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Modeled microgravity suppressed invasion and migration of human glioblastoma U87 cells through downregulating store-operated calcium entry

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ABSTRACT

Glioblastoma is the most common brain tumor and is characterized with robust invasion and migration potential resulting in poor prognosis. Previous investigations have demonstrated that modeled microgravity (MMG) could decline the cell proliferation and attenuate the metastasis potential in several cell lines. In this study, we studied the effects of MMG on the invasion and migration potentials of glioblastoma in human glioblastoma U87 cells. We found that MMG stimulation significantly attenuated the invasion and migration potentials, decreased thapsigargin (TG) induced store-operated calcium entry (SOCE) and downregulated the expression of Orai1 in U87 cells. Inhibition of SOCE by 2-APB or stromal interaction molecule 1 (STIM1) downregulation both mimicked the effects of MMG on the invasion and migration potentials in U87 cells. Furthermore, upregulation of Orai1 significantly weakened the effects of MMG on the invasion and migration potentials in U87 cells. Therefore, these findings indicated that MMG stimulation inhibited the invasion and migration potentials of U87 cells by downregulating the expression of Orai1 and sequentially decreasing the SOCE, suggesting that MMG might be a new potential therapeutic strategy in glioblastoma treatment in the future.

1. Introduction

Glioblastoma is the most common primary brain tumor in adults, which is characterized by uncontrolled cellular proliferation, extraordinary invasion and migration potentials and these features make this disease's treatment tough [1–3]. At present, though standard treatments of glioblastoma, including surgical resection, irradiation with adjuvant temozolomide and on recurrence, experimental chemotherapy, have gained improvements, the survival of patients remains poor and 2-year relative survival probability is low [2,3]. Inventing and improving novel therapeutic strategies are critically needed.

Recent years, magnetic fluid-modeled microgravity has been emerging as a new potential therapy for tumor treatment [4]. Considerable attentions were paid to the effects of microgravity on cellular behaviors, especially on tumor cells. Increasing investigations have indicated that microgravity has evident effects on the morphology, proliferation, apoptosis, invasion, migration, and even gene expression's variation [5,6]. Jing Li et al. found that microgravity insult would change the cytoskeleton and focal adhesions, and decrease migration in human breast carcinoma [7]. De Chang et al. found that microgravity treatment inhibited cell proliferation, migration, and invasion of human lung adenocarcinoma cell line [8]. Meanwhile, after microgravity insult, differentiation alteration and apoptosis increase have been found in human follicular thyroid carcinoma cells [9]. However, though a few studies have demonstrated that microgravity induced apoptosis in cultured glial cells [10], inhibited proliferation and enhanced chemical sensitivity of glioblastoma cells [11], the evident effects of

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microgravity on glioblastoma cells have been poor investigated, especially on the invasion and migration potentials.

In this study, we applied a clinostat to model microgravity on the ground to investigate the effects of modeled microgravity (MMG) on the invasion and migration potentials of U87 cells. We found that MMG stimulation could attenuate the invasion and migration potentials by downregulating the expression of Orai1 and decreasing the SOCE, indicating that MMG might be a new good perspective in glioblastoma therapy and much more mechanism investigations should be paid to it.

2. Materials and methods

2.1. Cell culture

The human glioblastoma U87 cells were obtained from the Institute of Biochemistry and Cell Biology, SIBS, CAS. The cell line was cultured in complete Dulbecco's modified Eagle medium (DMEM) (Gibco, MD, USA), supplemented with 10% fetal bovine serum (Gibco, MD, USA), 100 U/ml penicillin and 0.1 mg/ml streptomycin (Gibco, MD, USA), in a humidified incubator with 5% CO_2 and 95% air.

2.2. Plasmids and transfections

The STIM1-shRNA (sc-76589-SH) and scramble-shRNA (Scr-shRNA) (sc-108060) were purchased from Santa Cruz Biotech (Santa Cruz, CA, USA). Full-length cDNAs for Orai1 (BC015369) was synthesized and subcloned into pcDNA3.1 vector. U87 cells were transfected with plasmids using Lipofectamine 2000 transfection reagent according to the manufacturer's protocol (Invitrogen, CA, USA). Optimal transfection conditions were determined by using GFP-labeled nonspecific shRNA. Protein expression levels were detected to evaluate the efficiency of downregulation or upregulation 72 h after transfection.

