Cucurbitacin E inhibits the proliferation of glioblastoma cells via FAK/AKT/GSK3β pathway

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Abstract. Glioblastoma (GBM) is the most common primary intracranial tumor in the brain with high growth rate and high mortality rate. Cucurbitacin E (CUE), a tetracyclic triterpene compound derived from species of the genus *Cucurbita*, has been demonstrated to display significant antitumor effects on various malignancies. In the present study, the effects of CUE on GBM and its underlying molecular mechanisms were explored. The data revealed that CUE inhibited the proliferation of the GBM cell lines U87-MG and U251-MG in a dose- and time-dependent manner. Mechanistically, CUE reduced the phosphorylation of focal adhesion kinase (FAK), protein kinase B (AKT), and glycogen synthase kinase-3β (GSK3β) at both basal and epidermal growth factor (EGF)-induced levels. Moreover, CUE inhibited the proliferation of U87-MG and U251-MG cells by blocking EGF-induced phosphorylation of the FAK, AKT and GSK3β. Subsequently, CUE reduced the expression of cyclinD1 and cyclinB1. Collectively, these results indicated that CUE inhibited the proliferation of U87-MG and U251-MG cells by suppressing the FAK/AKT/GSK3β signaling pathway, which also suggested that CUE has potential application in treating GBM.

Introduction

Glioblastoma (GBM) is the most common malignant tumor of the brain, accounting for >50% of primary intracranial malignant tumors, and is also regarded as one of the most intractable early-death solid tumors in neurosurgery due to its strong aggression, rapid postoperative recurrence and high mortality (1). At present, GBM therapy continues to be a challenging medical issue. Multiple drug resistance and a high recurrence rate are the two main barriers to effective treatment; GBM therapy resistance has been attributed to tumor heterogeneity, hypermutation, hypoxia and immune-suppressive tumor microenvironment (2,3). Despite progress in the main treatment modalities for GBM, including surgery, radiotherapy and chemotherapy, the outcome for patients remains almost generally fatal, with a median survival of <2 years (4,5). Therefore, it is critically necessary to identify more effective therapeutic targets and improved therapeutic strategies for the treatment of GBM.

With the rapid advancement of natural medicines, the outstanding antitumor activity of traditional Chinese medicine monomers has steadily drawn more attention in recent years. Cucurbitacin E (CUE), a highly oxidized tetracyclic triterpene compound isolated from species of the genus *Cucurbita*, has been reported to exert anti-inflammatory and anti-analgesic properties (6). During the past few years, numerous studies have demonstrated that CUE has also anticancer effects, including inhibiting the proliferation of various cancer types, including gastric, liver, lung and colon cancers (7,8), as well as the capacity of inducing apoptosis and G2/M arrest in a number of cancer cells (9,10). CUE can also disrupt the cytoskeleton of actin and vimentin in prostate cancer cells, alter the morphology of tumor cells (11,12), and inhibit angiogenesis in human prostate tumors through the Janus kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3) signaling pathway mediated by vascular endothelial growth factor receptor 2 (VEGFR2) (13). Additionally, it has been revealed that CUE can inhibit Yes-associated protein signaling pathway and brain metastases of human non-small cell lung cancer (14), suppress the proliferation and invasion of osteosarcoma cells through phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway (15) and induce cellular senescence in colon cancer via modulating the miR-371b-5p/transcription factor AP-4 (TFAP4) axis (16). Of note, a previous study has shown that CUE inhibits the growth of GBM by arresting the

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cell cycle at G2/M phase through GADD45γ gene expression and blockade of cyclinB1/CDC2 complex (17). There is also evidence suggesting that CUE delays the onset of mitosis in GBM cells by upregulating GADD45β rather than downregulating the phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) (18). However, the underlying mechanisms by which CUE exerts its anticancer effects on GBM remain to be fully elucidated.

Receptor tyrosine kinases (RTKs), a family of cell surface receptors, play critical roles in cell proliferation, survival and migration (19). The epidermal growth factor receptor (EGFR), which belongs to the ErbB family of RTKs, is frequently mutated and/or overexpressed in numerous human cancers (20). Once activated, EGFR activates numerous downstream signaling pathways, including mitogen-activated protein kinase (MAPK), PI3K/AKT, JAK/STAT and protein kinase C (PKC), which are highly associated with the proliferation, migration, angiogenesis and apoptosis of tumor cells (21-23). Focal adhesion kinase (FAK) plays an important role in tumor development. Previous studies have revealed that FAK activation promotes breast cancer angiogenesis (24). In an FAK-deficient mouse model, the cell motility is reduced and focal adhesion contact formation is enhanced (25). In addition, inhibition of the FAK and EGFR signaling pathways synergistically promotes apoptosis in breast cancer cells (26).

Protein kinase B (AKT), a serine/threonine kinase, is dysregulated in human cancers and plays a crucial role in tumor growth (27). Once activated, it transmits signals to numerous downstream effectors, including glycogen synthase kinase-3β (GSK3β) and forkhead box protein O (FOXO) (27). Collected evidence suggests that the FAK/AKT/GSK3β pathway is essential for the proliferation, invasion and metastasis of numerous cancers, including GBM (28-32). Furthermore, it has been identified that dysregulation of the cell cycle checkpoint proteins, including cyclinB1 and cyclinD1, contributes to the uncontrolled cellular growth and tumorigenesis (33). For example, cyclinB1 and cyclinD1 are overexpressed in numerous cancers which are involved in neogenesis and progression (34,35). However, it is not clear whether these signaling cascades are involved in the antitumor effect of CUE.

In the present study, it was aimed to demonstrate the effects of CUE on the proliferation of GBM cells and reveal the possible underlying molecular mechanisms.

Materials and methods

Antibodies and reagents. CUE was purchased from MedChemExpress. Antibodies for western blot analysis, including phospho-FAK (Tyr397) (1:2,000; cat. no. 8556S), FAK (1:2,000; cat. no. 3285S), phospho-AKT (Ser473) (1:2,000; cat. no. 4058S), AKT (1:2,000; cat. no. 4691S), phospho-GSK3β (1:2,000; cat. no. 9336S), GSK3β (1:2,000; cat. no. 9315S; all from Cell Signaling Technology, Inc.), cyclinB1 (1:2,000; cat. no. ab181593), cylinD1 (1:10,000; cat. no. ab134175), cyclinA2 (1:2,000; cat. no. ab181591), cyclinE1 (1:5,000; cat. no. ab133266; all from Abcam), Proliferating Cell Nuclear Antigen (PCNA; 1:2,000; cat. no. 10205-2-AP; Proteintech Group, Inc.), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; monoclonal, rabbit anti-mouse; 1:5,000; cat. no. KC-5G4; Zhejiang Kangchen Biotech, Co., Ltd.), goat anti-rabbit IgG HRP-linked antibody (1:5,000; cat. no. 31460) and goat anti-mouse IgG HRP-linked antibody (1:5,000; cat. no. 31431; both from Thermo Fisher Scientific, Inc.). PF-562271 (FAK selective inhibitor, 10 μM) (cat. no. HY-10459) was purchased from MedChemExpress. EGF (cat. no. AF-100-15; 20 ng/ml) was purchased from PeproTech, Inc. Reagents used for cell culture including Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and trypsin-EDTA solution were purchased from Gibco; Thermo Fisher Scientific, Inc.

