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Original Research Article

Butein inhibits proliferation and migration of gastric cancer cells by regulating PI3K/AKT pathway

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Abstract

Purpose: *To investigate the possible effects of butein on the proliferation, apoptosis, and motility of gastric cancer cells, and the possible mechanism of action.*
Methods: *MTT assay was conducted to determine the effects of butein on gastric cancer cell proliferation. The effect of butein on gastric cancer cell apoptosis was evaluated by flow cytometry. In addition, wound closure and Transwell assays were performed to assess butein's effects on gastric cancer cell motility, while immunoblot assays were performed to assess the effect of butein on the expression of proteins in the PI3K/AKT pathway of gastric cancer cells.*
Results: *Butein treatment suppressed gastric cancer cell proliferation and stimulated apoptosis ($p < 0.001$). In addition, butein treatment reduced the motility of gastric cancer cells, but mediated the PI3K/AKT axis and, hence, influenced the proliferation, apoptosis, and motility of gastric cancer cells ($p < 0.001$).*
Conclusion: *Butein inhibits the proliferation and migration of gastric cancer cells by regulating PI3K/AKT pathway. Thus, this compound has potentials for development as an agent for the treatment.*

Keywords: *Butein, Gastric cancer, Proliferation, Apoptosis, Cell migration, PI3K/AKT pathway*

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INTRODUCTION

Gastric cancer is the second leading cause of cancer deaths, with a high incidence in countries such as China and Japan [1]. Gastric cancer is caused by a variety of congenital and acquired factors, including genetic, epigenetic, and molecular changes [2]. Metastasis of middle and advanced gastric cancer occurs frequently, and traditional radiotherapy and chemotherapy have poor therapeutic effects in patients with advanced gastric cancer [3]. To combat this disease, more effective therapeutic drugs are still badly needed.

Butein (2',3,4,4'-tetrahydroxychalcone) is a plant polyphenol and bioactive agent extracted from the heartwood of sandalwood geranifolium and the stem bark of cashew [4]. These plant extracts are reported to have a variety of pharmacological effects, have long been used as traditional herbs

in Asian countries, and have the potential to treat several chronic diseases, such as liver tuberculosis, obesity, diabetes, and hypertension [5]. Butein has inhibitory effects on a variety of cancers, such as non-small cell lung cancer [6]. Butein also induces apoptosis of ovarian cancer cells and inhibits proliferation of cervical cancer cells by inhibiting the PI3K/mTOR pathway [7]. However, the effects of butein on gastric cancer are still unclear.

Phosphatidylinositol 3-kinase (PI3K) is involved in the regulation of a variety of cellular processes [8]. Protein kinase B (AKT), the main downstream effector of PI3K, regulates various biological processes through the activation and phosphorylation of various enzymes, kinases, and transcription factors [9]. The PI3K/AKT pathway is activated in a variety of cancers [10]. Inhibition of the PI3K/AKT pathway inhibits the invasion and migration of cancer cells and promotes apoptosis of tumor cells [11]. Several herbal extracts could suppress gastric cancer progression via targeting of this pathway. In this study, the effect of butein on the progression of gastric cancer cells was investigated.

EXPERIMENTAL

Antibodies and drugs

The following antibodies were used: Bax antibody (1:500 dilution, ab32503, Abcam), Bcl-2 antibody (1:1000 dilution, ab32124, Abcam), PI3K antibody (1:500 dilution, ab191606, Abcam), p-PI3K antibody (1:1000 dilution, ab182651, Abcam), AKT antibody (1:500 dilution, ab8805, Abcam), p-AKT antibody (1:500 dilution, ab38449, Abcam), and beta-actin antibody (1:3000 dilution, ab8226, Abcam). Butein (CAS: 487-52-5) was purchased from Sigma-Aldrich (Cat. No: 72795).

Cell culture and treatment

The human gastric cancer cell line AGS was purchased from the ATCC and cultured in DMEM supplemented with 10 % fetal bovine serum (FBS) at 37 °C in 5 % CO_2. AGS cells were treated with butein at concentrations of 10, 20, and 30 μM for 24 h.

