ± 1355.6[△△]
Compare with model group: [△]P<0.05,[△△]P<0.01		

Separation of human primary glioma cells

After digestion by trypsin, the cells of the glioma were initially round and dispersed in the culture medium. After 4 hours of culture in the cell suspension, the cells adhered to the wall and showed oval shape. Some cells formed colonies. After 24 hours, the cells expanded. After 48 hours, the number of cells was small and the density was low. However, the proliferation of cells was vigorous. The tumor cells varied in shape and size, mainly fusiform, and the body extended upward Protrude and stretch around. The primary glioma cells cultured successfully proliferated rapidly, and the cells after subculture grew vigorously, in good condition and stable character(Fig. 4).

Effect of borneol on the growth of primary cultured human glioma cells

According to Fig. 5, compared with the control group, the cell survival rate of the treatment group has a downward trend, when the concentration of borneol reaches 50 μg/ml or above, it shows a significant difference($P < 0.05$).

Apoptosis detection in primary cultured human glioma cells

After the cells were treated with 4 different concentrations (10, 20, 40, 80 μg/ml) of borneol for 24 hours, the apoptosis rate showed an upward trend (8.27% − 15.81%), and there was a concentration dependence. The higher the concentration of borneol, the greater the apoptosis rate (Fig. 6). When the borneol concentration reached 40 μg/ml, there was a significant difference between the two groups ($P < 0.05$).

The effect of Borneol on the expression of HIF-1 α, mTORC1, eIF4E, Bcl-2, Bax and Caspase-3 in human glioma cells by qRT-PCR

HIF-1 α, mTORC1, eIF4E and Bcl-2 were treated with 4 different concentrations (10, 20, 40, 80 μg/ml) of borneol for 24 hours MRNA expression level showed a downward trend, there was a certain concentration dependence, the higher the borneol concentration, the lower the mRNA expression level; Bax, caspase-3 mRNA expression level showed an upward trend in each group of cells, and there was a concentration dependence, the higher the borneol concentration, the higher the mRNA expression level (Fig. 7). When the borneol concentration reached 40 μg/ml, there was a significant difference between the two groups ($P < 0.05$ or $P < 0.01$).

The effects of Borneol on the expression of HIF-1 α, mTORC1, eIF4E, p-eif4e, Bcl-2, Bax and Caspase-3 in human glioma cells by Western blot analysis

After four different concentrations (10, 20, 40, 80 μ g /ml) of borneol were treated for 24 hours, the expression of HIF-1 α, mTORC1, eIF4E, p-eif4e and bcl-2 protein showed a downward trend, and there was a concentration dependence. The higher the concentration of borneol, the lower the protein expression level; the expression of Bax and caspase-3 protein showed an upward trend, and there was a concentration dependence, borneol concentration showed a downward trend The higher the expression

level, the higher the protein expression level (Fig. 8). When the borneol concentration reached 40 μg/ml, there was a significant difference between the two groups ($P < 0.05$ or $P < 0.01$).

Regulation of HIF-1 α expression by silencing mTORC1/eIF4E in human primary glioma cells

The expression levels of eIF4E and HIF-1 α in primary human glioma cells transfected with mTORC1 siRNA were detected by immunofluorescence. As can be seen in Fig. 9A, the expression of eIF4E in mTORC1 siRNA transfected cells was significantly lower than that in the control group ($P < 0.01$). In Fig. 1B, it is visible that the expression of HIF-1 α in the cells transfected with eIF4E siRNA was significantly lower than that in the control group ($P < 0.01$). Western blot results revealed significantly lower expression of eIF4E in mtorc1-sirna group than that in the control group ($P < 0.01$). We established that the protein expression level of HIF-1 α in the eIF4E siRNA group was significantly ($P < 0.01$) lower than that in the control group (Fig. 9D). The expression level of eIF4E mRNA in the mtorc1-sirna group significantly decreased ($P < 0.05$, compared with the control group) after the transfection of human glioma cells with mTORC1 siRNA by qPCR (Fig. 9E). Additionally, we found that the expression level of HIF-1 α mRNA in the eIF4E siRNA group was significantly lower ($P < 0.01$) than that in the control group (Fig. 9F). These results indicated that mTORC1/eIF4E silencing downregulated the expression of HIF-1α.

Discussion

High-grade gliomas (WHO III and IV glioblastomas) are the most common intracranial malignancies in adults. These tumors do not have an intact envelope, grow invasively, and are not clearly distinguishable from normal brain tissue. Hence their remove complete surgical removal is difficult, and postoperative radiotherapy and chemotherapy are important adjuvant treatment methods which can significantly improve patient survival [23, 24]. However, clinically, the desired effect of postoperative radiotherapy or chemotherapy are often difficult to achieve mainly due to the frequent development of tolerance to radiation or chemotherapy in tumor cells.

Previous research has shown that borneol can open the blood-brain tumor barrier by regulating the expression of TJPs protein[25]. In addition, studies have revealed the role of borneol as a new type of chemotherapy sensitizer inducing mitochondrial dysfunction and oxidative damage, which promotes the anticancer effect of temozolomide on human glioma cells [26]. However, it is still unclear whether borneol could exert an apoptosis effect on malignant gliomas. Therefore, in this study, we evaluated the inhibitory effect of borneol treatment on SD rat brain C6 glioma transplantation model and human primary glioma cell proliferation. Results obtained confirm that borneol could inhibit the proliferation of malignant glioma to some extent. Further, we found that borneol induced human primary glioma cell apoptosis with a concentration dependence. Li Feifei[27]reported that borneol (Natural Borneol) can cause apoptosis of human nasopharyngeal carcinoma, squamous cell carcinoma, adenocarcinoma and breast cancer cells.