Cell culture. Human GBM cell lines U87-MG (derived from GBM of unknown origin; American Type Culture Collection no. HTB-14; cat. no. TCHu 138) and U251-MG (cat. no. TCHu 58) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) in 2018. Authentication testing of U87-MG and U251-MG cell lines was performed by Shanghai Biowing Applied Biotechnology Co. Ltd. via STR profiling. STR profiles match the standards recommended for U87-MG and U251-MG cell lines authentication. U87-MG and U251-MG cells were cultured in DMEM containing 4.5 g/l glucose supplemented with 10% FBS. Cells were maintained at 37˚C in an incubator with 5% CO_2.

Cell viability. A total of $2.0x10^3$ cells/well were seeded onto 96-well plates and incubated at 37˚C in an incubator with 5% CO_2. After adherence, the cells were incubated with different concentration of CUE (0, 0.01, 0.025, 0.25, 2.5 and 25 μM) for 24 h. Then, 10 μl Cell Counting Kit-8 (CCK-8; cat. no. CK04; Dojindo Laboratories, Inc.) reagent was used to detect the cell viability, and the optical density (OD) at 450 nm was measured. Cell survival was expressed as fold of the control group. The optimal treatment concentration was 2.5 μM and thus was selected for the following experiments.

Western blot analysis. Cells were cultured overnight at 37˚C in an incubator. After treatment, the cells were washed with PBS, and cell lysis buffer (cat. no. R0010; Beijing Solarbio Science & Technology Co., Ltd.) was added at 4˚C for 25 min. The lysate was centrifuged at 12,000 x g at 4˚C for 15 min. The protein concentration was measured by bicinchoninic acid protein assay. The equivalent amounts of protein (20 μg) were loaded into 10% SDS-PAGE gel (30% acrylamide) and separated at 110 V voltage. The proteins in the gel were transferred to a PVDF membrane and blocked in 5% skim milk for 2 h at room temperature. Subsequently, the membrane was incubated with different primary antibodies including phospho-FAK, FAK, phospho-AKT, AKT, phospho-GSK3β, GSK3β, cyclinB1, cylinD1, cyclinA2, cyclinE1 and GAPDH overnight at 4˚C and then incubated with HRP-conjugated anti-rabbit IgG secondary antibody (1:5,000) or HRP-conjugated anti-mouse IgG secondary antibody (1:5,000) for 90 min at room temperature. Immunoreactive bands were visualized by enhanced chemiluminescence (Pierce; Thermo Fisher Scientific, Inc.) and semi-quantified using ImageJ software (version 1.47t; National Institutes of Health).

Immunofluorescence confocal microscopy. A total of $6.0x10^4$ cells/well were seeded onto 12-well plates and treated

