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Original Article

Bufalin inhibits glycolysis-induced cell growth and proliferation through the suppression of Integrin β2/FAK signaling pathway in ovarian cancer

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Abstract: Bufalin is the major digoxin-like component of the traditional Chinese medicine Chansu and has obvious anti-tumor effect in major malignancies, but the role of bufalin in glucose metabolism in ovarian cancer remains illustrated. Here, we sought to elucidate the regulatory function of bufalin on cell glucose metabolism in ovarian cancer. The treatment of bufalin on ovarian cancer cells effectively inhibited glucose uptake and lactate production in ovarian cancer cells. The expression levels of glycolysis-related proteins, including GLUT4, LDHB and HK2, were decreased by the treatment of bufalin detected by qRT-PCR and immunoblotting. Mechanistically, bufalin exerted its anti-tumor effect by targeting ITGB2/FAK signaling pathway *in vitro* and *in vivo*, which could be rescued by the introduction of ITGB2 cDNA in ovarian cancer cells. These findings provide evidence that bufalin inhibited cellular glycolysis-induced cell growth and proliferation through repression of the ITGB2/FAK pathway, indicating that bufalin may be developed as a chemotherapeutic agent to treat ovarian cancer.

Keywords: Bufalin, glucose metabolism, ITGB2, FAK, ovarian cancer

Introduction

Epithelial ovarian carcinoma is the fifth most prevalent cancer and the most malignant tumor in female reproductive system worldwide with nearly 22,440 new cases and 14,080 deaths in 2017 [1, 2]. Since ovarian tumors locate deep in the pelvic cavity and have no specific symptoms at the early stage, more than 60% of ovarian cancer patients have been already at the advanced stage when diagnosed. For advanced ovarian cancer, one of the main causes of treatment failure is drug resistance, so revealing the mechanisms of ovarian cancer and finding new therapeutic agents are of important practical significance for improving the survival rate of ovarian cancer.

Bufalin is extracted from traditional Chinese medicine Chansu and its active ingredient is the digoxin-like component [3, 4]. Recent studies have demonstrated the anticancer effect of bufalin in various types of cancers, such as lung, breast, liver and pancreatic cancer, by inhibiting cell proliferation, invasion and metastasis, inducing cell apoptosis and cell cycle arrest, increasing chemosensitivity and mediating the immune response [5-9]. It has been reported that bufalin exerts its anti-proliferation by interrupting the synthesis of DNA [10], blocking the transduction of cell signaling pathway [11], changing the cell cycle distribution of cancer cells [12] and inhibiting the growth of cancer stem cells. Although the anti-cancer property of bufalin on cell proliferation has been widely established, the effect of bufalin on glucose metabolism in cancer cells still remains elusive.

Integrins, formed by the combination of 18 kinds of α-subunits and 8 kinds of β-subunits, are probably the most important class of cell-

adhesion receptors. By regulating cell-cell and cell-matrix contacts, integrins participate in cell proliferation, invasion and migration, differentiation and survival [13-16]. Due to lack of kinase activity, integrins function by recruiting and activating kinases, such as focal adhesion kinases (FAKs) and Src family kinases (SFKs). Activated FAK-Src aims to promote cell motility, cell cycle progression and cell survival [17, 18]. Increasing studies have confirmed that integrins are frequently overexpressed in malignant tumors [19-22], indicating that integrin-related signaling pathway might be effective targets to treat various cancers. Several preclinical studies concerning on various integrin inhibitors have shown their potentials in blocking ovarian cancer progression such as targeting α5β1 [23], αvβ3 [24] and αvβ5 [25].

In this study, the effect of bufalin on cell glucose metabolism was determined to explore the possible mechanism of bufalin-induced proliferative inhibition of ovarian cancer cells. We showed that bufalin inhibited cell growth and proliferation of ovarian cancer through suppressing cancer cell glucose metabolism by targeting ITGB2/FAK signaling pathway, which provided the new evidence that bufalin may be developed as a potential therapeutic agent to treat ovarian cancer patients.

Materials and methods

Cell lines and cell culture

The established human ovarian cancer cell line A2780 and Hey were obtained from American Type Culture Collection (ATCC). All cells were maintained in Dulbecco's modified Eagle's medium (DMEM, HyClone, Thermo Scientific, USA) supplemented with 10% fetal bovine serum (Gibco, Life technologies, USA), 100 U/ml penicillin (Biowest, Nuaillé, France), and 100 U/ml streptomycin (Biowest, Nuaillé, France) and incubated at 37°C in a humidified atmosphere with 5% CO_2.

Drug treatment

Bufalin was purchased from Sigma-Aldrich (St Louis, MO) and its store concentration was 2 mM. All ovarian cells were treated with different concentrations (nmol/L) bufalin for 48 h.

Cell viability assay

To evaluate cell viability rate, we plated 8 × 10^3 cancer cells per well in 96-well plates with 100 μl maintenance medium. The next day, the cells were treated with various concentrations of bufalin. Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan) was used to monitor cell viability after 48 hours and the number of viable cells was assessed by measurement of absorbance at 450 nm after two-hour incubation by a Microplate Reader (BioTek Instruments, Winooski, VT, USA). The viability rate was calculated as experimental OD value/control OD value.

