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Effects of β-carboline alkaloids from *Peganum harmala* on the FAK/PI3K/AKT/Mtor pathway in human gastric cancer cell line SGC-7901 and tumor-bearing mice

Yuxiang Fan[1, 2], Fanye Zeng[1, 2], Lili Ma[3] and Hongliang Zhang[1, 2]*

[1]The Second Department of Oncology, the Fourth Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang, PR China
[2]The Second Department of Oncology, Xinjiang Traditional Chinese Medicine Hospital, Urumqi, Xinjiang, PR China
[3]Department of Oncology, People's Hospital of Xinjiang Uygur Autonomous Region, Urumqi, Xinjiang, PR China

Abstract: This study investigates the effects of β-carboline alkaloids from *Peganum harmala* on FAK/PI3K/AKT/mTOR pathway in gastric cancer cell line SGC-7901 and tumor-bearing mice. Western blot, immunohistochemistry and RT-PCR were performed to detect protein and mRNA expressions of BCL-2, Bax, FAK, PI3K, AKT and mTOR. Mice model of gastric tumor was established with SGC-7901 cells. TUNEL assay was used to detect apoptosis. HE staining was used to observe morphological changes. *In vitro*, the protein and mRNA expressions of FAK, PI3K, AKT and mTOR in β-carboline alkaloids groups were significantly lower than those in control and fluorouracil groups ($P<0.05$). BCL-2 decreased while Bax increased. *In vivo*, the tumor weights of β-carboline alkaloids and fluorouracil groups were significantly lower than those of control group ($P<0.05$). FAK, PI3K, AKT and mTOR proteins in tumor tissues of β-carboline alkaloids and fluorouracil groups were significantly lower than control group ($P<0.05$). Additionally, β-carboline alkaloids treatment *in vivo* caused obvious cell necrosis and apoptosis. Conclusively, β-carboline alkaloids can reduce FAK, PI3K, AKT and mTOR expressions at both protein and mRNA levels in SGC-7901 cells and tumor tissues formed by SGC-7901 cells. They may be targets of β-carboline in FAK/PI3K/AKT/mTOR pathway.

Keywords: β-carboline alkaloid, gastric cancer, AKT, mTOR, *Peganum harmala*

INTRODUCTION

Gastric cancer is one of the most common tumors of the digestive tract (Canseco-Avila *et al*, 2018; Martinson *et al*, 2018), and the second most common tumor in China. The number of cases and deaths of gastric cancer in China account for 42.6% and 45.0% of the global incidence and death of gastric cancer, respectively, ranking 5th in morbidity and 6th in mortality in the world (Park *et al*, 2018; Sitarz *et al*, 2018). The gastric cancer-related mortality is expected to continue to rise (Zuo *et al*, 2017). Traditional treatments for gastric cancer include surgery, chemotherapy and radiation therapy. However, there are often different degrees of side effects during radiotherapy and chemotherapy. Therefore, development of low-toxic and high-efficient anti-tumor drugs is an urgent demand.

The β-carboline alkaloids could be extracted from the total alkaloids of *Peganum harmala L.*, by combining ion exchange chromatography with other column chromatography techniques. The obtained β-carboline alkaloids have purity greater than 90% (Gao *et al*, 2017). In a previous study, we confirmed by *in vitro* experiments that β-carboline alkaloids could induce apoptosis of human gastric cancer cell line SGC-7901 (Fan *et al*, 2015). Some *in vivo* experiments found that β-carboline alkaloids could reduce the weight of tumor formed by SGC-7901 (Fan *et al*, 2015; Yuxiang *et al*, 2015), but the underlying mechanism is still unclear.

The mammalian target of rapamycin (mTOR) is involved in the physiological and pathological processes of many tumors (Atsushi, 2015). It is the hub of many important signaling pathways in the cell and its dysfunction is closely related to tumorigenesis and development. Ras, PI3K and mTOR are protein kinases that are closely related to cell proliferation. Ras and PI3K signals play a key role in regulating cell growth by regulating the downstream molecule mTOR.

In this study, the effects of β-carboline alkaloids on expressions of the apoptosis-related and mTOR upstream proteins in human gastric cancer SGC-7901 cells and tumor-bearing mice were investigated.

MATERIALS AND METHODS

Cell culture and treatment

SGC-7901 cell line was purchased from KeyGen Biotech Co. Ltd. (Nanjing, China). Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin at 37^{o}C humidified atmosphere with 5% CO_2. For treatment, the cells were treated with β-carboline alkaloids (10μg/mL) or fluorouracil (40μg/mL) (positive control) for 24h. Cells without treatment were used as negative control.