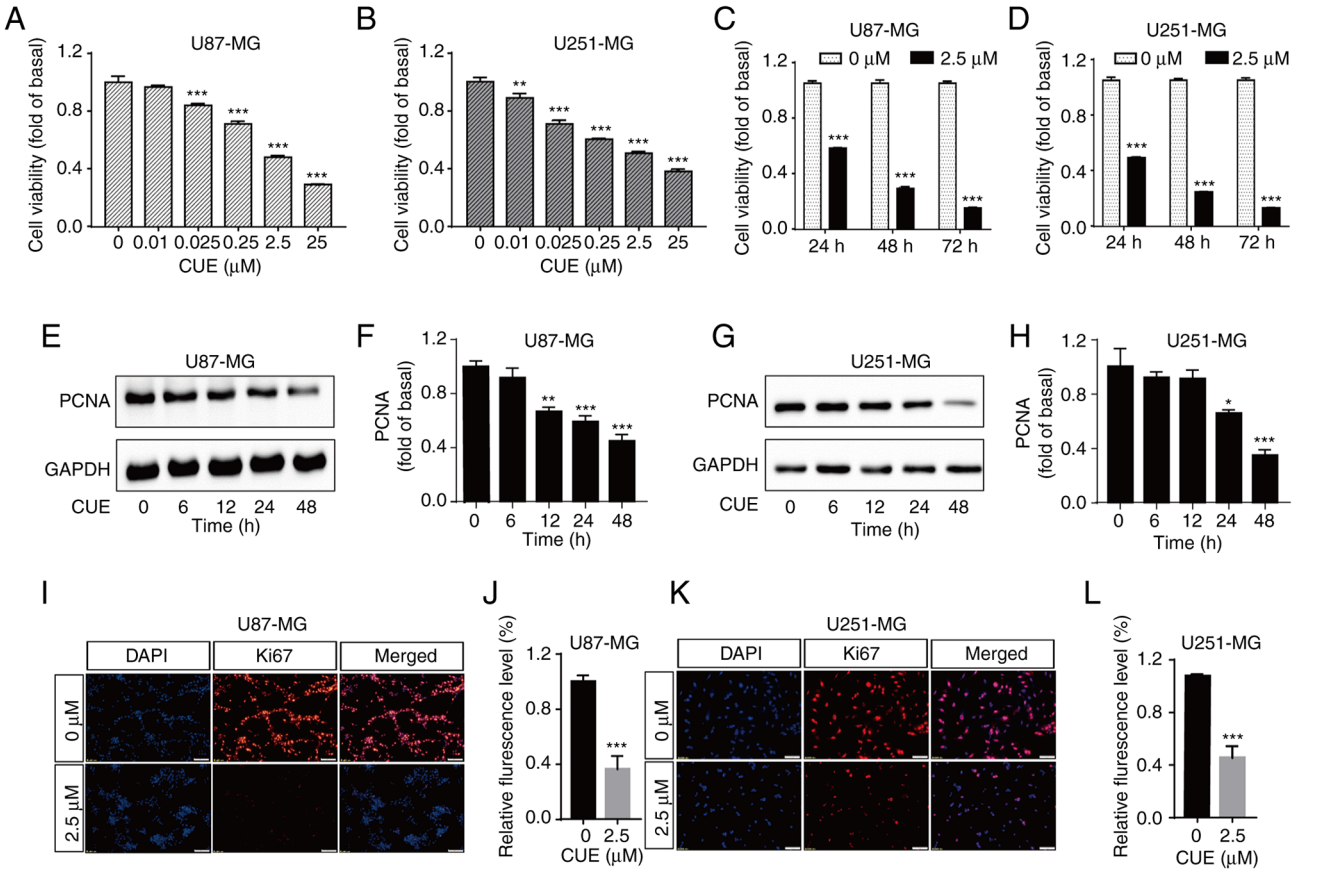

Figure 1. CUE inhibits the proliferation of glioblastoma cells. (A and B) Dose-dependent effects of CUE on the proliferation of glioblastoma cells. (A) U87-MG and (B) U251-MG cells were treated with increasing concentrations of CUE as indicated for 24 h and the cell viability was measured using Cell Counting Kit-8 assay. (C and D) Time-dependent effects of CUE on the proliferation of glioblastoma cells. (C) U87-MG and (D) U251-MG cells were treated with CUE (2.5 μM) for 24, 48 and 72 h. (E and G) The expression of PCNA in glioblastoma cells was measured by western blot after CUE treatment. (F and H) Quantitation of western blot results shown in panels E and G. (I and K) The expression of Ki67 in glioblastoma cells was measured by immunofluorescence after CUE treatment (Scale bar, 50 μm; 20X objective). (J and L) Quantitation of fluorescence level results demonstrated in panels I and K. Data are presented as the mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001 vs. the control group. CUE, Cucurbitacin E.

with 2.5 μM CUE for 24 h. After washed with pre-cooled PBS, the cells were fixed with 4% paraformaldehyde for 15 min followed by permeabilization with 0.2% Triton X-100 in PBS for 10 min. Subsequently, the cells were blocked in PBST (0.1% Tween-20) containing 1% bovine serum albumin (cat. no. A8020; Beijing Solarbio Science & Technology Co., Ltd.) for 40 min. After washing with pre-cooled PBS, the cells were incubated with anti-human rabbit Ki67 antibody (1:1,000; cat. no. 27309-1-AP; Proteintech Group, Inc.) overnight at 4˚C in a wet box. Next, the cells were then washed with pre-cooled PBS and incubated with anti-rabbit Alexa Fluor® 594 secondary antibody [(1:500; cat. no. Ab150080; Abcam), Excitation wavelength: 590 nm; Emission wavelength: 617 nm] for 1 h. The nucleus was stained with DAPI (cat. no. AR1176, no dilution; Boster Biological Technology Co., Ltd.), and the fluorescence signal was detected under an inverted fluorescent microscope.

Statistical analysis. Data are presented as the mean ± SEM of at least three independent experiments and were analysed via GraphPad Prism 7.0 (Dotmatics). Unpaired Student's t-test (two groups) or one/two-way ANOVA with Bonferroni's multiple comparison tests (more than two groups) were used for comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

CUE inhibits the proliferation of GBM cells. The effect of CUE on the growth of GBM cells was first examined using CCK-8 assay. Compared with the control group, CUE significantly inhibited the proliferation of U87-MG and U251-MG cells in a dose-dependent manner (Fig. 1A and B). The half maximal inhibitory concentration was ~2.5 μM. To further detect the influence of CUE on cell viability at different treatment times, the cells were exposed to 2.5 μM CUE for 24, 48 or 72 h, respectively. The results demonstrated that CUE significantly inhibited the proliferation of GBM cells in a time-dependent manner (Fig. 1C and D).

To further confirm the effect of CUE on the proliferation of GBM cells, the expression of PCNA and Ki67, which are important indicators of tumor cells proliferation, was then detected. The cells were treated with 2.5 μM CUE, and then the expression of Ki67 and PCNA were measured by immunofluorescence and western blot assays, respectively. It was observed that the expression of PCNA was significantly down-regulated in CUE-treated cells as compared with the control group (Fig. 1E-H). In addition, red fluorescence intensity of the experimental group was significantly decreased, indicating that CUE-treated cells had less ki67 expressed than untreated