Cell proliferation assay

To evaluate cell proliferation rate, we plated 1 × 10^3 cells per well in 96-well plates with 100 μl maintenance medium. Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan) was used to monitor cell growth at 1-7 day and the number of viable cells was assessed by measurement of absorbance at 450 nm by a Microplate Reader (BioTek Instruments, Winooski, VT, USA). The proliferation index was calculated as experimental OD value/control OD value. Cell numbers were calculated with the following equation, cell number = proliferation index × 1000.

Plasmids construction and viral infection

To selectively overexpress ITGB2, the recombinant plasmid pENTER-ITGB2 containing human full cDNA sequence of ITGB2 was purchased from Vigene Biosciences (Jinan, China). We subcloned the cDNA sequence of ITGB2 into lentivirus vector pCDHCMV-MCS-EF1-PURO, generating the recombitant plasmid pCDH/ITGB2 cDNA. Lentivirus carrying ITGB2 cDNA were generated and harvested as described previously. Both A2780 and Hey cells were infected and the positive clones were selected with puromycin. Control cell lines were generated by infection with viruses containing the empty vector by following the same protocol.

RT-PCR and real-time PCR

Total RNAs of A2780 and Hey cells were isolated using Trizol reagent (Invitrogen, Life technologies, USA) respectively and reversely transcribed into cDNA using PrimeScript TM RT reagent Kit (Takara Biotechnology, Shiga, Japan). PCR products were amplified with TaKaRa Taq TM with reactions of 30 cycles of (94°C, 30 s; 58°C, 30 s and 72°C, 1 min) using the Mastercycler 1 eprealplex (eppendorf AG,

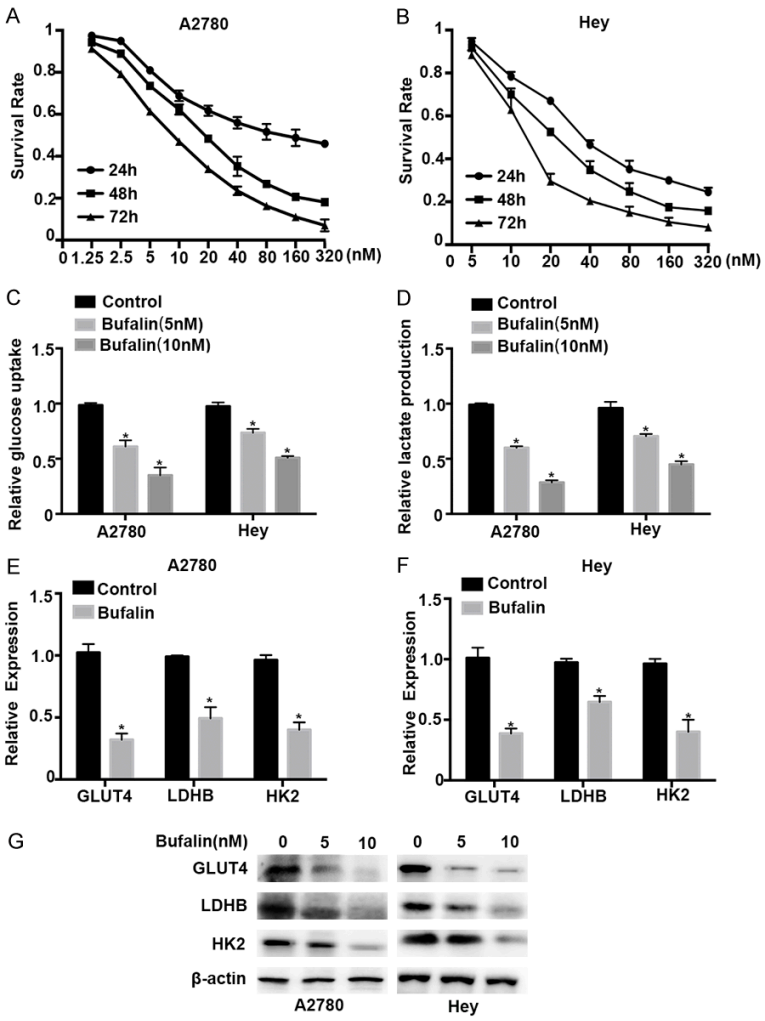

Figure 1. Bufalin suppresses cell proliferation by inhibiting cell glycolysis. A, B. In A2780 and Hey cell lines, exposure to bufalin resulted in significant decrease of cell viability compared with control in a dose-dependent manner. C. Glucose uptake dramatically decreased in A2780 and Hey cells when compared with controls ($P < 0.05$). D. Lactate production dramatically decreased in A2780 and Hey cells when compared with controls ($P < 0.05$). E-G. The results of real-time PCR and Western blot exhibited that mRNA level and protein level of GLUT4, LDHB and HK2 were down-regulated while FBP1 was up-regulated ($P < 0.05$).