The occurrence of hypoxia in the tumor microenvironment changes the transcriptional activity of some genes in tumor cells. The changes in the expression products of tumor cells lead to the adaptation of tumor cells to the hypoxic microenvironment, promoting tumor growth, angiogenesis, metastasis,

apoptosis or apoptosis impairment. The transcription factor HIF-1 plays a central role in this process. The HIF-1α gene is the rate-limiting factor of HIF-1 that determines the activity of HIF-1[28, 29]. Here, we found that eIF4E siRNA suppressed the expression of HIF-1α, whereas the expression of eIF4E was inhibited by the mTORC1 siRNA, suggesting that the mTORC1/eIF4E pathway participates in regulating the expression of HIF-1α in primary human glioma culture cells under hypoxia. It hypoxia has been shown to promote the migration and invasion of malignant glioma cells, which can be achieved by activating PI3K / Akt / mTOR / HIF-1 α pathway [30]. Moreover, evidence revealed that borneol enhanced paclitaxel-induced apoptosis of human esophageal squamous cell carcinoma (ESCC) cells by inhibiting the activity of PI3K / Akt [31]. In order to clarify the exact mechanism of borneol induced apoptosis in malignant glioma, this study further evaluated the effect of Borneol on HIF-1α, mTORC1, eIF4E expression. Both in SD rat brain C6 glioma transplantation model and human primary glioma cell model, results showed that borneol could inhibit the expression of mTORC1/eIF4E/HIF-1a pathway, implying that borneol could induce primary glioma cells apoptosis by suppressing the mTORC1 / eIF4E / HIF-1 α signaling pathway.

Apoptosis is a cascade of active cell death process, which is regulated by many genes and closely related to Caspase-3 family and BCL-2 family. The interaction of apoptosis, proliferation and anti-apoptosis of tumor cells determines the degree of malignant progression of tumor biological behavior. Therefore, this study further evaluated the effect of borneol on apoptosis-related regulatory factors. We found that borneol could downregulate the expression of Bcl-2 and upregulation the expression of Bax and caspase-3,suggesting that borneol could further regulate the expression of apoptosis-related factors and induce the apoptosis of glioma cells.

Conclusion

The findings of the present study suggest that HIF-1α may be a key factor in apoptosis of glioma cells, and mTORC1 / eIF4E / HIF-1a pathway is involved in the apoptosis induced by borneol in malignant glioma. Our results not only reveal the target and molecular mechanism and action of borneol leading to promote apoptosis in glioma cells, but also provide experimental basis and theoretical support for the clinical application of borneol.

Declarations

Ethics approval and consent to participate

This study was approved by the Medical Ethical Committee of Zhejiang Cancer Hospital.

Consent for publication

Not applicable

Availability of data and materials

All data, models, and code generated or used during the study appear in the submitted article.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

All authors read and approved the final manuscript. Conceptualization and methodology by Guonong Yang and Xinjun Cai; Experiments, Liang Xia, Caixing Sun, Qiong Wang; Writing – Original Draft, Qinglin Li; Writing – Review & Editing, Zeng Wang.

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None

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Figures

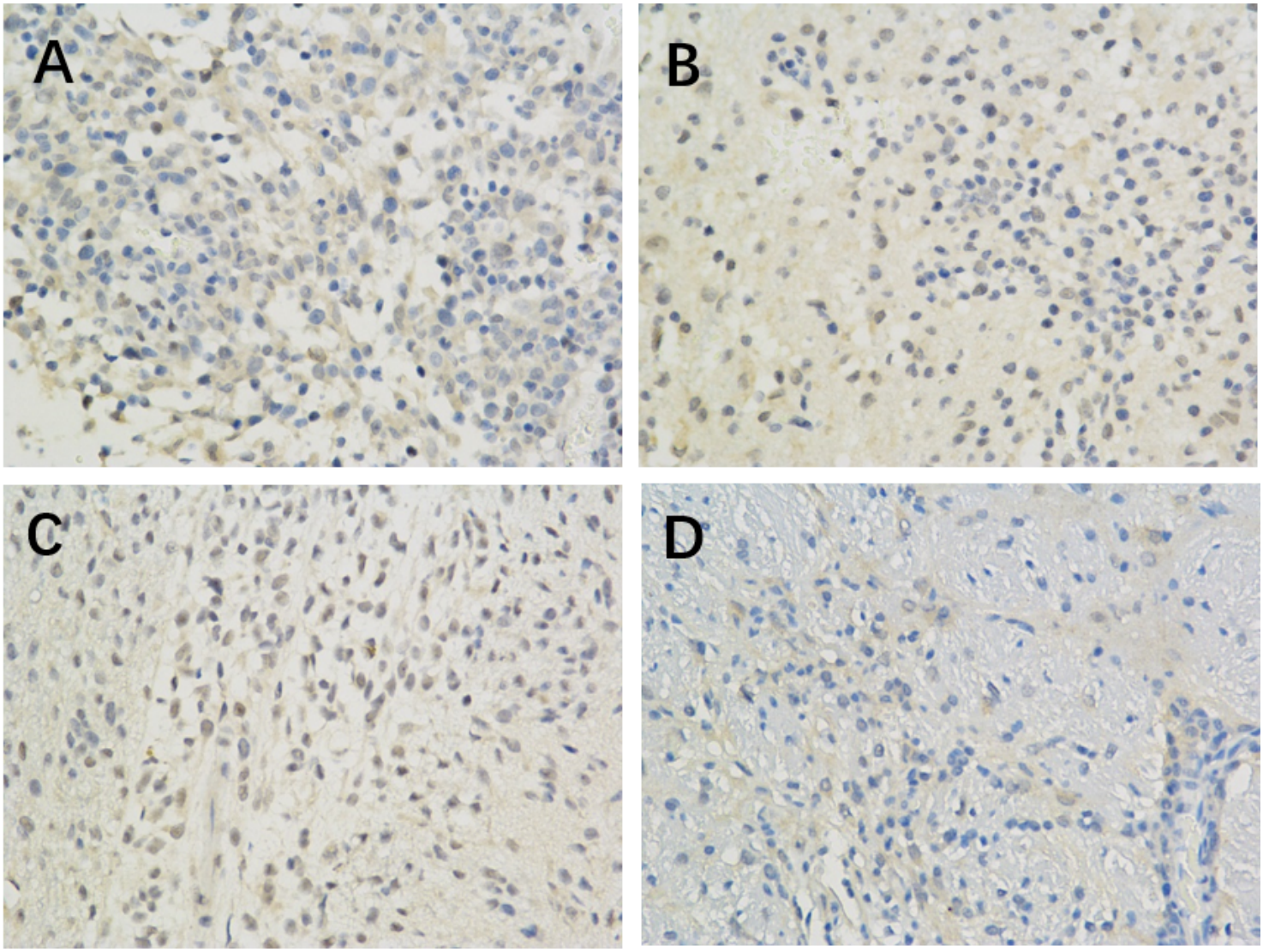


Figure 1

The effect of Borneol on the expression of HIF-1 protein in C6 rat brain glioma model (400 times) A. Model Group (400 times) B. Low dose group of Borneol (400 X) C. Medium dose group of Borneol (400 x) D. High dose Group of BORNEOL (400 x)

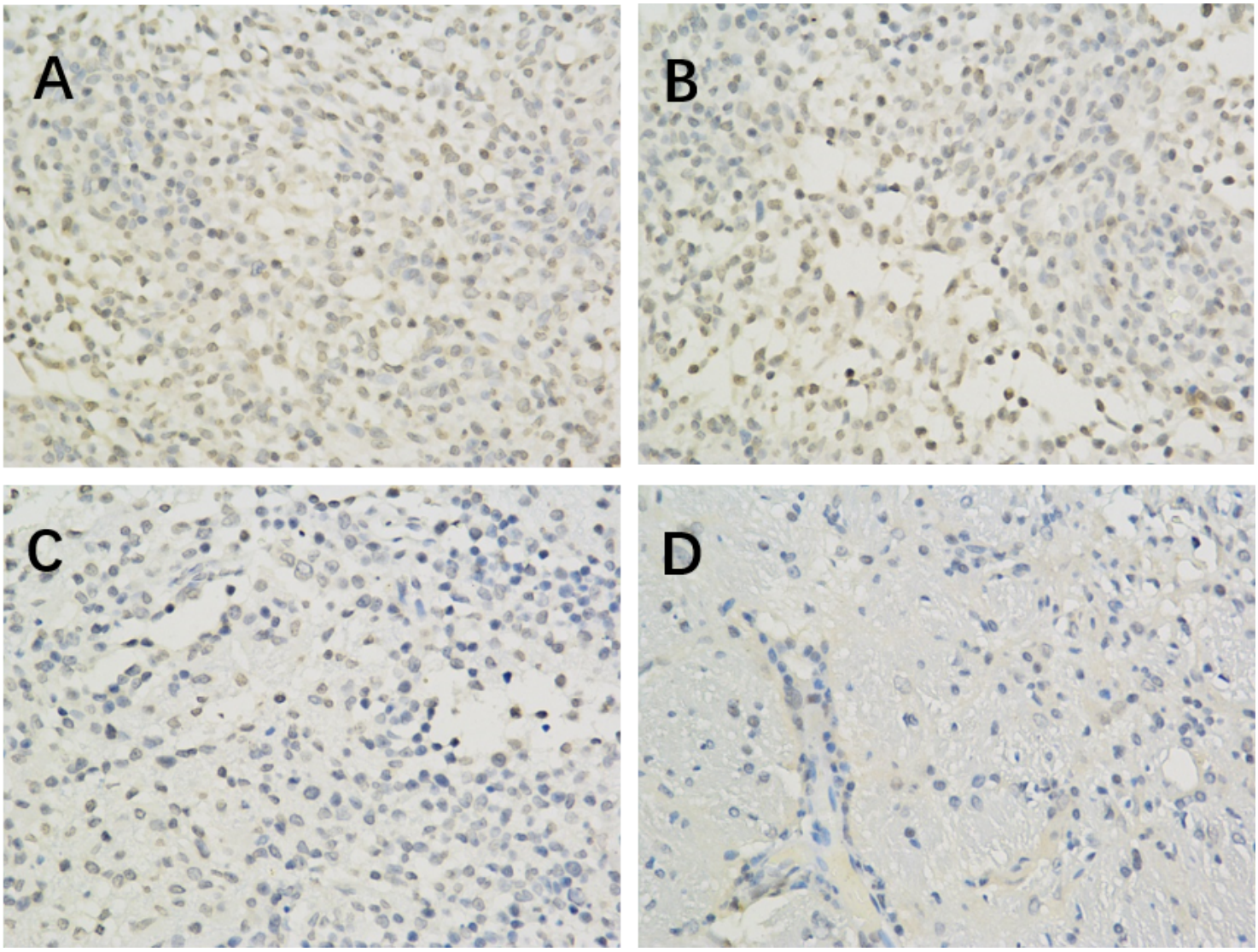


Figure 2

The effect of Borneol on the expression of MTORC1 protein (400-fold) in C6 rat intracranial transplanted glioma Model A. Low dose group of Borneol (400 X) C. Medium dose group of Borneol (400 x) D. High dose Group of BORNEOL (400 x)