Immunoblot assay

All cell and tissue samples were lysed using RIPA buffer (9800; Cell Signaling) to extract the proteins. The proteins were separated using 10% SDS-PAGE gels, then transferred onto PVDF membranes. Subsequently, membranes were blocked with 5% fat-free milk in TBST buffer. All membranes were then incubated with primary antibodies for 2 h at room temperature. Subsequently, the membranes were incubated with secondary antibodies for 1 h. Blots were imaged using chemiluminescence.

MTT assay

AGS cells were plated in 96-well plates at a cell density of 1000, and maintained for 48 h upon the indicated treatment. Cells were then incubated with MTT for 4 h, and the stained cells were resuspended in DMSO. The OD value was measured at wavelength 490 nm.

Flow cytometry (FCM) assay

AGS cells were resuspended and incubated with annexin V-FITC and propidium iodide for 20 min. Subsequently, flow cytometry of the samples was conducted using a FACS Calibur flow cytometer, and the apoptotic cells were analyzed and compared among different groups.

Scratch wound assay

AGS cells treated with butein were grown to confluency. Then, scratches were made with a 10 μL-pipette tip, followed by washing with PBS buffer. Subsequently, serum-free culture medium was refreshed to induce wound closure. Images were photographed to analyze cell migration ability.

Transwell assays

Cells treated with butein were plated in the upper chamber of transwell units, in culture medium without serum. Then, complete culture medium containing 10 % FBS was added to the bottom chambers to stimulate cell invasion. After 24 h, cells in the upper chamber were removed, and the remaining cells were stained using 0.2 % crystal violet and quantified.

Statistical analysis

GraphPad 5.0 software was used for statistical analysis. Data are presented as mean ± SEM. Student's *t*-test was used for comparisons between two groups, and $p < 0.05$ was considered statistically significant.

RESULTS

Butein inhibits gastric cancer cell proliferation

To uncover the possible effects of butein on gastric cancer cells, the gastric cancer cell line

AGS was treated with different doses of butein for 24 h. The chemical structure of butein is shown in Figure 1 A. MTT assays were performed to reveal the effects of butein at concentrations of 10, 20, and 30 μM, on the viability of gastric cancer cells. Interestingly, a decrease in OD value upon treatment with butein was found (Figure 1B). Therefore, butein suppressed the proliferation of gastric cancer cells.

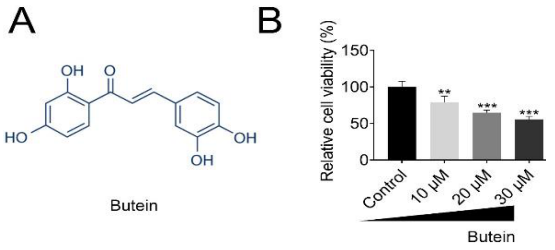

Figure 1: Butein inhibits the proliferation of gastric cancer cells. (A) Chemical structure of butein. (B) MTT assays were used to measure the proliferative capacity of AGS cells upon treatment with butein at concentrations of 10, 20, and 30 μM for 24 h. The relative proliferation (OD value) was measured. Data are presented as mean ± SEM; $^{**}p < 0.01$, $^{***}p < 0.001$.

Butein stimulates the apoptosis of gastric cancer cells

Through FCM assays, the apoptotic capacity of AGC cells was determined. Butein treatment dramatically stimulated apoptosis of gastric cancer cells (Figure 2 A). Through immunoblot assays, the effects of butein on the expression of Bax and Bcl-2 in AGC cells were also determined. The expression of Bax was increased, whereas Bcl-2 expression was decreased, upon butein treatment of AGC cells, in a dose-dependent manner (Figure 2 B). Therefore, these results confirmed that butein stimulated apoptosis of gastric cancer cells.