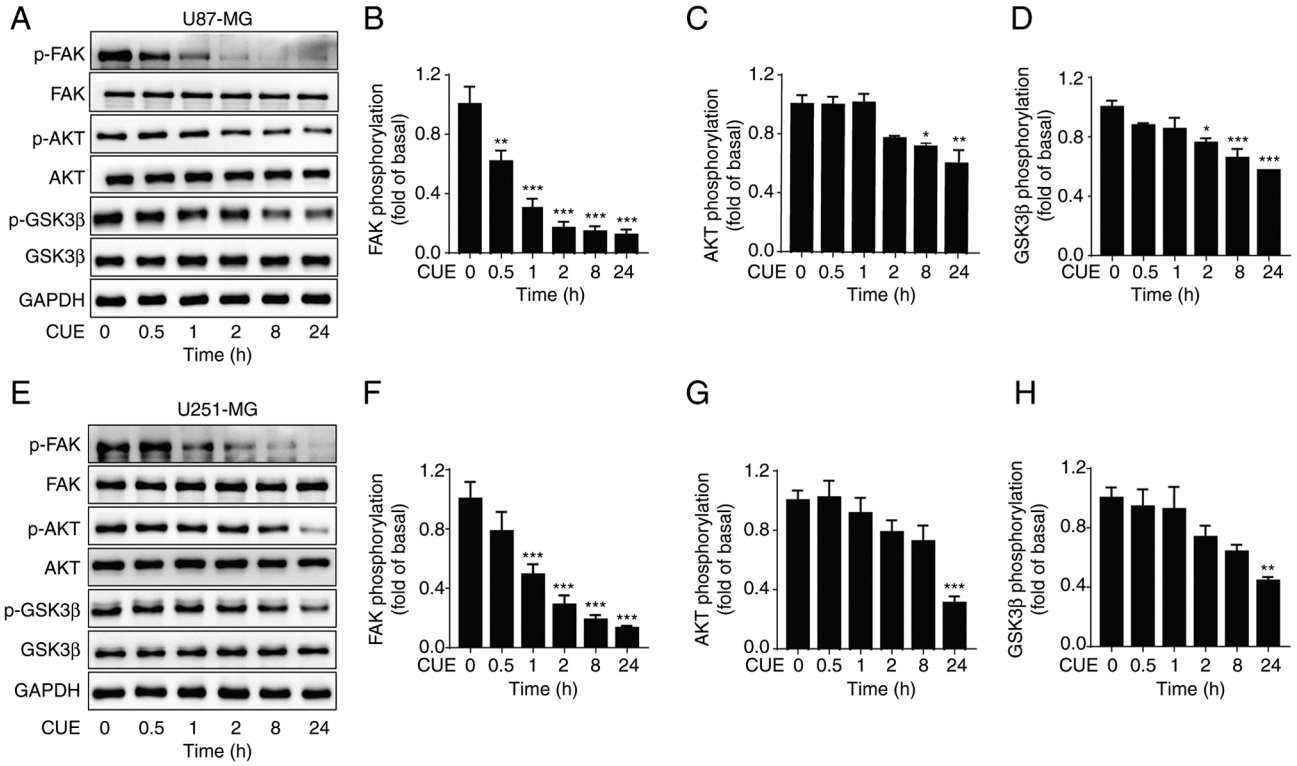

Figure 2. CUE downregulates the phosphorylation level of FAK, AKT and GSK3β in glioblastoma cells. (A and E) (A) U87-MG cells and (E) U251-MG were treated with CUE (2.5 μM) for the indicated time intervals. FAK, AKT and GSK3β phosphorylation were determined using western blot analysis. (B-D and F-H) Quantitation of the western blot results as shown in panels A and E. Data are presented as the mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001 vs. the control group. CUE, Cucurbitacin E; FAK, focal adhesion kinase; p-, phosphorylated.

cells (Fig. 1I-L). Taken together, these results suggested that CUE significantly inhibits the proliferation of GBM cells (Fig. 1E-L).

CUE downregulates the phosphorylation level of FAK, AKT and GSK3β in GBM cells. FAK is a tyrosine kinase, which is closely related to the occurrence and development of tumors. Numerous studies have shown that downregulating the expression of FAK greatly inhibits the proliferation, migration and invasion of tumor cells (32,36,37). To evaluate whether the FAK-mediated signal transduction pathways, including AKT and GSK3β, were engaged in the antitumor effect of CUE, the phosphorylation level of FAK, AKT and GSK3β was measured in GBM cells following 2.5 μM CUE treatment at various time-points (0, 0.5, 1, 2, 8 and 24 h). The results revealed that CUE significantly decreased the phosphorylation level of FAK, AKT and GSK3β in U87-MG cells (Fig. 2A-D). Similar results were observed in U251-MG cells (Fig. 2E-H). These results suggested that CUE may inhibit the proliferation of GBM cells through FAK/AKT/GSK3β signaling pathway.

CUE blocks EGF-induced FAK, AKT and GSK3β phosphorylation in GBM cells. It has been reported that EGF upregulates the phosphorylation level of FAK, AKT and GSK3β in GBM cells (38-40). The effects of CUE on EGF-induced FAK, AKT and GSK3β phosphorylation were then explored. It was demonstrated that EGF (20 ng/ml) significantly increased the phosphorylation of FAK, AKT and GSK3β in both GBM cell lines, which were significantly blocked by CUE pre-treatment (Fig. 3A-D). In order to confirm whether AKT and GSK3β were in the downstream of FAK, the impact of PF-562271 (10 μM), a selective inhibitor of FAK, was examined on EGF-induced AKT and GSK3β phosphorylation in GBM cells. The results showed that PF-562271 significantly inhibited EGF-induced AKT and GSK3β phosphorylation (Fig. 3E-H), suggesting that AKT and GSK3β were downstream of FAK.

CUE inhibits the proliferation of GBM cells through the EGF-mediated FAK/AKT/GSK3β signaling pathway. After confirming that CUE inhibited the proliferation of GBM cells and blocked EGF-induced FAK/AKT/GSK3β phosphorylation, it was then investigated whether CUE inhibits the proliferation of GBM cells through the FAK/AKT/GSK3β signaling pathway. To this end, the effect of CUE on EGF-induced cell proliferation was first explored. It was found that CUE (2.5 μM) significantly inhibited the proliferation of EGF-induced GBM cells after treating the cells with it, independently of EGF (20 ng/ml) being present or absent (Fig. 4A and B). Similarly, FAK-specific inhibitor PF-562271 (10 μM) also significantly inhibited EGF-induced proliferation of GBM cells (Fig. 4C and D). Collectively, these results indicated that FAK/AKT/GSK3β signaling pathway is involved in the anti-proliferative effect of CUE in GBM cells.

CUE inhibits the expression of cyclinB1 and cyclinD1 in GBM cells. The effects of CUE on the expression of cyclinB1 and