2.3. Clinostat to model microgravity (MMG)

A 2D-clinostat device from the Fourth Military Medical University was applied in this study [12]. Cells were initially seeded on coverslips which were 2.55 × 2.15 cm^2. After the cells adhering to the coverslips for 6 h, the coverslips were inserted into the fixtures of chambers, and then the chambers were filled completely with DMEM with 10% FBS without any air bubbles existing to avoid the cell surface shearing. Finally, these chambers underwent rotation at 30 rpm around the vertical axis for 24, 48 or 72 h respectively. Normal gravity (NG) group was static control culture which was kept in the same clinostat conditions without rotation. The average gravitational force acting on the cells generated by the 2D-clinostat is reduced to about 10^{-3} G, compared with the NG, namely 1 G. After treatments, the cells were applied in the follow experiments.

2.4. Wound healing assay

The wound healing assay was carried out to evaluate the migration potential of the U87 cells. Cells were initially seeded on coverslips at a density of 5×10^4/well. After the cells adhering to the coverslips for 6 h, a line was scratched with a sterile 200 μl pipette tip across the coverslips and the coverslips were inverted into the chamber. Then, both NG and MMG groups were obtained corresponding treatments as described above. The wounded areas were photographed at the predicted times by inverted phase contrast microscope (Olympus, Tokyo, Japan). The wound healing effect was calculated by the equation ((the wound healing area/the cell-free area of the initial scratch) × 100%).

2.5. Transwell invasion assay

Transwell invasion assay was conducted with Transwell chamber (24-well insert; pore size, 8 μm; Merck Millipore, Darmstadt, Germany). Matrigel Matrix (BD Biosciences, CA, USA) was diluted with DMEM at the ratio of 1:8 on ice, and 100 μl mixture was added into each chamber. Then, the chambers were kept at 37 °C overnight. On the second day, 750 μl DMEM containing 10% FBS was added to the bottom of the chambers in the well and 200 μl cell suspension at the density of 1×10^5/ml was seeded into the top chamber. After a culture of 24 h, the non-invading cells on the top chamber were removed with cotton swab. Then the chambers were washed twice with PBS, fixed with 75% alcohol for 10 min, stained with 0.5% Crystal Violet for 5 min at room temperature (RT) and photographed in four independent ×10 fields/well. The stained cells were counted to quantify the invasive cells.

2.6. Calcium imaging

Briefly, U87 cells grown on coverslips were loaded with Fura-2AM (5 μM) (Molecular Probes, OR, USA) in Hanks Balanced Salt Solution supplemented with 20 mM D-glucose and 10 mM HEPES (HBSS) (Gibco, MD, USA) for 45 min, and equilibrated for 30 min in dark at RT. Then, cells were tightly mounted on open-bath imaging chamber containing HBSS. Using the Nikon inverted fluorescence microscope, cells were excited at 345 and 385 nm and the emission fluorescence at 510 nm was recorded in an XYT-plane fashion. Images were collected and analyzed with the MetaFluor image-processing software (Universal Imaging Corp, PA, USA). Standard "Ca^{2+}-off and Ca^{2+}-on" protocol was used to detect the SOCE. After the baseline recording for 120 s in Ca^{2+}-free HBSS, the recording buffer was changed with Ca^{2+}-free HBSS supplemented with TG (2 μM) and the calcium signaling was recorded for 720 s. Then, the buffer was changed with HBSS supplemented with TG (2 μM) and the calcium signaling was recorded for 360 s. The results were calculated and shown as F/F_0 and area under curve (AUC).

2.7. Western blot

Protein was extracted from U87 cells with RIPA lysis buffer. Protein concentration was quantified by using a BCA protein kit (Thermo Scientific, USA). 30 μg protein was loaded on 10% SDS-PAGE gels. After electrophoresis, the proteins were transferred to nitrocellulose membranes. Then the membranes were blocked with 5% skim milk and incubated at 4 °C overnight with the appropriate primary antibodies: STIM1 (1:1000) (CST, Danvers, MA, USA), Orai1 (1:500) (Santa Cruz Biotech, Santa Cruz, CA, USA) and β-Actin (1:2000) (Abcam, MA, USA). Immunoreactivity was detected by incubation with horseradish peroxidase-conjugated secondary antibodies (1:20000, CST, MA, USA) followed by chemiluminescent substrate development (Thermo Scientific, USA). Optical densities of the bands were calculated using a MiVnt image analysis system (Bio-Rad, CA, USA).

2.8. Quantitative RT-PCR (qRT-PCR)

A 2 μg template RNA was used to synthesize the first strand of cDNA using a reverse transcription kit (Takara, Dalian, China). Real-time PCR of cDNA was performed using the forward and reverse primer sequences: STIM1: forward: 5′-AGG AGC CTC ATC CTA ATC TCA CTC A-3′; reverse: 5′-GGC ATC CAC TCA TGC TCC AA-3′; STIM2: