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UNBS5162 Inhibits U251 Glioma Cells Proliferation and Metastasis by Promoting Apoptosis

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glioma cells proliferation, migration and invasion. Cell viability, migration and invasion capabilities of U251 cells were examined by Cell Counting Kit-8 (CCK-8) and transwell assays. The cell apoptosis was tested by Annexin V-FITC/PI and þow cytometry. Apoptosis-associated proteins and the related-markers of Phosphatidylinositol 3-kinase (PI3K) were examined by western blot analysis. The CCK-8 test suggested that UNBS5162 markedly suppressed the viability of glioma cell line U251. UNBS5162 markedly repressed glioma cell migration and invasion, which was shown from results of transwell assay. Further, UNBS5162 promoted glioma cell apoptosis rate, accompanied by reduced expression level of anti-apoptotic protein Bcl-2 and elevated expression level of pro-apoptotic protein Active Caspase-3 and Bax in U251 cell line administrated with UNBS5162. Additionally, UNBS5162 suppressed the Phosphatidylinositol 3-kinase (PI3K) signaling pathways in U251 cell line. Therefore, the current ýnding unveils that UNBS5162 can repress glioma cell viability and induce glioma cell apoptosis by regulating the PI3K signaling pathway, suggesting it might be a potentially promising drug target for clinical glioma therapy.

UNBS5162; Glioma; Proliferation; Apoptosis

Glioma is recognized as one of the commonest and the most deadly human intracranial tumors, and it accounts for more than 40% of primary central nervous system tumors [1,2]. e life expectancy of glioblastoma (GBM) patients is 6 to 12 months. Even in the most advantageous factors, the patients will die within 2 years [3]. According to the World Health Organization's neuropathological classi cation, glioma is divided into astrocytoma, GBM, oligodendrocytoma and choroid papilloma and so on. e degree of malignancy of glioma is

A er cell treated with UNBS5162 for 24 h, cell migration and invasion were tested using the transwell assay in accordance with producer's instructions. For examining cell migration, 300 µl serum-free medium containing with 1 × 10^5 cell was plated into the top of transwell chamber (Millipore Corp., Billerica, MA, USA) and 500 µl medium with 10% FBS was plated into the low-storied chamber. A er cultivation of 12 h, cells on the upper chamber were eliminated using cotton swabs and cells on the low-storied chamber were xed in 4% paraformaldehyde, then stained with 0.1% crystal violet. Next, ve random elds were chosen under microscope, photographed and counted. For examining cell invasion, Matrigel (BD Biosciences, San Diego, CA, USA) dissolved with serum-free DMEM diluted at 1:6 overnight in advance and paved to the upper chamber of transwell, the remaining steps resembled migration assay [17].

Cell proteins were extracted with RIPA lysis bu er and inhibitors speci c to protease and phosphatase (Beyotime). Proteins concentrations were tested by bicinchoninic acid (BCA) (Beyotime) kit. en, protein with equal amount (20 µg) was separated in 10% sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene di uoride (PVDF) membranes. A er blocking with 5% BSA for 1.5 h, membranes were cultivated with primary antibodies at 4°C overnight and then incubated with secondary antibodies in dark place at room temperature for 2 h. An enhanced-chemiluminescence (ECL, Beyotime) detection system was applied to visualize the bands. e gray value was scanned by Quantity One (Bio-Rad). GAPDH was used as the internal control. Membranes were incubated with the antibodies as follows: primary antibody rabbit anti-human, AKT, p-AKT, mTOR, p-mTOR, p-P70S6K, Bcl-2, Bax, Active Caspase-3 (at dilute of 1 : 1000, Cell Signaling Technology, Danvers, MA, USA), GAPDH (at dilute of 1:5000, Proteintech Group, Inc, Rosemont, IL, USA); corresponding horseradish peroxidase (HRP)-labelled secondary antibody (at dilute of 1:5000, Proteintech Group, Inc).

A er 10 µM UNBS5162 treating U251 cells for 24 h, cells apoptosis percentage was tested by Annexin V-FITC/Propidium Iodide (PI) and ow cytometry analyses in light of producer's manuals. A er cell were collected, centrifuged and resuspended, 1 × binding bu er were added to resuspend cells, and cells density were adjusted to 3 × 10^6/ml. Cells were stained with 5 µl Annexin V-FITC and 10 µl PI (Beyotime) in dark place for 20 min. Finally, cells were detected and analyzed using a FACS Calibur instrument (BD Biosciences).

SPSS18.0 statistical analysis so ware (SPSS Inc., Chicago, IL) and GraphPad Prism 6.0 (GraphPad So ware, Inc., LaJolla, CA, USA) were utilized to analyze the experimental data. e data are exhibited as the mean ± standard deviation (SD). e Student's t test, ANOVA and post hoc Dunnett were used to analyze statistical correlation of data. $p<0.05$ indicated statistically remarkable di erence.

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To assess the impact of UNBS5162 on GBM, we chose glioma cell U251 and the normal cell HEB using CCK-8 assay. 10 µM and 100 µM UNBS5162 treatment were e ective on U251 cells (Figure 1A, $p<0.01$). e viability of HEB cells was repressed only in 100 µM UNBS5162

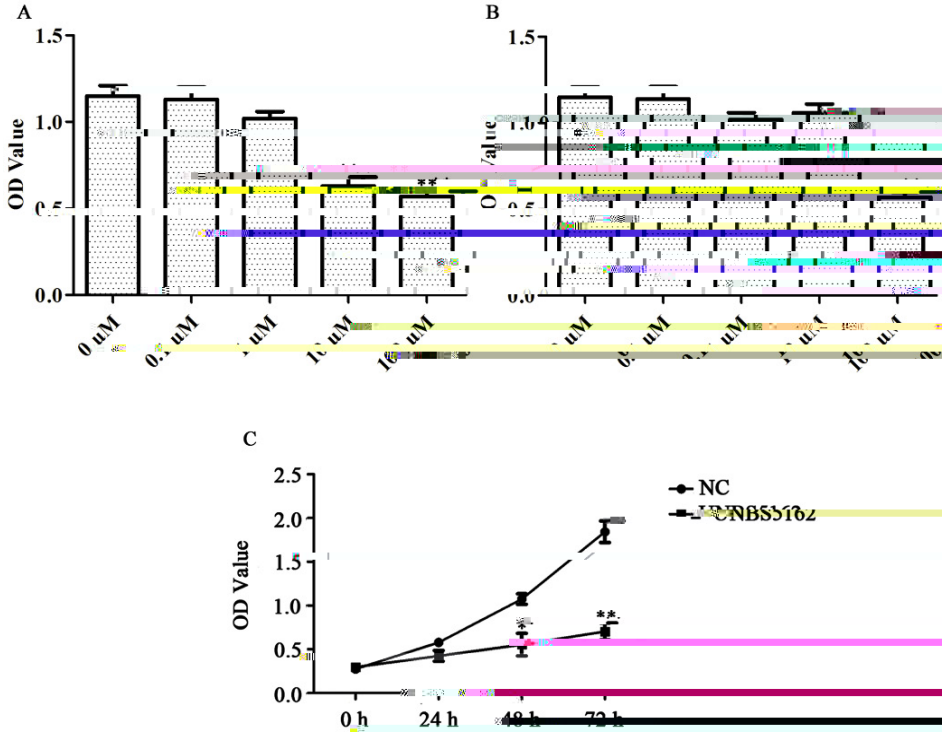

Figure 1: UNBS5162 inhibited U251 cell proliferation by the CCK-8 assay (*p<0.05).

treated group (Figure 1B, $p<0.01$). So in the following experiments, 10 μM UNBS5162, a highly-e ective and low-toxicity dose, was used. And the results also show that UNBS5162 obviously decreased the OD value of U251 cells in a time-dependent mode (Figure 1C, $p<0.05$, $p<0.01$).

ese data indicated that UNBS5162 hampered glioma cell U251 viability e ectively.