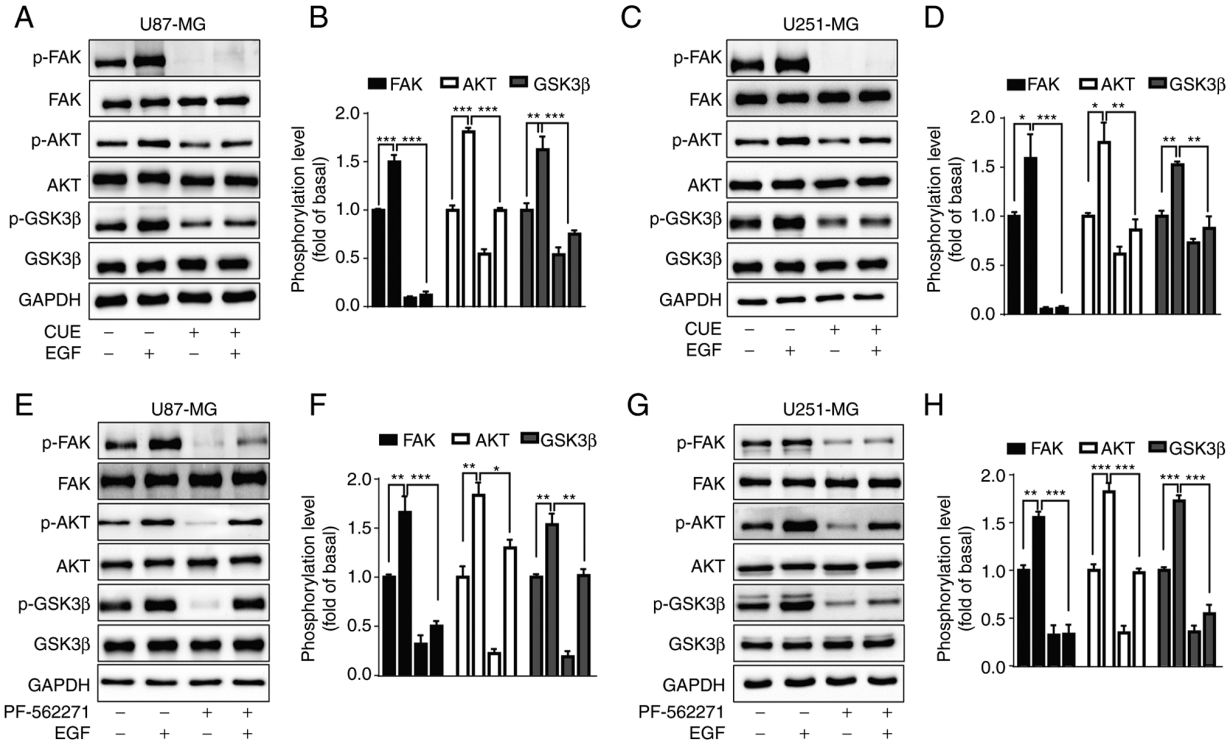

Figure 3. CUE blocks EGF-induced FAK, AKT and GSK3β phosphorylation in glioblastoma cells. (A and C) Effects of CUE on EGF-induced FAK, AKT and GSK3β phosphorylation in (A) U87-MG cells and (C) U251-MG cells. Cells were pre-treated with CUE (2.5 μM) for 24 h and then together treated with EGF (20 ng/ml) for 10 min. (B and D) Quantitation of western blot results as shown in panels A and C. (E and G) Effects of PF-562271 on EGF-induced FAK, AKT and GSK3β phosphorylation in (E) U87-MG cells and (G) U251-MG cells. Cells were pre-treated with PF-562271 (10 μM) for 30 min and then together treated with EGF for 10 min. (F and H) Quantitation of western blot results as shown in panels E and G. Data are presented as the mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001 vs. the control group. CUE, Cucurbitacin E; FAK, focal adhesion kinase; p-, phosphorylated.

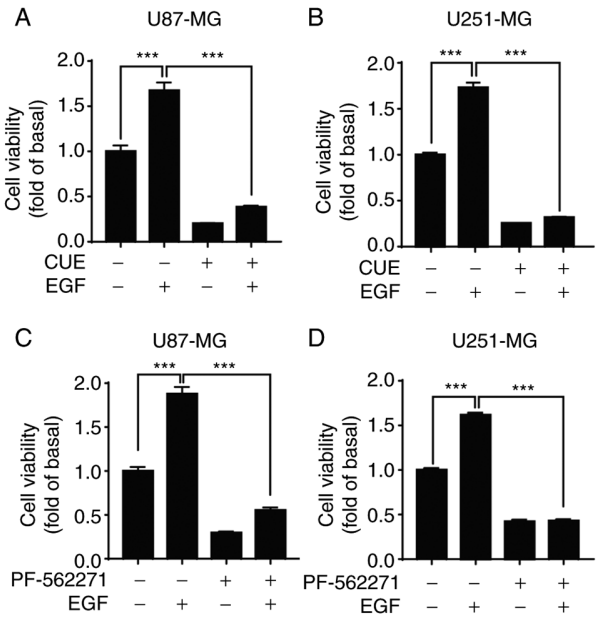

Figure 4. CUE inhibits proliferation of glioblastoma cells through the EGF-mediated FAK/AKT/GSK3β signaling pathway. (A and B) Effects of CUE on EGF-induced cell proliferation in (A) U87-MG cells and (B) U251-MG cells. Cells were treated with CUE (2.5 μM) in the absence or presence of EGF (20 ng/ml) for 72 h and then cell viability was measured using CCK-8 assay. (C and D) Effects of PF-562271 on EGF-induced cell proliferation in (C) U87-MG cells and (D) U251-MG cells. Cells were treated with PF-562271 (10 μM) in the absence or presence of EGF (20 ng/ml) for 72 h and then cell viability was determined using CCK-8 assay. Data are presented as the mean ± SEM. ***P<0.001 vs. the control group. CUE, Cucurbitacin E; CCK-8, Cell Counting Kit-8.

cyclinD1, two cyclins which play crucial roles in the proliferation of tumor cells, were also evaluated. In GBM cells treated with 2.5 μM CUE, cyclinB1 and cyclinD1 expression significantly decreased as compared with the control group (Fig. 5A-F). Notably, in contrast to cyclinB1 and cyclinD1, CUE had no discernible effect on cyclinA2 and cyclinE1 expression (Fig. S1).

Discussion

In the present study, the effect of CUE on GBM cell proliferation and its underlying molecular mechanisms were investigated. The results demonstrated that CUE reduced the expression of cyclinB1 and cyclinD1, downregulated the FAK/AKT/GSK3β signaling pathway and consequently inhibited the proliferation of GBM cells.

Traditional Chinese medicine offers a number of drug candidates for cancer treatment. CUE, a member of the *Cucurbitaceae* family, inhibits the growth of multiple cancers (7,8). Previous studies have stated that CUE inhibited breast tumor metastasis (41) and induced autophagy of cancer cells by decreasing mTORC1 signaling and increasing Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) activity (42). In addition to its anticancer properties, CUE also improved liver fibrosis and inhibited the production of inflammatory factors (43,44). However, the effect of CUE on GBM is rarely reported except in two previous studies showing that CUE could induce mitosis delay in GBM cells by upregulating GADD45β and inhibit GBM

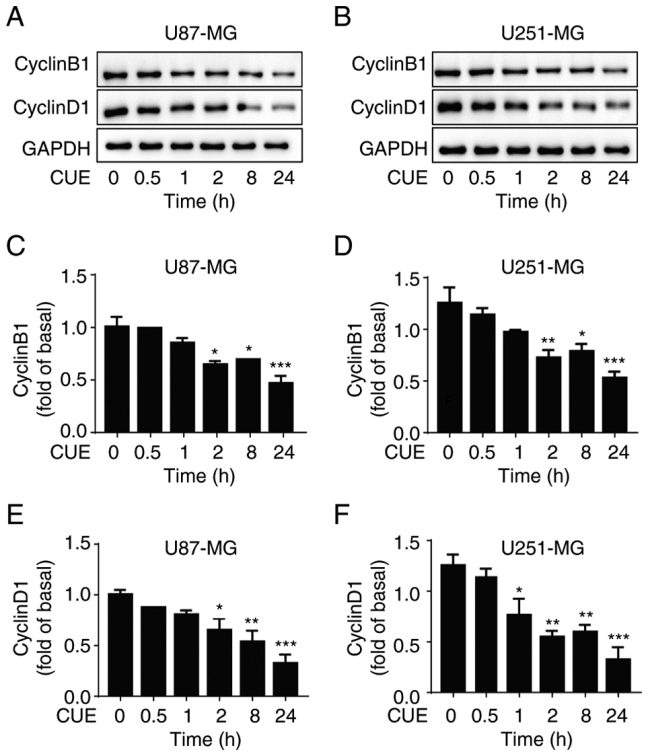

Figure 5. CUE inhibits the expression of cyclinB1 and cyclinD1 in glioblastoma cells. (A) U87-MG and (B) U251-MG cells were treated with CUE (2.5 μM) for the indicated time intervals. Expression levels of cyclinB1 and cyclinD1 were measured using western blot analysis. (C-F) Quantitation of western blot results as shown in panels A and B. Data are presented as the mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001 vs. the control group. CUE, Cucurbitacin E.