Hamburg, Germany). Five microliters of PCR products were analyzed by electrophoresis on 1.5% agarose gel containing ethidium bromide and visualized under UV illumination. Real-time PCR was carried out in the Applied Biosystems Prism 7900 system (Applied Biosystems, Life technologies, USA) using ExScipt Syber green QPCR kit (Takara Biotechnology, Shiga, Japan) in the following conditions: an initial denaturation of 95°C for 30 s, one cycle; 95°C for 5 s; 55°C for 30 s; and 72°C for 30 s, 40 cycles followed by a melting curve analysis to check the specificity of amplification. Each sample was tested in triplicate, and primers to Glyceraldehyde 3-phosphatedehydrogenase (GAPDH) were used in parallel reactions as internal control. Three independent experiments were done for final analyses using the 2-ΔΔCT relative quantification method. The primer pairs of GAPDH were 5'-GGCCTC CAAGGAGTAAGACC-3' (forward primer) and 5'-CAAGGGGTCTACATGGCAAC-3' (reverse primer). The primer pairs of ITGB2 were 5'-TC-CGTTTGGGCAGAAACCAT-3' (forward primer) and 5'-CACTCCTGAGAGAGGACGCA-3' (reverse primer). The primer pairs of FAK used were 5'-GCGGCCCAGGTTTACTGAA-3' (forward primer) and 5'-GGCCTGTCTTCTGGACTCCA-3' (reverse primer). The primer pairs of HK2 used were 5'-CCTGAGGACATCATGCGAGG-3' (forward primer) and (5'-TGAGACCAGGAAACTCTCGTC-3') (reverse primer). The primer pairs of LDHB were 5'-GGAAGGAAGTGCATAAGATGGTGG-3' (forward primer) and 5'-CCCCTTTACCATTGTTGACACG-3' (reverse primers). The primer pairs of GLUT4 were 5'-TGGAAGGAAAAGGGCCATGCTG-3' (forward primer) and 5'-CAATGAGGAATCGTCCAAGGATG-3' (reverse primers).

Glycolysis analysis

Glucose Uptake Colorimetric Assay Kit (Biovision, U.S.A.) and Lactate Colorimetric Assay Kit (Biovision, USA) were purchased to examine the glycolysis process in ovarian cancer cells according to the manufacturer's protocol.

Western blot analysis

Western blot analysis was performed to determine the expression levels of various proteins in cells. Cells were pre-treated with bufalin or DMSO (diluent) for 48 h. Then Cells were collected and lysed in RIPA lysis buffer (Beyotime)

and protease inhibitor cocktail (Roche Diagnostics). The total protein concentration was determined by BCA protein assay kit (Beyotime). Equal amounts (30 μg per load) of protein samples were subjected to SDS-PAGE electrophoresis and transferred on to polyvinylidene fluoride (PVDF) membranes (Millipore). The blots were blocked in 5-8% non-fat milk, and incubated with primary antibodies, followed by incubation with secondary antibodies conjugated with horseradish peroxidase (HRP). The protein bands were developed with the chemiluminescent reagents (Millipore). Antibody to ITGB2, GLUT4, LDHB, HK2 were from Proteintech, and antibody to β-Actin was purchased from Sigma-Aldrich. FAK and phospho-FAK Y397 were purchased from Cell Signaling Technology.

Colony formation assay

Cells treated with bufalin or DMSO (diluent) at single concentration for 48 h were seeded in six-well plates at a density of 500 per well. The cells were cultured with fresh medium and allowed to grow at least for 1 week before being fixed with ice-cold methanol and stained with Crystal violet.

In vivo tumor growth assay

Animal experiments were approved by the Ethics Committee at FUSCC. Briefly, female BALB/c nude (Shanghai Slac Laboratory Animal Co. Ltd, 4-6 weeks) were injected subcutaneously with A2780 cells (5×10^6 suspended in 0.1 mL PBS for each mouse). Once reaching an average tumor volume of 100 mm^3, mice were randomized into groups (n = 5). Then they were intraperitoneally treated with bufalin (10 mg/kg) thereafter. Administration of vehicle or agents and measurement of tumor growth with a digital caliper were done once every other day. After treated with bufalin, all the mice were subjected to perform positron emission tomography/computedtomography (PET/CT) scan. The glucose uptake of tumor was evaluated by the standard uptake value (SUV). Tumor volumes were calculated with the following equation: $V = L \times W^2 \times 0.52$. V represented the volume, L represented length, and W was the width. Mice were sacrificed and the tumors were dissected and weighed one week after the last bufalin injection. RT-PCR of xenograft tumor were done according to the protocol above.

Statistical analysis

All experiments were performed in triplicate. Results in this study were calculated using Graph Pad Prism and reported as mean ± S.E. Comparisons between controls and treated groups were determined by t test or one-way ANOVA followed by Tukey's multiple comparison tests. *P* value less than 0.05 was taken as statistical significance.

Results

Bufalin suppresses cell glycolysis in ovarian cancer cells

Ovarian cancer cells, A2780 and Hey, were treated with a series of concentration of bufalin (shown in **Figure 1A**, **1B**) for 24, 48 and 72 h, respectively. In both cancer cell lines, exposure to bufalin resulted in significant decrease of cell viability compared with control in a dose-dependent and time-dependent manner ($p < 0.05$, **Figure 1A**, **1B**). The half-maximal inhibitory concentration (IC50) value for A2780 and Hey cells were 21.47 (95% CI 18.43-25.01) nM and 25.59 (95% CI 21.04-31.12) nM, respectively. Concentrations used in subsequent experiments were calculated from above dose-response curves.