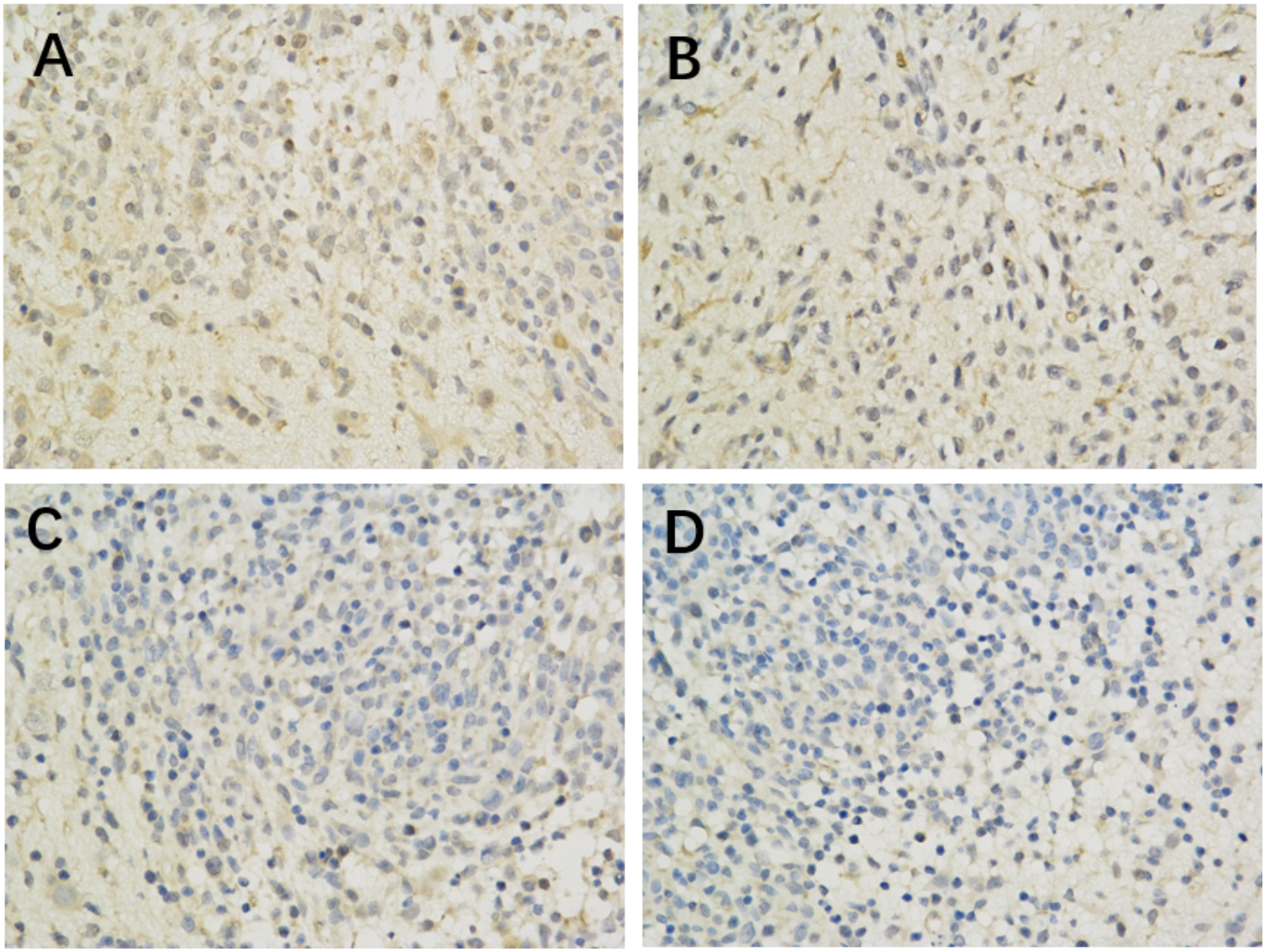
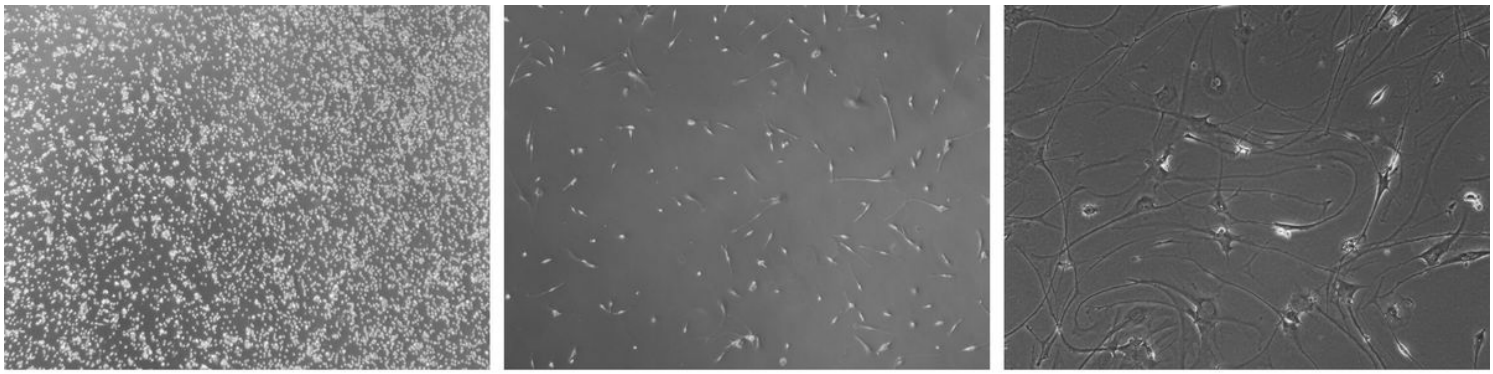