growth via arresting the cell cycle at G2/M phase (17,18). In the present study, it was found that CUE significantly decreased the cell viability of GBM cell lines U87-MG and U251-MG in a dose-dependent and time-dependent manner. This finding was further confirmed by measuring the expression of Ki67 and PCNA, which are nuclear antigens linked to dividing cells, and both can be used to assess the level of cell proliferation (45). Using an immunofluorescence assay, it was identified that the amount and intensity of Ki67 fluorescence in cells treated with CUE were significantly reduced. Furthermore, it was revealed by western blot analysis that CUE suppressed PCNA expression. These findings indicated that CUE significantly inhibits the proliferation of U87-MG and U251-MG cells.

FAK, which is overexpressed and phosphorylated in various advanced solid tumors, is essential for tumor growth, proliferation and metastasis (46,47). Thus, FAK has become a potential target for cancer therapy (48). AKT is one of the best-characterized kinases known to regulate multiple cellular functions through phosphorylation of various substrates. Previous studies have demonstrated its critical role in the survival and death of cancer cells (49). As a downstream target of AKT, GSK3β signaling pathway is one of the crucial signal transduction pathways implicated in the development of numerous cancers (50). Although it has been established that AKT and GSK3β can be downstream signaling molecules of FAK, it is unknown if the FAK/AKT/GSK3β signaling pathway contributes to the GBM development process. In the present study, it was identified that CUE significantly downregulated the phosphorylation of FAK, AKT and GSK3β in GBM cells, but the exact molecular mechanism of how CUE affects the phosphorylation of FAK, AKT and GSK3β remains to be further studied. Notably, it was also found that CUE significantly blocked EGF-induced phosphorylation of FAK, AKT and GSK3β. Considering that numerous evidences have indicated that EGFR is frequently overexpressed in human cancers and overactivation of EGFR signaling cascades are highly associated with the occurrence and development of tumors (51,52), the aforementioned finding further confirmed the potential value of CUE in tumor therapy. Notably, in the present study, it was not explored which enzymes phosphorylate the FAK/AKT/GSK3β. It is worth noting that previous studies reported that FAK is downstream of EGFR. Once activated, EGFR transmits signals to the downstream Src/FAK pathway and the phosphorylation of the Src/FAK complex can initiate the activation of the MAPK or PI3K/AKT pathway (53). Furthermore, it has been reported that Src-3Δ4 mediates the interaction of EGFR with FAK and leads to EGF-induced FAK phosphorylation (54). Such evidence suggests that EGFR and Src-3Δ4 may be promising candidate enzymes that phosphorylate the FAK/AKT/GSK3β.

Another important finding in the present study was that CUE reduced the expression of cyclinB1 and cyclinD1 in GBM cells. Both cyclinB1 and cyclinD1 are important cell cycle-driven proteins. CyclinB1 regulates the G2/M phase transition of the cell cycle (33), while cyclinD1 regulates the G1/S phase transition of the cell cycle (55). Numerous studies have demonstrated that downregulation of cyclinB1 or cyclinD1 would lead to mitotic block and would inhibit the proliferation of numerous tumor cells (56-59). However, whether the reduced expression of cyclinB1 or cyclinD1 is related to the antiproliferation effect of CUE, and whether FAK/AKT/GSK3β pathway participated in this process remains to be further investigated.

While the current study revealed that CUE inhibited the proliferation of U87-MG and U251-MG cells by modulating the FAK/AKT/GSK3β signaling pathway, certain important questions remain to be answered. For example, in the present study, the antitumor effect of CUE on cultured cell lines was only evaluated; thus, it would be interesting to further validate the antitumor effect of CUE on the growth of GBM using tumor xenograft animal models, and then isolate the tumor tissues to detect the phosphorylation of FAK, AKT and GSK3β to further elucidate the molecular mechanisms. In addition, whether CUE has any effect on GBM migration, invasion and apoptosis, and whether the FAK/AKT/GSK3β signaling pathway is involved in these processes remains unknown. Answering these important questions will help us fully understand the antitumor effect of CUE.

In conclusion, the present study demonstrated that CUE exerts a distinct antitumor effect on GBM cells. CUE may inhibit the proliferation of GBM cells through the FAK/AKT/GSK3β signaling pathway. The present finding provides a promising basis for the development of effective new drugs for GBM therapy.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