To evaluate whether bufalin regulated cell glucose metabolism in ovarian cancer, we treated A2780 and Hey cells with bufalin with low toxic dosages and then performed glucose uptake and lactate production assays. As is shown in **Figure 1C**, **1D**, both glucose uptake and lactate production dramatically decreased in A2780 and Hey cells, compared with the corresponding controls ($P < 0.05$). Furthermore, we used qRT-PCR and Western blotting to detect the expression levels of glycolysis-related proteins in A2780 and Hey cells after bufalin treatment. Compared with their controls, the results of qRT-PCR and Western blotting exhibited that mRNA and protein expression levels of GLUT4, LDHB and HK2 were decreased ($P < 0.05$) (**Figure 1E-G**).

Bufalin regulates glucose metabolism by inhibiting the expression of ITGB2 in ovarian cancer cells

Integrins have been demonstrated to play vital roles in cell glucose metabolism in vertical cancer, so we inferred that the members of integrin

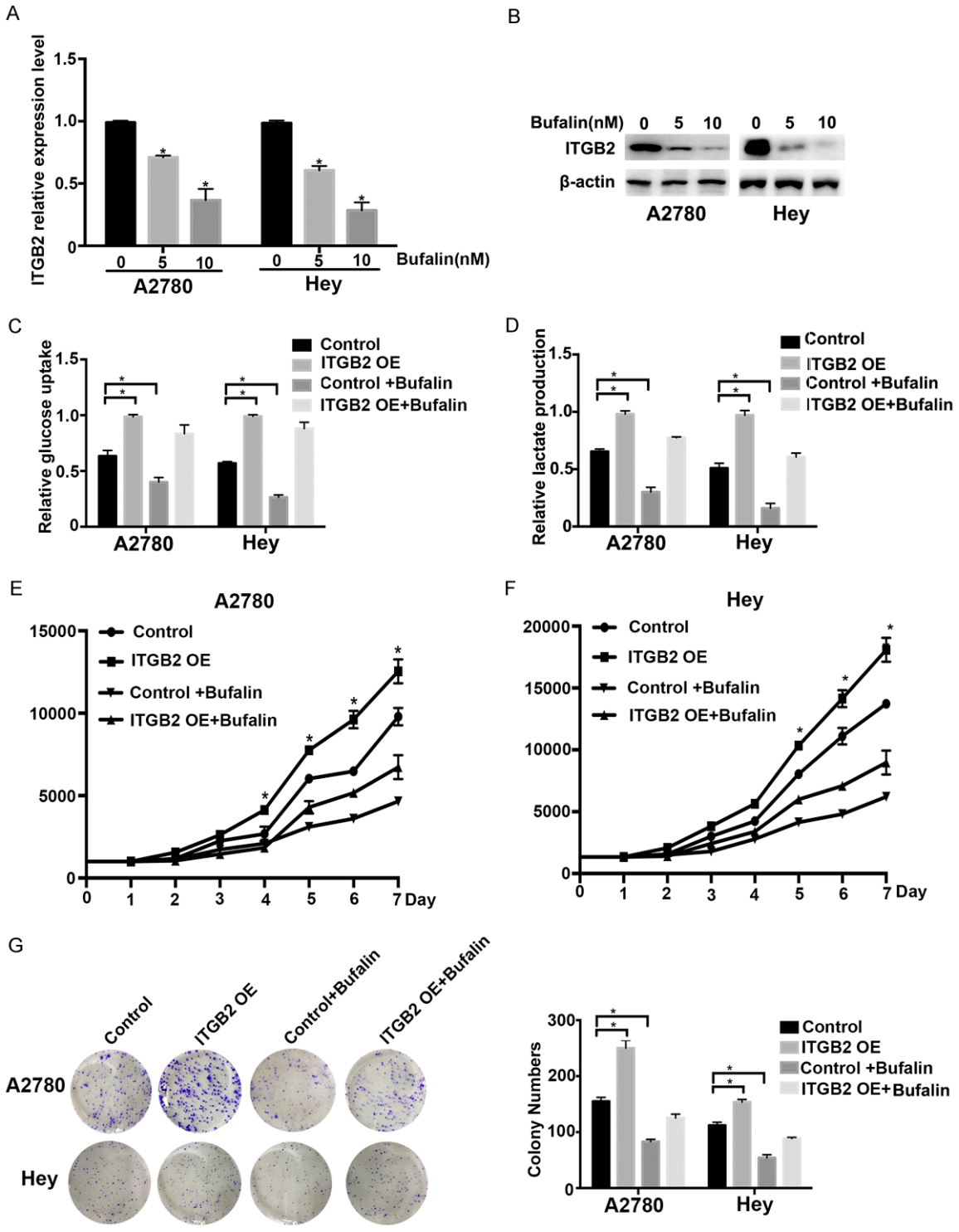

Figure 2. ITGB2 restores the effect of bufalin on cell growth and proliferation by upregulating cell glycolysis. A, B. The mRNA level and protein level of ITGB2 was suppressed after cisplatin treatment in a dose-dependent manner. C. overexpression of ITGB2 rescued the effect of bufalin on cells in glucose uptake assays when compared with their controls treated with bufalin ($P < 0.05$). D. Overexpression of ITGB2 rescued the effect of bufalin on cells in lactate production assays when compared with their controls treated with bufalin ($P < 0.05$). E, F. The results of CCK8 exhibited that bufalin's inhibitory effect on ovarian cancer cell growth and proliferation was obviously weakened by the induction of ITGB2 when compared with controls ($P < 0.05$, E-G). G. The results of Colony formation assay exhibited that bufalin's inhibitory effect on colony formation ability was obviously weakened by the induction of ITGB2 when compared with controls ($P < 0.05$).