Figure 3

The effect of Borneol on the expression of eIF4E protein in the C6 rat model of intracranial transplanted Glioma (400 fold) A. Low dose group of Borneol (400 X) C. Medium dose group of Borneol (400 x) D. High dose of Borneol (400 x)



Primary culture cells of human glioma

Figure 4

Separation of human primary glioma cells

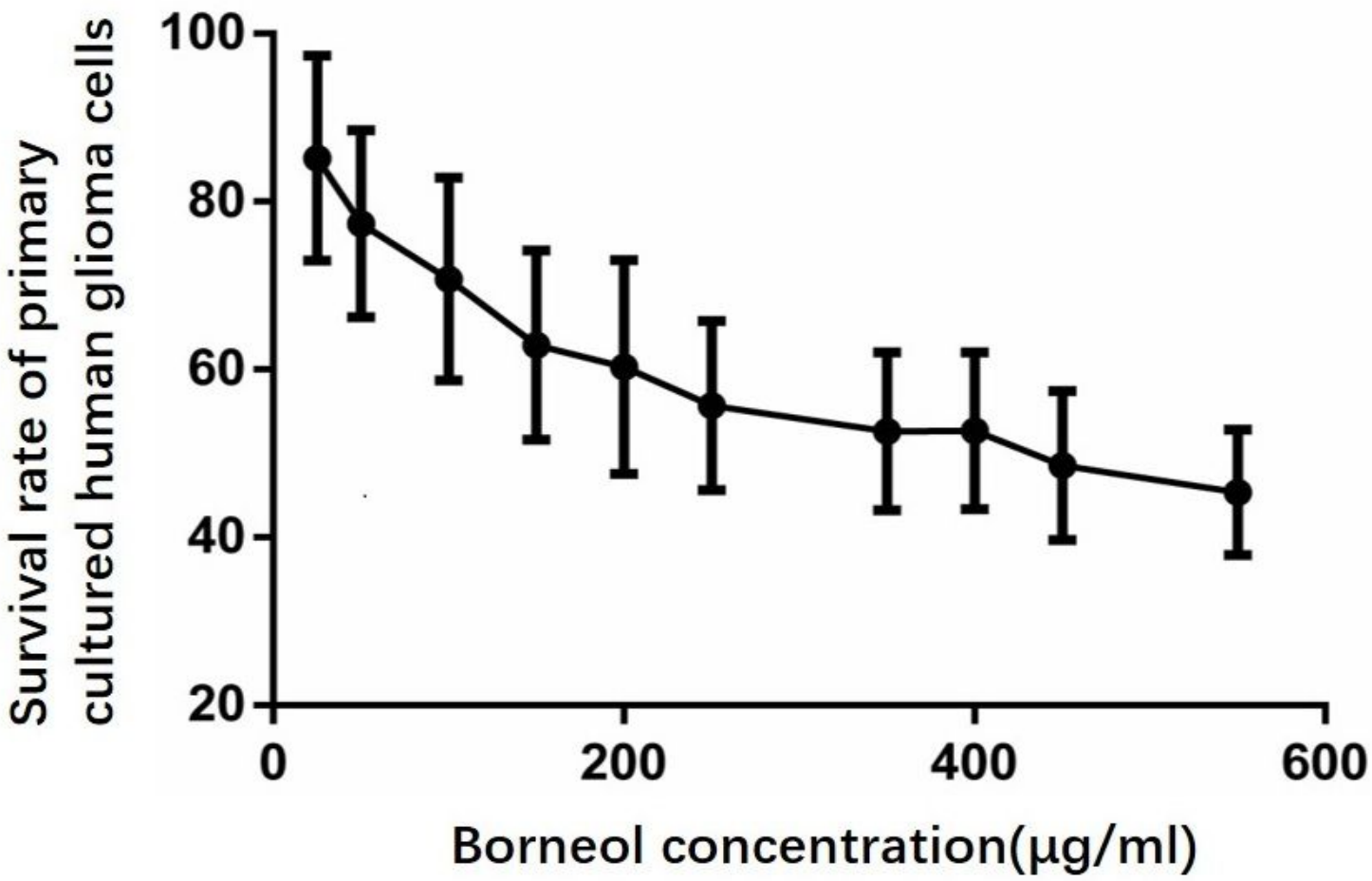


Figure 5

Effect of borneol on the growth of primary cultured human glioma cells

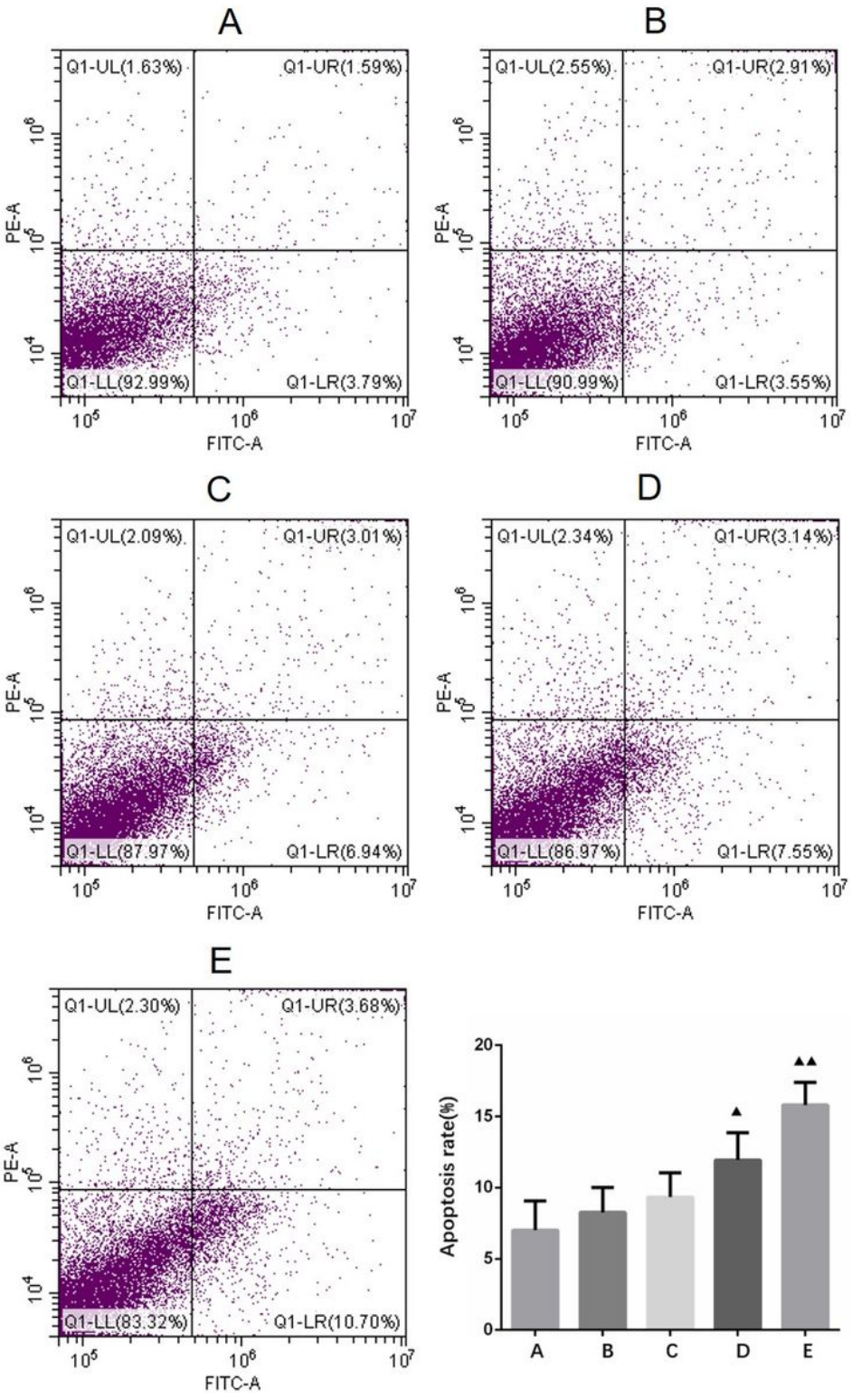


Figure 6

Apoptosis detection in primary cultured human glioma cells A. control B. Borneol 10μg/ml C. Borneol 20μg/ml D. Borneol 40μg/ml E. Borneol 80μg/ml comparison with the control group▲ P<0.05▲▲ P <0.01