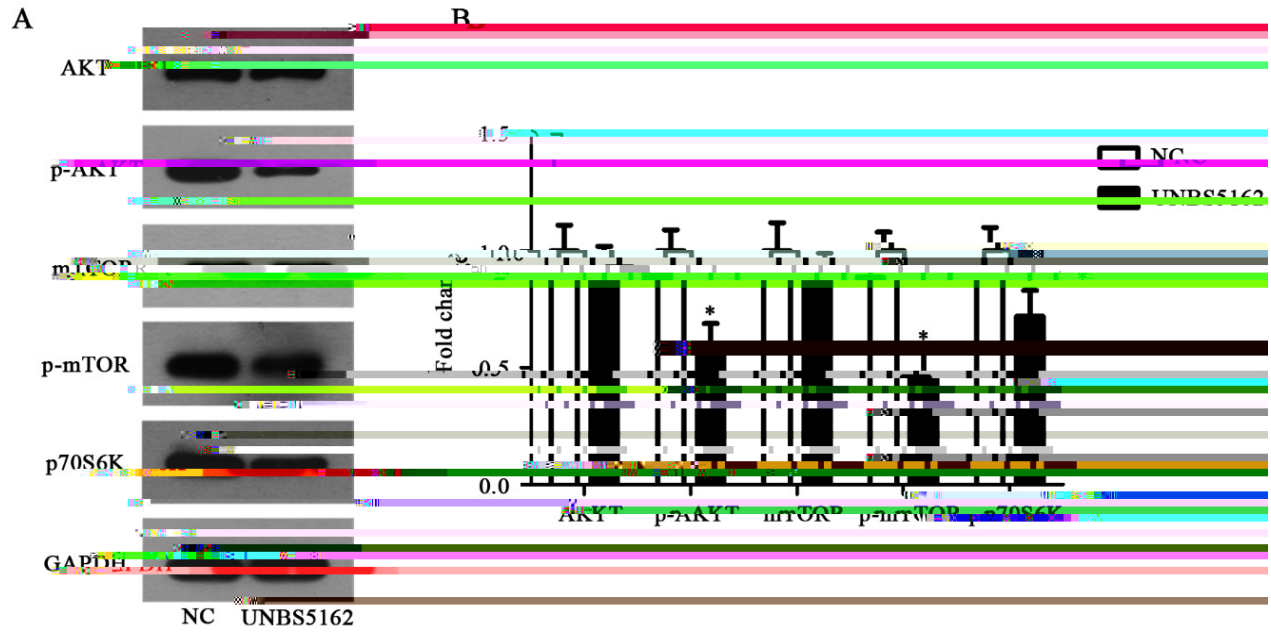

Figure 4: Functional role of UNBS5162 on the PI3K signaling in U251 cells. (A) Expression levels of PI3K signaling related proteins were tested in the U251 cells treatment with UNBS5162 by western blot analysis. (B-F) Protein bands were in contrast with control group (*p<0.05).

Figure 5: A diagram showing our ýndings associated with tumor-inhibitory role of UNBS5162 on glioma cell.

esophageal, showing it has an e ect on anti-angiogenesis. ymus, bone marrow, gastrointestinal tract and so on have been determined as targets for UNBS5162. Meanwhile, researches have suggested that *o* incubation of UNBS5162 with human cancer cells obviously reduced the expression of the proangiogenic chemokines (e.g. CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, and CXCL8). Because of these features, UNBS5162, as an antitumor agent, has considerable prospects to provide a new drug target for clinical cancer treatment and research. Researches have shown that UNBS5162 treatment can obviously reduce vascular surface area in human prostate cancer xenogra s *o*. But the functional action of UNBS5162 in other cancers remains unknown. In this work, we performed western blot assay, CCK8 proliferation test, transwell invasion/migration analyses and ow cytometry detection with U251 cell lines under di erent administration conditions. Our results suggested that UNBS5162 signi cantly repressed cell proliferation, migration/invasion capabilities and promoted cell apoptosis. In the future, UNBS5162 may become a potential molecular marker for therapy of glioma.

In our report, transwell assay showed that UNBS5162 inhibited glioma cell U251 invasion and migration. Furthermore, UNBS5162 could promote cell apoptosis and upregulate pro-apoptotic protein Active Caspase-3 and Bax, as well as downregulate anti-apoptotic protein Bcl-2. Bax/Bcl-2 and activation of Caspase-3, identi ed as a pivotal mechanism of action of anti-tumoral drug, are involved in cell death and apoptosis of tumor cells, which is developed as an important determining factor of chemotherapy e ectiveness. Studies have shown that changes in tumor cell apoptosis are mainly due to abnormal expression of apoptosis-related factors and abnormal of cell apoptotic signal transduction pathways [21]. PI3K, known as a family of lipid kinases, plays an essential part in cell growth by inhibiting apoptosis in various cancers [22]. Our results showed that U251 cell apoptosis was associated with the PI3K pathway. In addition, PI3K pathway was involved in the development of cancer through AKT, mTOR and p-P70S6K, which suggests PI3K pathway to be the action pathways of UNBS5162 a ecting on cancer. e hallmarks p-AKT and p-mTOR in the pathway exert an important action in the proliferation and metastasis of tumor cells. Activation of Akt antagonizes angiogenesis, tumor metastasis and invasion and phosphorylates protein kinase mTOR [23]. Researchers have suggested that PI3K/AKT/mTOR pathway facilitates the resis

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