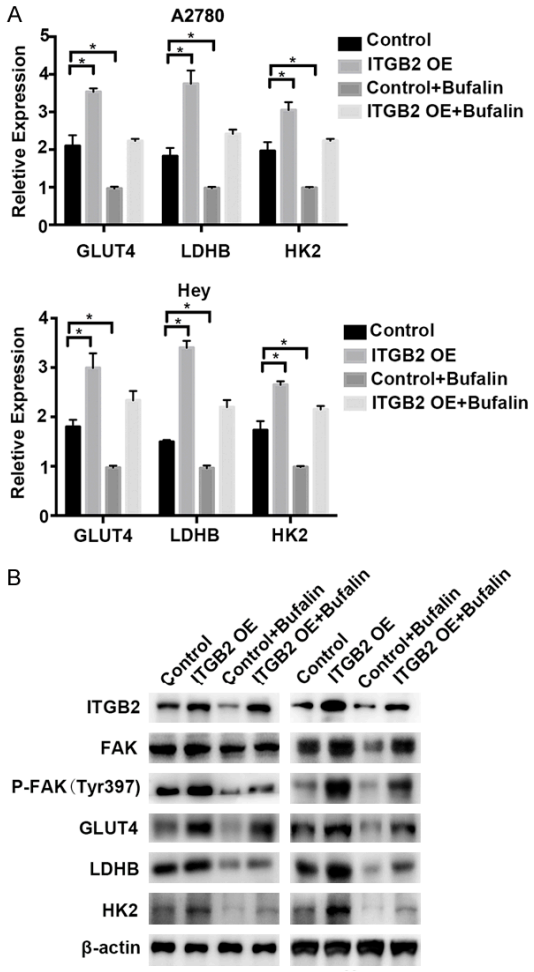

Figure 3. Bufalin disrupts ITGB2/FAK (Tyr397) signaling pathway to regulate ovarian cancer cell glycolysis. A. Bufalin suppressed the expression of focal adhesion kinase (FAK) and phosphorylated FAK (p-FAK) ($P < 0.05$). B. ITGB2 overexpression attenuated bufalin-induced suppression on FAK and p-FAK ($P < 0.05$).

family, including α or β subunits, may be affected by the treatment of bufalin. We found that, shown in **Figure 2A**, **2B**, the mRNA and protein level of ITGB2 were decreased after bufalin treatment in a dose-dependent manner, demonstrating ITGB2 might be a downstream effector of bufalin in A2780 and Hey cells ($P < 0.05$).

To further confirm the role of ITGB2 in bufalin-induced glycolysis inhibition, we established A2780/ITGB2 OE and Hey/ITGB2 OE cells stably expressing ITGB2 cDNA. We found that overexpression of ITGB2 effectively rescued the effect of bufalin on ovarian cancer cells in glucose uptake and lactate production when compared with their controls ($P < 0.05$, **Figure 2C**, **2D**). The results of CCK8 and colony formation assay also showed that bufalin's inhibitory effect on ovarian cancer cell growth and proliferation was obviously weakened by the induction of ITGB2 when compared with controls ($P < 0.05$, **Figure 2E-G**). Furthermore, we found that ITGB2 overexpression rescued the expression levels of GLUT4, LDHB and HK2 in A2780 and Hey cells treated with bufalin ($P < 0.05$, **Figure 3A-C**).

Bufalin disrupts ITGB2/FAK signaling pathway to regulate cell glycolysis in ovarian cancer

It is well established that integrins can recruit and activate FAK and its subsequent downstream signaling pathway in order to regulate cell growth and proliferation. In our study, we also found that bufalin suppressed the expression of focal adhesion kinase(FAK) and phosphorylated FAK (Tyr397) ($P < 0.05$, **Figure 3A**, **3B**). Furthermore, the induction of ITGB2 attenuated bufalin-induced suppression on FAK and p-FAK ($P < 0.05$, **Figure 3A**, **3B**).

Bufalin inhibits cell proliferation and glycolysis of ovarian cancer cells in vivo

To test the antitumor effect of bufalin *in vivo*, we injected A2780 cells into nude mice to observe subcutaneous tumor formation. After the volume of the tumor reached 100 mm^3, the mice were subjected to bufalin treatment every other day. As shown in **Figure 4A**, **4B**, the treatment with bufalin obviously slowed the growth speed of xenografts *in vivo,* compared with the corresponding controls. The tumor volume and tumor weight in the mouse treated by bufalin were significantly lower than those in control group ($P < 0.05$, **Figure 4C**, **4D**). In addition, based on the detection results of PET-CT, we found that bufalin can significantly suppressed the glucose uptake in ovarian cancer *in vivo* and resulted in a lower SUVmax value (**Figure 4E**, **4F**). To determine whether the bufalin inhibited the expression of ITGB2, FAK, p-FAK, GLUT4, LDHB and HK2 *in vivo*, we performed qRT-PCR to detect these genes expression in tumor tissues from the mouse with or without the treatment of bufalin. As shown in **Figure 4G**, the expression levels of ITGB2, FAK, GLUT4, LDHB and HK2 were largely reduced, compared with the their controls.