PH and WC designed the study. WC conducted the majority of the experiments. FL, XL and LL contributed to the data collection and statistical analysis. WTC performed parts of the western blot experiments. TZ and YL performed immunofluorescence experiments. LN and YZ analysed the western blot data. WC and PH wrote the manuscript. All authors read and approved the final manuscript. PH and WC confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Perus LJM and Walsh LA: Microenvironmental heterogeneity in brain malignancies. Front Immunol 10: 2294, 2019.
2. Goenka A, Tiek D, Song X, Huang T, Hu B and Cheng SY: The many facets of therapy resistance and tumor recurrence in glioblastoma. Cells 10: 484, 2021.
3. Guo G, Sun Y, Hong R, Xiong J, Lu Y, Liu Y, Lu J, Zhang Z, Guo C, Nan Y and Huang Q: IKBKE enhances TMZ-chemoresistance through upregulation of MGMT expression in glioblastoma. Clin Transl Oncol 22: 1252-1262, 2020.
4. Jackson CM, Choi J and Lim M: Mechanisms of immunotherapy resistance: Lessons from glioblastoma. Nat Immunol 20: 1100-1109, 2019.
5. Rong L, Li N and Zhang Z: Emerging therapies for glioblastoma: Current state and future directions. J Exp Clin Cancer Res 41: 142, 2022.
6. Alghasham AA: Cucurbitacins-a promising target for cancer therapy. Int J Health Sci (Qassim) 7: 77-89, 2013.
7. Si W, Lyu J, Liu Z, Wang C, Huang J, Jiang L and Ma T: Cucurbitacin E inhibits cellular proliferation and enhances the chemo-response in gastric cancer by suppressing AKt activation. J Cancer 10: 5843-5851, 2019.
8. Feng H, Zang L, Zhao ZX and Kan QC: Cucurbitacin-E inhibits multiple cancer cells proliferation through attenuation of Wnt/β-catenin signaling. Cancer Biother Radiopharm 29: 210-214, 2014.
9. He X, Gao Q, Qiang Y, Guo W and Ma Y: Cucurbitacin E induces apoptosis of human prostate cancer cells via cofilin-1 and mTORC1. Oncol Lett 13: 4905-4910, 2017.
10. Kong Y, Chen J, Zhou Z, Xia H, Qiu MH and Chen C: Cucurbitacin E induces cell cycle G2/M phase arrest and apoptosis in triple negative breast cancer. PLoS One 9: e103760, 2014.
11. Duncan KL, Duncan MD, Alley MC and Sausville EA: Cucurbitacin E-induced disruption of the actin and vimentin cytoskeleton in prostate carcinoma cells. Biochem Pharmacol 52: 1553-1560, 1996.
12. Momma K, Masuzawa Y, Nakai N, Chujo M, Murakami A, Kioka N, Kiyama Y, Akita T and Nagao M: Direct interaction of cucurbitacin E isolated from Alsomitra macrocarpa to actin filament. Cytotechnology 56: 33-39, 2008.
13. Dong Y, Lu B, Zhang X, Zhang J, Lai L, Li D, Wu Y, Song Y, Luo J, Pang X, *et al*: Cucurbitacin E, a tetracyclic triterpenes compound from Chinese medicine, inhibits tumor angiogenesis through VEGFR2-mediated Jak2-STAT3 signaling pathway. Carcinogenesis 31: 2097-2104, 2010.
14. Hsu PC, Tian B, Yang YL, Wang YC, Liu S, Urisman A, Yang CT, Xu Z, Jablons DM and You L: Cucurbitacin E inhibits the Yes-associated protein signaling pathway and suppresses brain metastasis of human non-small cell lung cancer in a murine model. Oncol Rep 42: 697-707, 2019.
15. Wang Y, Xu S, Wu Y and Zhang J: Cucurbitacin E inhibits osteosarcoma cells proliferation and invasion through attenuation of PI3K/AKT/mTOR signalling pathway. Biosci Rep 36: e00405, 2016.
16. Yang P, Lian Q, Fu R, Ding GB, Amin S, Li Z and Li Z: Cucurbitacin E triggers cellular senescence in colon cancer cells via regulating the miR-371b-5p/TFAP4 signaling pathway. J Agric Food Chem 70: 2936-2947, 2022.
17. Hsu YC, Chen MJ and Huang TY: Inducement of mitosis delay by cucurbitacin E, a novel tetracyclic triterpene from climbing stem of *Cucumis melo* L., through GADD45γ in human brain malignant glioma (GBM) 8401 cells. Cell Death Dis 5: e1087, 2014.
18. Cheng AC, Hsu YC and Tsai CC: The effects of cucurbitacin E on GADD45β-trigger G2/M arrest and JNK-independent pathway in brain cancer cells. J Cell Mol Med 23: 3512-3519, 2019.
19. Lemmon MA and Schlessinger J: Cell signaling by receptor tyrosine kinases. Cell 141: 1117-1134, 2010.
20. Sigismund S, Avanzato D and Lanzetti L: Emerging functions of the EGFR in cancer. Mol Oncol 12: 3-20, 2018.
21. Rajaram P, Chandra P, Ticku S, Pallavi BK, Rudresh KB and Mansabdar P: Epidermal growth factor receptor: Role in human cancer. Indian J Dent Res 28: 687-694, 2017.
22. Lui VW, Thomas SM, Zhang Q, Wentzel AL, Siegfried JM, Li JY and Grandis JR: Mitogenic effects of gastrin-releasing peptide in head and neck squamous cancer cells are mediated by activation of the epidermal growth factor receptor. Oncogene 22: 6183-6193, 2003.
23. Mishra R, Hanker AB and Garrett JT: Genomic alterations of ERBB receptors in cancer: Clinical implications. Oncotarget 8: 114371-114392, 2017.
24. Mitra SK, Mikolon D, Molina JE, Hsia DA, Hanson DA, Chi A, Lim ST, Bernard-Trifilo JA, Ilic D, Stupack DG, *et al*: Intrinsic FAK activity and Y925 phosphorylation facilitate an angiogenic switch in tumors. Oncogene 25: 5969-5984, 2006.
25. Ilić D, Furuta Y, Kanazawa S, Takeda N, Sobue K, Nakatsuji N, Nomura S, Fujimoto J, Okada M and Yamamoto T: Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature 377: 539-544, 1995.
26. Golubovskaya V, Beviglia L, Xu LH, Earp HS III, Craven R and Cance W: Dual inhibition of focal adhesion kinase and epidermal growth factor receptor pathways cooperatively induces death receptor-mediated apoptosis in human breast cancer cells. J Biol Chem 277: 38978-38987, 2002.
27. Revathidevi S and Munirajan AK: Akt in cancer: Mediator and more. Semin Cancer Biol 59: 80-91, 2019.
28. Xie Y, Du J, Liu Z, Zhang D, Yao X and Yang Y: MiR-6875-3p promotes the proliferation, invasion and metastasis of hepatocellular carcinoma via BTG2/FAK/Akt pathway. J Exp Clin Cancer Res 38: 7, 2019.
29. Chen X, Guo ZQ, Cao D, Chen Y and Chen J: MYC-mediated upregulation of PNO1 promotes glioma tumorigenesis by activating THBS1/FAK/Akt signaling. Cell Death Dis 12: 244, 2021.
30. Fan Z, Xu Q, Wang C, Lin X, Zhang Q and Wu N: A tropomyosin-like meretrix meretrix linnaeus polypeptide inhibits the proliferation and metastasis of glioma cells via microtubule polymerization and FAK/Akt/MMPs signaling. Int J Biol Macromol 145: 154-164, 2020.