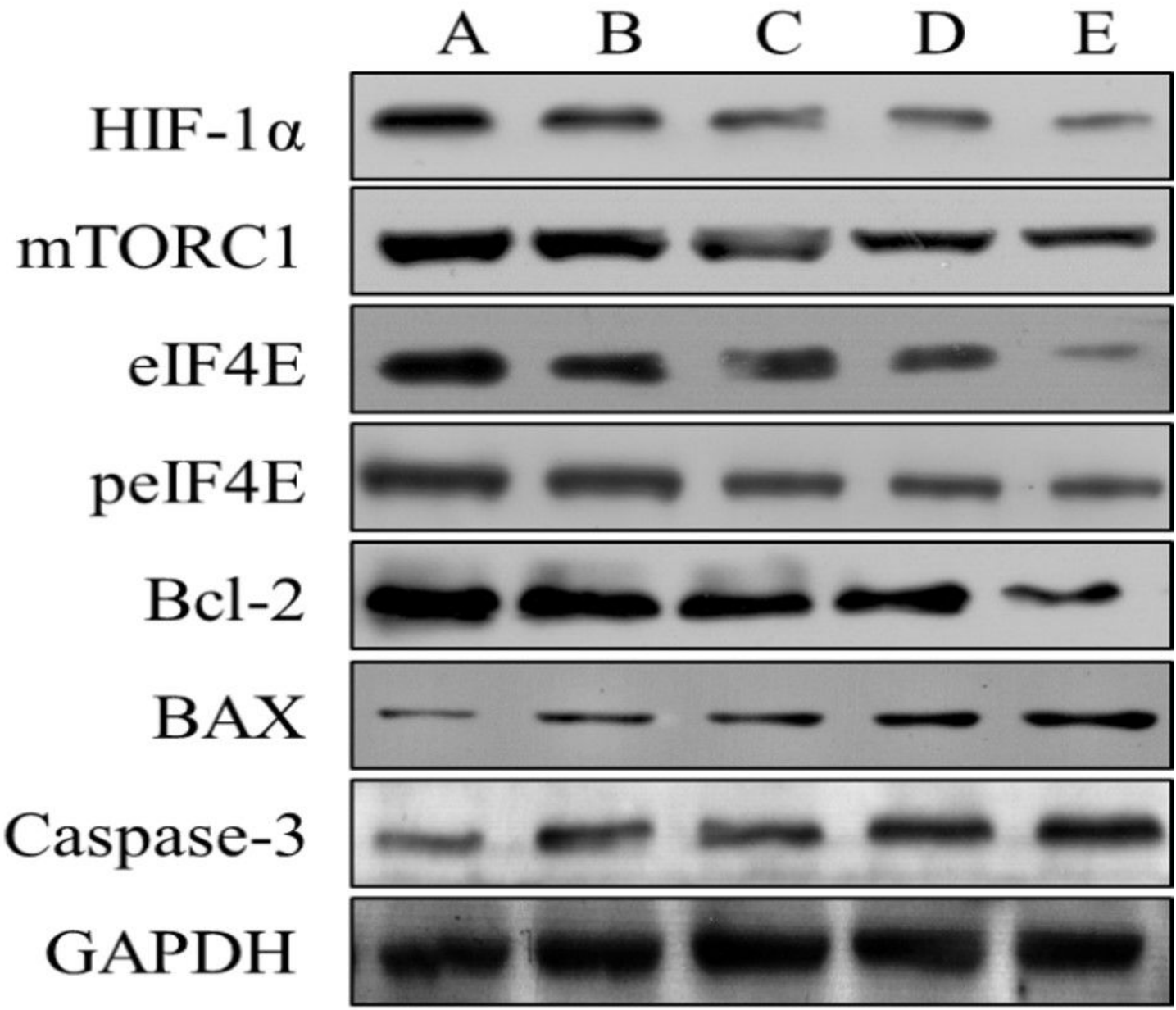


Figure 7

The effect of Borneol on the expression of HIF-1 α, mTORC1, eIF4E, Bcl-2, Bax and Caspase-3 in human glioma cells by qRT-PCR A. control B. Borneol 10μg/ml C. Borneol 20μg/ml D. Borneol 40μg/ml E. Borneol 80μg/ml comparison with the control group▲ P<0.05▲▲ P <0.01

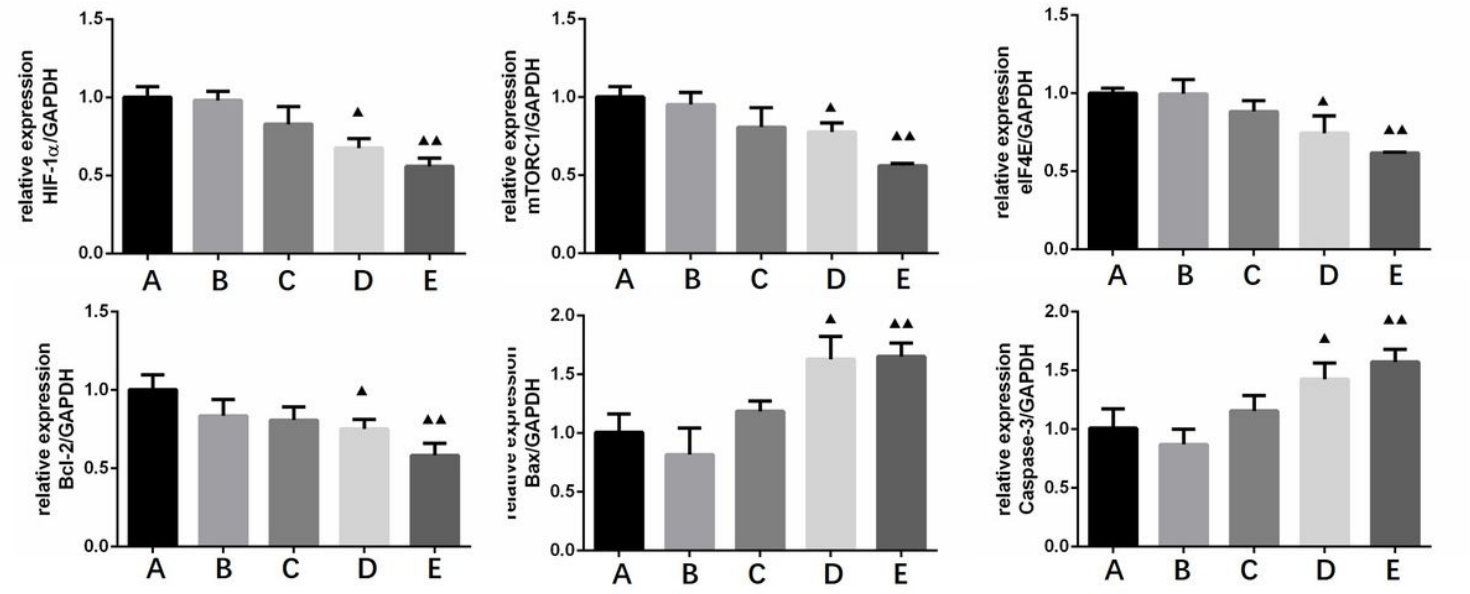


Figure 8

The effects of Borneol on the expression of HIF-1 α, mTORC1, eIF4E, p-eif4e, Bcl-2, Bax and Caspase-3 in human glioma cells by Western blot analysis A. control B. Borneol 10μg/ml C. Borneol 20μg/ml D. Borneol 40μg/ml E. Borneol 80μg/ml