Discussion

In the present study, we described the functional role of bufalin in the regulation of glucose metabolism in ovarian cancer cells by blocking

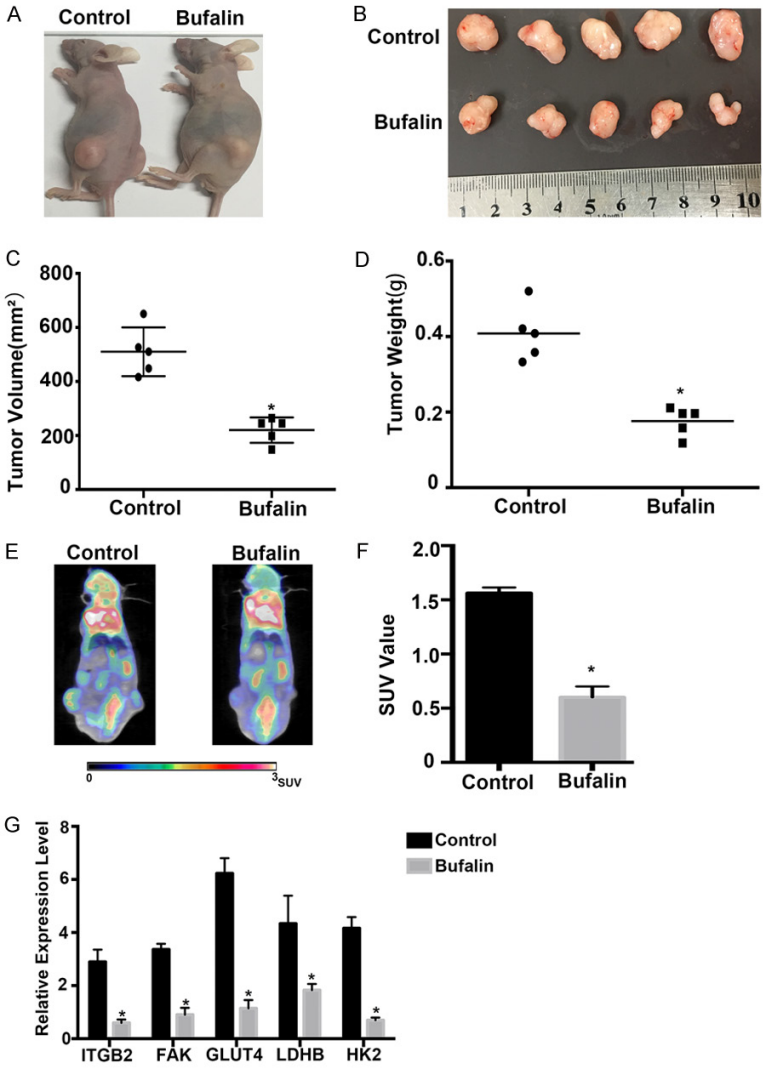

Figure 4. Bufalin inhibits cell proliferation and glycolysis of ovarian cancer cells through suppressing ITGB2 in vivo A, B. Treatment with bufalin slowed the speed of tumor growth *in vivo* ($P < 0.05$). C, D. The tumor volume and tumor weight in the experimental group were significantly lower than those in control group ($P < 0.05$). E, F. Bufalin can significantly suppressed the glucose uptake in ovarian cancer *in vivo* and resulted in a lower SUV ($P < 0.05$). G. We performed real-time PCR on tumor sections from the tumors. The expression levels of ITGB2, FAK, p-FAK, GLUT4 and LDHB were largely reduced while FBP1 increased ($P < 0.05$).

ITGB2/FAK signaling, which provides a deep understanding of cytotoxic mechanism of bufalin and offers more potential biomarkers for diagnosis and treatment of ovarian cancer in future.

Growing evidence has shown that tumor cells harbor a higher rate of aerobic glycolysis compared with oxidative phosphorylation. Rounds of anticancer agents leads to inefficient ATP synthesis thus becomes a hinder for cell growth and proliferation [26]. Currently, we assessed the impact of bufalin on glucose metabolism in ovarian cancer cells and found that bufalin, known as a sodium-potassium ATPase inhibitor, also could be acted as an anti-metabolic agent.

Integrins modulated a wide variety of cell-cell and cell-matrix interactions that lead to cell migration, proliferation, differentiation and survival. β-integrins is an important subunit of integrin. It has been reported that intergrin β subunits, such as β1, β3 and β5, mediated cancer cell growth, proliferation, invasion and migration [13, 27]. We also showed that bufalin exerted its anti-tumor effects through the regulation of ITGB5 in cervical cancer [28]. Herein, we found that the expression level of ITGB2 was obviously decreased in ovarian cancer cells treated with bufalin. Meanwhile, the induction of ITGB2 cDNA into ovarian cancer cells partly attenuated the anti-tumor effect of bufalin. We also demonstrated that ITGB2 not only enhanced glucose uptake and lactate production to boost cancer cell growth and proliferation, but also offset bufalin-induced cytotoxicity to ovarian cancer cells, indicating ITGB2 as a novel therapeutic target for ovarian cancer patients. Therefore, therapy targeting ITGB2 could further benefit more drug-resistant, recurrent and metastatic patients.