Butein suppressed the motility of gastric cancer cells *in vitro*

Because butein affected the proliferation and apoptosis of gastric cancer cells, its effects on the motility of gastric cancer cells were investigated though wound closure and Transwell assays. Butein treatment at concentrations of 10, 20, and 30 μM dramatically reduced the migration of AGS cells (Figure 3 A). Subsequently, transwell assays were performed, and the results revealed that butein treatment at concentrations of 10, 20, and 30 μM suppressed gastric cancer cell invasion (Figure 3 B). Thus, butein suppressed the motility of gastric cancer cells *in vitro*.

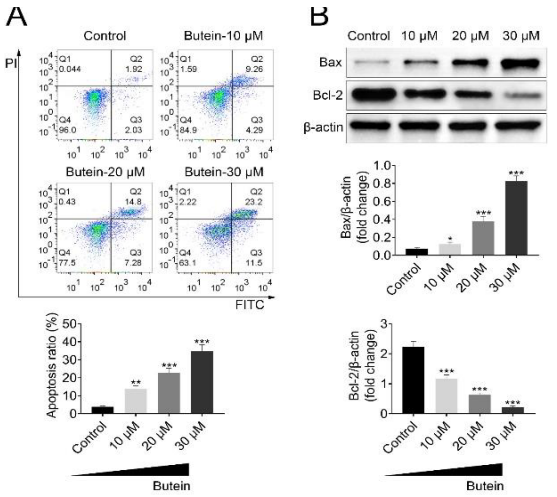

Figure 2: Butein stimulates apoptosis of gastric cancer cells. (A) FCM assays showing apoptosis of AGS cells upon treatment with butein at concentrations of 10, 20, and 30 μM for 24 h. (B) Immunoblot assays showing the expression of Bax and Bcl-2 in AGS cells upon treatment with butein at concentrations of 10, 20, and 30 μM for 24 h. Data are presented as mean ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

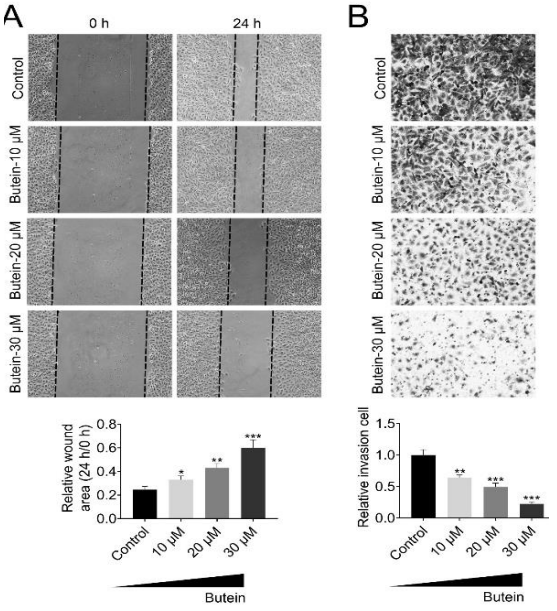

Figure 3: Butein suppresses the motility of gastric cancer cells *in vitro*. (A) Wound closure assays showed the migration capacity of AGS cells upon treatment with butein (10, 20, and 30 μM) for 24 h. The wound widths were measured at 0 and 24 h time points. (B) Transwell assays showed the invasive capacity of AGS cells upon treatment with butein (10, 20, and 30 μM) for 24 h. The cells were counted. Data are presented as mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Butein mediated PI3K/AKT pathway in gastric cancer cells

Butein treatment at concentrations of 10, 20, and 30 μM decreased the phosphorylation levels of PI3K and AKT in AGS cells (Figure 4). Therefore, butein mediated the PI3K/AKT pathway in gastric cancer cells.