31. Wang JF, Chen YY, Zhang SW, Zhao K, Qiu Y, Wang Y, Wang JC, Yu Z, Li BP, Wang Z and Chen JQ: ITGA5 promotes tumor progression through the activation of the FAK/AKT signaling pathway in human gastric cancer. Oxid Med Cell Longev 2022: 8611306, 2022.
32. Benelli R, Monteghirfo S, Venè R, Tosetti F and Ferrari N: The chemopreventive retinoid 4HPR impairs prostate cancer cell migration and invasion by interfering with FAK/AKT/GSK3beta pathway and beta-catenin stability. Mol Cancer 9: 142, 2010.
33. Xie X, Lin W, Zheng W, Chen T, Yang H, Sun L, Huang F, Wang Z, Lin H, Chen L, *et al*: Downregulation of G2/mitotic-specific cyclinB1 triggers autophagy via AMPK-ULK1-dependent signal pathway in nasopharyngeal carcinoma cells. Cell Death Dis 10: 94, 2019.
34. Yuan J, Yan R, Krämer A, Eckerdt F, Roller M, Kaufmann M and Strebhardt K: Cyclin B1 depletion inhibits proliferation and induces apoptosis in human tumor cells. Oncogene 23: 5843-5852, 2004.
35. Qi Y, Wang D, Huang W, Wang B, Huang D, Xiong F, Chen X and Chen Y: CyclinD1 inhibits dicer and crucial miRNA expression by chromatin modification to promote the progression of intrahepatic cholangiocarcinoma. J Exp Clin Cancer Res 38: 413, 2019.
36. Bian ZQ, Luo Y, Guo F, Huang YZ, Zhong M and Cao H: Overexpressed ACP5 has prognostic value in colorectal cancer and promotes cell proliferation and tumorigenesis via FAK/PI3K/AKT signaling pathway. Am J Cancer Res 9: 22-35, 2019.
37. Zhang B, Ma X, Li Y, Li S and Cheng J: Pleuromutilin inhibits proliferation and migration of A2780 and Caov-3 ovarian carcinoma cells and growth of mouse A2780 tumor xenografts by down-regulation of pFAK2. Med Sci Monit 26: e920407, 2020.
38. Nuñez RE, del Valle MM, Ortiz K, Almodovar L and Kucheryavykh L: Microglial cytokines induce invasiveness and proliferation of human glioblastoma through Pyk2 and FAK activation. Cancers (Basel) 13: 6160, 2021.
39. Toyama M, Hamaoka Y and Katoh H: EphA3 is up-regulated by epidermal growth factor and promotes formation of glioblastoma cell aggregates. Biochem Biophys Res Commun 508: 715-721, 2019.
40. Zou Q, Hou Y, Shen F and Wang Y: Polarized regulation of glycogen synthase kinase-3β is important for glioma cell invasion. PLoS One 8: e81814, 2013.
41. Zhang T, Li J, Dong Y, Zhai D, Lai L, Dai F, Deng H, Chen Y, Liu M and Yi Z: Cucurbitacin E inhibits breast tumor metastasis by suppressing cell migration and invasion. Breast Cancer Res Treat 135: 445-458, 2012.
42. Zha QB, Zhang XY, Lin QR, Xu LH, Zhao GX, Pan H, Zhou D, Ouyang DY, Liu ZH and He XH: Cucurbitacin E induces autophagy via downregulating mTORC1 signaling and upregulating AMPK activity. PLoS One 10: e0124355, 2015.
43. Wu YL, Zhang YJ, Yao YL, Li ZM, Han X, Lian LH, Zhao YQ and Nan JX: Cucurbitacin E ameliorates hepatic fibrosis in vivo and in vitro through activation of AMPK and blocking mTOR-dependent signaling pathway. Toxicol Lett 258: 147-158, 2016.
44. Jia Q, Cheng W, Yue Y, Hu Y, Zhang J, Pan X, Xu Z and Zhang P: Cucurbitacin E inhibits TNF-α-induced inflammatory cytokine production in human synoviocyte MH7A cells via suppression of PI3K/Akt/NF-κB pathways. Int Immunopharmacol 29: 884-890, 2015.
45. Juríková M, Danihel Ľ, Polák Š and Varga I: Ki67, PCNA, and MCM proteins: Markers of proliferation in the diagnosis of breast cancer. Acta Histochem 118: 544-552, 2016.
46. Zhang J and Hochwald SN: The role of FAK in tumor metabolism and therapy. Pharmacol Ther 142: 154-163, 2014.
47. Sulzmaier FJ, Jean C and Schlaepfer DD: FAK in cancer: Mechanistic findings and clinical applications. Nat Rev Cancer 14: 598-610, 2014.
48. Yoon H, Dehart JP, Murphy JM and Lim STS: Understanding the roles of FAK in cancer: Inhibitors, genetic models, and new insights. J Histochem Cytochem 63: 114-128, 2015.
49. Sharma AK, Kline CL, Berg A, Amin S and Irby RB: The Akt inhibitor ISC-4 activates prostate apoptosis response protein-4 and reduces colon tumor growth in a nude mouse model. Clin Cancer Res 17: 4474-4483, 2011.
50. Gao F, Huang W, Zhang Y, Tang S, Zheng L, Ma F, Wang Y, Tang H and Li X: Hes1 promotes cell proliferation and migration by activating Bmi-1 and PTEN/Akt/GSK3β pathway in human colon cancer. Oncotarget 6: 38667-38680, 2015.
51. Liu X, Wang P, Zhang C and Ma Z: Epidermal growth factor receptor (EGFR): A rising star in the era of precision medicine of lung cancer. Oncotarget 8: 50209-50220, 2017.
52. Yarden Y: The EGFR family and its ligands in human cancer. Signalling mechanisms and therapeutic opportunities. Eur J Cancer 37 (Suppl 4): S3-S8, 2001.
53. Laurent-Puig P, Lievre A and Blons H: Mutations and response to epidermal growth factor receptor inhibitors. Clin Cancer Res 15: 1133-1139, 2009.
54. Long W, Yi P, Amazit L, LaMarca HL, Ashcroft F, Kumar R, Mancini MA, Tsai SY, Tsai MJ and O'Malley BW: SRC-3Delta4 mediates the interaction of EGFR with FAK to promote cell migration. Mol Cell 37: 321-332, 2010.
55. Li M, Zheng W and Wang C: CyclinD1 promotes lymph node metastasis by inducing lymphangiogenesis in human ovarian carcinoma. Int J Clin Exp Pathol 11: 3726-3731, 2018.
56. Tang Y, Xie M, Jiang N, Huang F, Zhang X, Li R, Lu J, Liao S and Liu Y: Icarisid II inhibits the proliferation of human osteosarcoma cells by inducing apoptosis and cell cycle arrest. Tumour Biol 39: 1010428317705745, 2017.
57. Wei Y, Huang C, Wu H and Huang J: Estrogen receptor beta (ERβ) mediated-cyclinD1 degradation via autophagy plays an anti-proliferation role in colon cells. Int J Biol Sci 15: 942-952, 2019.
58. Rattanaburee T, Tipmanee V, Tedasen A, Thongpanchang T and Graidist P: Inhibition of CSF1R and AKT by (±)-kusunokinin hinders breast cancer cell proliferation. Biomed Pharmacother 129: 110361, 2020.
59. Hu Y, Cheng Y, Jiang X, Zhang Y, Wang H, Ren H, Xu Y, Jiang J, Wang Q, Su H, *et al*: PCGF3 promotes the proliferation and migration of non-small cell lung cancer cells via the PI3K/AKT signaling pathway. Exp Cell Res 400: 112496, 2021.