FAK located at the intersection of various signaling pathways controlling cancer growth and metastasis. This includes kinase-dependent control of cell proliferation [29]. Integrin-FAK signaling has been shown to activate a number of signaling pathways through phosphorylation

and protein-protein interactions to promote tumorigenesis [30]. Currently, Small molecule FAK inhibitors emerge as a novel chemotherapeutic agent in cancer treatment and have already exhibited enhanced effect in combination with cytotoxic drugs [31, 32] or agents targeting angiogenesis [33, 34]. In this study, FAK, as the downstream regulatory protein of ITGB2, was efficaciously inhibited by the treatment of bufalin, suggesting the combination treatment of bufalin and FAK inbihitors might bring better treatment effect through more effectively blocking ITGB2/FAK signaling.

Platium-based chemotherapy is the first line chemotherapeutic agent in the current treatment of ovarian cancer. In addition to persistent accumulation of irreparable DSBs, glycolysis inhibition may be another mechanism for cisplatin treatment [35]. Therefore, combined treatment of bufalin and cisplatin hold promise for the treatment of chemoresistant cancer cells [36] and contributed to overcome the resistance of cisplatin-based regimen after further validation.

In summary, we demonstrated that bufalin inhibited cellular glycolysis-induced cell growth and proliferation through the repression of the ITGB2/FAK signal pathway. Our results not only provide the new insight into the underlying mechanism of bufalin-induced glycolysis, but also offer the important implications for the development of therapeutic approaches using bufalin to prevent metastasis in various cancers, including ovarian cancer.

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Disclosure of conflict of interest

None.

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References

[1] Cannistra SA. Cancer of the ovary. N Engl J Med 2004; 351: 2519-2529.
[2] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.
[3] Zhao H, Zhao D, Tan G, Liu Y, Zhuang L and Liu T. Bufalin promotes apoptosis of gastric cancer by down-regulation of miR-298 targeting bax. Int J Clin Exp Med 2015; 8: 3420-3428.
[4] Chang Y, Zhao Y, Zhan H, Wei X, Liu T and Zheng B. Bufalin inhibits the differentiation and proliferation of human osteosarcoma cell line hMG63-derived cancer stem cells. Tumour Biol 2014; 35: 1075-1082.
[5] Zhai XF, Fang FF, Liu Q, Meng YB, Guo YY and Chen Z. MiR-181a contributes to bufalin-induced apoptosis in PC-3 prostate cancer cells. BMC Complement Altern Med 2013; 13: 325.
[6] Gu W, Liu L, Fang FF, Huang F, Cheng BB and Li B. Reversal effect of bufalin on multidrug resistance in human hepatocellular carcinoma BEL-7402/5-FU cells. Oncol Rep 2014; 31: 216-222.
[7] Jiang L, Zhao MN, Liu TY, Wu XS, Weng H, Ding Q, Shu YJ, Bao RF, Li ML, Mu JS, Wu WG, Ding QC, Cao Y, Hu YP, Shen BY, Tan ZJ and Liu YB. Bufalin induces cell cycle arrest and apoptosis in gallbladder carcinoma cells. Tumour Biol 2014; 35: 10931-10941.
[8] Qiu YY, Hu Q, Tang QF, Feng W, Hu SJ, Liang B, Peng W and Yin PH. MicroRNA-497 and bufalin act synergistically to inhibit colorectal cancer metastasis. Tumour Biol 2014; 35: 2599-2606.
[9] Wang H, Zhang C, Xu L, Zang K, Ning Z, Jiang F, Chi H, Zhu X and Meng Z. Bufalin suppresses hepatocellular carcinoma invasion and metastasis by targeting HIF-1alpha via the PI3K/AKT/mTOR pathway. Oncotarget 2016; 7: 20193-20208.
[10] Pastor N and Cortes F. Bufalin influences the repair of X-ray-induced DNA breaks in Chinese hamster cells. DNA Repair (Amst) 2003; 2: 1353-1360.
[11] Jiang Y, Zhang Y, Luan J, Duan H, Zhang F, Yagasaki K and Zhang G. Effects of bufalin on the proliferation of human lung cancer cells and its molecular mechanisms of action. Cytotechnology 2010; 62: 573-583.
[12] Takai N, Ueda T, Ishii T, Kira N, Nishida M, Nishida Y, Nasu K and Narahara H. Effects of bufalin on the proliferation of human chorio-