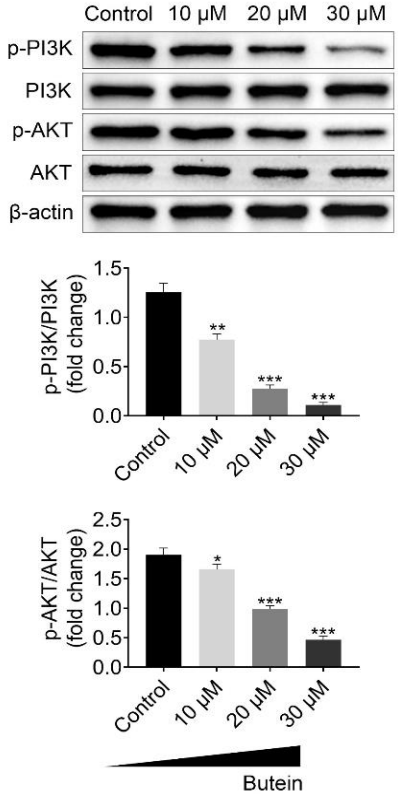

Figure 4: Butein mediated the PI3K/AKT pathway in gastric cancer cells. Immunoblot assays showing the expression of p-PI3K, PI3K, p-AKT, and AKT in AGS cells upon treatment with butein at concentrations of 10, 20, and 30 μM for 24 h. Data are presented as mean ± SEM; $^{**}p < 0.01$, $^{***}p < 0.001$

DISCUSSION

Gastric cancer is the most common malignant tumor of the digestive tract in China and the third most common cancer in the whole body [12]. Some symptoms of gastric cancer occur only after complications or malignant changes because of tumor growth [13]. Conventional treatment methods, such as surgical resection, radiotherapy, and chemotherapy, have a significant effect in early gastric cancer, while the effect in advanced gastric cancer is poor [14]. Because of frequent metastasis and recurrence of advanced gastric cancer, new and more effective drugs are needed [15]. In this study, a plant polyphenol and bioactive agent, butein, was shown to have the potential to combat advanced gastric cancer. The effects of butein on gastric cancer cell proliferation, apoptosis, and motility, as well as the mechanism, were uncovered.

Butein has wide range of biological activities and functions [16]. It was reported that butein induced cellular senescence in osteosarcoma cells [17]. And showed that butein inhibited lipogenesis in *Caenorhabditis elegans* [17]. Butein promoted commitment of bone marrow-derived stem cells to the osteoblast lineage through targeting of the ERK pathway [18]. In addition, butein attenuated the cytotoxic effects of LPS-induced microglia on a neuronal cell line [19]. These studies showed the different effects of butein in a variety of cellular processes via different mechanisms and targets. This study also found that butein affected gastric cancer progression via the PI3K/AKT pathway.

The effects of butein on tumor progression have also been widely reported [17]. Butein suppressed cell proliferation and TNF-α-induced CCL2 release in triple negative breast cancer cells in a racially dependent manner [20]. Butein mediated non-small-cell lung cancer (NSCLC) apoptosis and cell cycle arrest via targeting of the PERK/CHOP axis. Another study showed the effects of butein on oxidative stress and p38 activation in NSCLC cells [21]. Herein, the effects of butein on gastric cancer cell proliferation, apoptosis, and motility were revealed. These studies suggest that butein could serve as a promising therapeutic drug for the treatment of cancer.

Overactivation of PI3K and AKT induces multiple downstream cell pathways, contributing to cancer development [22]. Multiple proteins promote the progression and development of gastric cancer via the PI3K/AKT pathway. Xanthoceraside induced cell apoptosis through downregulation of the PI3K/AKT pathway in human bladder cancer cells [2]. A recombinant measles virus vaccine strain, rMV-Hu191, inhibited colorectal cancer growth through inducing autophagy and apoptosis, mediated by the PI3K/AKT pathway [23]. Apatinib suppressed the motility and angiogenesis of hepatocellular carcinoma cells by blocking the PI3K/AKT pathway [24]. Thus, the present work indicates that butein affected gastric cancer via PI3K/AKT pathway which may serve as a therapeutic target for gastric cancer treatment.

CONCLUSION

The findings of this study show that butein treatment suppresses the proliferation and

motility of gastric cancer cells but promotes the apoptosis of the cells, possibly by regulating PI3K/AKT pathway. Thus, butein coulis is a promising drug for gastric cancer treatment.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Kunhua Lin and Qiongjiao Lu designed the study and supervised the data collection, Shougang Wang analyzed and interpreted the data, and Yina Lin and Tao Cui prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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