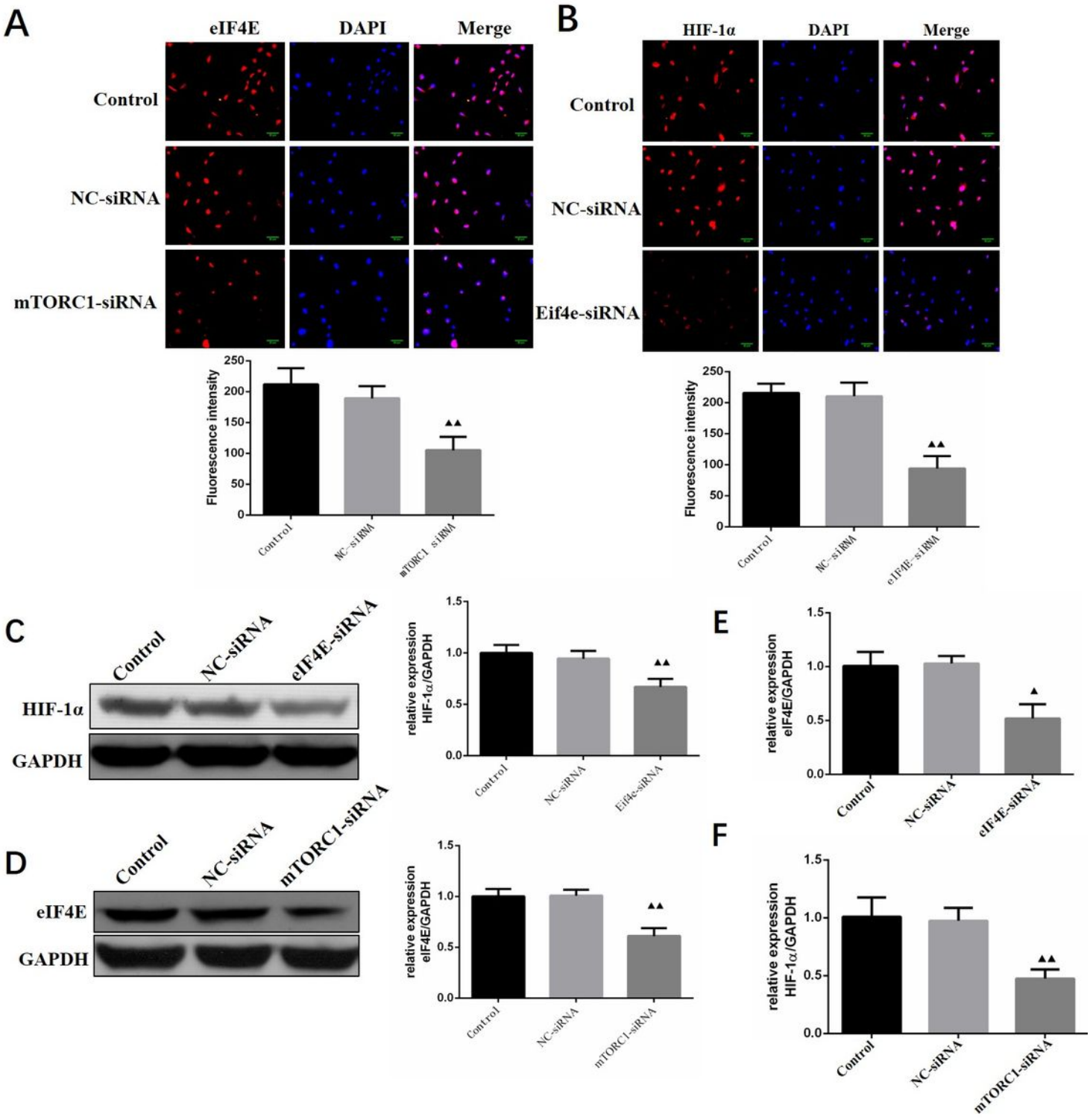


Figure 9

Regulation of HIF-1 α expression by silencing mTORC1 / eIF4E in human primary glioma cells A. EIF4E expression in cells of each group ($\bar{x}$±S,n=3） comparison with the control group）▲ P<0.05）▲▲ P <0.01）B. Expression of HIF-1 α in cells of each group）$\bar{x}$±S,n=3）comparison with the control group）▲ P<0.05）▲▲ P <0.01）C. Expression level of eIF4E protein in each group after mTORC1 siRNA transfection）$\bar{x}$±S, n=3） comparison with the control group）▲ P <0.05）▲▲ P <0.01） D. Expression level of eIF4E mRNA in each

group after mTORC1 siRNA transfection（$\bar{x}$±S, n=3）comparison with the control group（▲ P <0.05）▲▲ P <0.01）E. Expression of HIF-1 α protein in each group after eIF4E siRNA transfection（$\bar{x}$±S, n=3）comparison with the control group（▲ P <0.05）▲▲ P <0.01） F. Expression of HIF-1 α mRNA in each group after eIF4E siRNA transfection（$\bar{x}$±S, n=3）comparison with the control group（▲ P <0.05）▲▲ P <0.01.