carcinoma cells. Int J Gynecol Cancer 2011; 21: 1105-1109.
[13] Shimaoka M and Springer TA. Therapeutic antagonists and conformational regulation of integrin function. Nat Rev Drug Discov 2003; 2: 703-716.
[14] Duminuco R, Noble JW, Goody J, Sharma M, Ksander BR, Roskelley CD, Cox ME and Mills J. Integrin-linked kinase regulates senescence in an Rb-dependent manner in cancer cell lines. Cell Cycle 2015; 14: 2924-2937.
[15] Walker JA, Beck GA, Campbell JH, Miller AD, Burdo TH and Williams KC. Anti-alpha4 integrin antibody blocks monocyte/macrophage traffic to the heart and decreases cardiac pathology in a SIV infection model of AIDS. J Am Heart Assoc 2015; 4.
[16] Tennant BR, Chen J, Shih AZ, Luciani DS and Hoffman BG. Myt3 mediates laminin-V/integrin-beta1-induced islet-cell migration via tgfbi. Mol Endocrinol 2015; 29: 1254-1268.
[17] Desgrosellier JS and Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. Nat Rev Cancer 2010; 10: 9-22.
[18] Mitra SK and Schlaepfer DD. Integrin-regulated FAK-Src signaling in normal and cancer cells. Curr Opin Cell Biol 2006; 18: 516-523.
[19] Kurokawa A, Nagata M, Kitamura N, Noman AA, Ohnishi M, Ohyama T, Kobayashi T, Shingaki S, Takagi R, Oral MP and Surgery G. Diagnostic value of integrin alpha3, beta4, and beta5 gene expression levels for the clinical outcome of tongue squamous cell carcinoma. Cancer 2008; 112: 1272-1281.
[20] Wang WW, Wang YB, Wang DQ, Lin Z and Sun RJ. Integrin beta-8 (ITGB8) silencing reverses gefitinib resistance of human hepatic cancer HepG2/G cell line. Int J Clin Exp Med 2015; 8: 3063-3071.
[21] Ju L and Zhou C. Integrin beta 1 enhances the epithelial-mesenchymal transition in association with gefitinib resistance of non-small cell lung cancer. Cancer Biomark 2013; 13: 329-336.
[22] Song Y, Wang L, Yang F, Li G, Duan Q and Gong Z. Increased expressions of integrin subunit beta1, beta2 and beta3 in patients with cancer ------correlation analysis between risk factors of VTE and expression of core proteins. Int J Clin Exp Med 2015; 8: 2772-2777.
[23] Almokadem S and Belani CP. Volociximab in cancer. Expert Opin Biol Ther 2012; 12: 251-257.
[24] Huang J, Zhang J, Li H, Lu Z, Shan W, Mercado-Uribe I and Liu J. VCAM1 expression correlated with tumorigenesis and poor prognosis in high grade serous ovarian cancer. Am J Transl Res 2013; 5: 336-346.
[25] Gilbert MR, Kuhn J, Lamborn KR, Lieberman F, Wen PY, Mehta M, Cloughesy T, Lassman AB, Deangelis LM, Chang S and Prados M. Cilengitide in patients with recurrent glioblastoma: the results of NABTC 03-02, a phase II trial with measures of treatment delivery. J Neurooncol 2012; 106: 147-153.
[26] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.
[27] Bianchi A, Gervasi ME and Bakin A. Role of beta5-integrin in epithelial-mesenchymal transition in response to TGF-beta. Cell Cycle 2010; 9: 1647-1659.
[28] Liu F, Tong D, Li H, Liu M, Li J, Wang Z and Cheng X. Bufalin enhances antitumor effect of paclitaxel on cervical tumorigenesis via inhibiting the integrin alpha2/beta5/FAK signaling pathway. Oncotarget 2016; 7: 8896-8907.
[29] Sulzmaier FJ, Jean C and Schlaepfer DD. FAK in cancer: mechanistic findings and clinical applications. Nat Rev Cancer 2014; 14: 598-610.
[30] Zhao J and Guan JL. Signal transduction by focal adhesion kinase in cancer. Cancer Metastasis Rev 2009; 28: 35-49.
[31] Kang Y, Hu W, Ivan C, Dalton HJ, Miyake T, Pecot CV, Zand B, Liu T, Huang J, Jennings NB, Rupaimoole R, Taylor M, Pradeep S, Wu SY, Lu C, Wen Y, Huang J, Liu J and Sood AK. Role of focal adhesion kinase in regulating YB-1-mediated paclitaxel resistance in ovarian cancer. J Natl Cancer Inst 2013; 105: 1485-1495.
[32] Halder J, Lin YG, Merritt WM, Spannuth WA, Nick AM, Honda T, Kamat AA, Han LY, Kim TJ, Lu C, Tari AM, Bornmann W, Fernandez A, Lopez-Berestein G and Sood AK. Therapeutic efficacy of a novel focal adhesion kinase inhibitor TAE226 in ovarian carcinoma. Cancer Res 2007; 67: 10976-10983.
[33] Bagi CM, Christensen J, Cohen DP, Roberts WG, Wilkie D, Swanson T, Tuthill T and Andresen CJ. Sunitinib and PF-562,271 (FAK/Pyk2 inhibitor) effectively block growth and recovery of human hepatocellular carcinoma in a rat xenograft model. Cancer Biol Ther 2009; 8: 856-865.
[34] Hutchinson L. Ovarian cancer: FAK-new target for antiangiogenic therapy. Nat Rev Clin Oncol 2016; 13: 328.
[35] Zhao JG, Ren KM and Tang J. Overcoming 5-Fu resistance in human non-small cell lung cancer cells by the combination of 5-Fu and cisplatin through the inhibition of glucose metabolism. Tumour Biol 2014; 35: 12305-12315.
[36] Ai Z, Lu Y, Qiu S and Fan Z. Overcoming cisplatin resistance of ovarian cancer cells by targeting HIF-1-regulated cancer metabolism. Cancer Lett 2016; 373: 36-44.