**Corresponding author:* e-mail: zhl2019doctor@126.com

Animals

A total of 50 Kunming (KM) mice (including 25 male and 25 female mice; weight 18±3.1g) were obtained from the Animal Experiment Center of Xinjiang Medical University. All mice were acclimatized in SPF environment (22±2°C, 50-60% of relative humidity and 12h light-dark cycle) for one week before the experiments. Immunosuppression was performed using a previously reported method (Li *et al*, 2015). All animal experiments were conducted according to the ethical guidelines of Xinjiang Medical University.

Establishment of tumor-bearing mice

The SGC-7901 cells of 1×10^7/mL in RPMI 1640 medium (200μL) were inoculated on the back of the mice. Ten days after the inoculation, the inoculation site was probed using ultrasound, and uneven echoes under the skin indicated successful inoculation (Qi *et al*, 2010).

Animal treatment and grouping

The mice were randomly divided into 5 groups, including Control group, Fluorouracil group, β-carboline alkaloids High, Medium and Low dose groups (n=10 for each group). The Control group was intraperitoneally injected (i.p.) with normal saline (1mL/day) for 14 days. The Fluorouracil group was injected (i.p.) with 267mg/kg fluorouracil for 2 days (determined according to FOLFOX chemotherapy). For the β-carboline alkaloids High, Medium and Low dose groups, the mice were orally administered with 2.5mg/kg, 5mg/kg and 7.5mg/kg β-carboline alkaloids for 14 days, respectively, according to previous studies (Fan *et al*, 2015; Xi *et al*, 2018). The mice were weighed during the administration and observed for general conditions such as mental state, activity and diet. All mice were sacrificed on Day 21 and the tumor tissues were collected. The tumors were weighed.

Reverse transcription-polymerase chain reaction (RT-PCR)

The mRNA expressions of BCL-2, Bax, Focal adhesion kinase (FAK), PI3K, AKT and mTOR in cells and tumors were detected by RT-PCR. The RNA of each group was extracted by Trizol agent (Transgen Biotech, Beijing, China) and then reverse transcribed into cDNA. The mRNA expression was quantified by qPCR using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). The PCR condition was as follows: pre-denaturation at 95°C for 2 min, 40 cycles of denaturation at 95°C for 5s and annealing/extension at 60°C for 30s. β-actin was used as internal control. The sequences of the used primers were listed in table 1.

Western blot

Total protein was extracted from SGC-7901 cells and tumor tissues. Then, the proteins were subjected to SDS-PAGE. The protein bands were transferred onto a PVDF membrane, blocked with 5% skim milk and washed with TBST. The membrane was then incubated with anti-Bcl-2 (1:500; Cat# Ab692; Abcam), anti-Bax (1:500; Cat# Ab32503; Abcam), anti-PI3K P85 antibody (1: 1000; Cat# CSB-PA003765; Cusabio, Wuhan, China), anti-FAK antibody (1: 300; Cat# CSB-PA995146; Cusabio), anti-AKT antibody (1: 2000; cat #4691; Cell Signaling), anti-mTOR (1:100; Cat# 70-ab40045-100; MultiSciences) and anti-β-actin (1: 1000; Cat# D110001; Sangon Biotech, Shanghai, China) antibody for 1h at room temperature. After that, the membrane was incubated with HRP labeled goat anti-rabbit IgG secondary antibody (cat# ab205718; Abcam) for 1h at room temperature. After color development, the membrane was photographed with ChemiScope mini chemiluminescence imaging system (Clinx Science Instruments, Shanghai, China).

Hematoxylin-eosin (HE) staining

The tumor samples were fixed with 10% paraformaldehyde, embedded in paraffin and dehydrated. The samples were sliced into 5μm sections and treated different concentrations of xylene, toluene and ethanol successively. Thereafter, the sections were treated with hematoxylin and eosin, then treated with absolute ethanol, xylene and finally sealed with resin and observed under a microscope.

Immunohistochemistry

The fixed tumor tissues were sliced into 5 μm sections as described above. The sections were incubated with appropriate primary antibodies of anti-Bcl-2 (1:50; Cat# Ab692; Abcam), anti-Bax (1:50; Cat# Ab32503; Abcam), AKT (1: 100; cat #4691; Cell Signaling), FAK (1: 25; Cat# CSB-PA995146; Cusabio), m-TOR (1: 25; Cat# 70-ab40045-100; MultiSciences) and PI3K P85 (1: 50; Cat# CSB-PA003765; Cusabio) overnight at 4°C. After washing with PBS for 3 times, HRP-labeled secondary antibody was added to incubate for 20min at room temperature. The sections were then colored with DAB, washed with tap water and counter stained with hematoxylin. The sections were observed under a microscope and 10 fields were counted in each section for the brown-yellow positive cells. The gray value of positive cells was analyzed. The smaller the gray value, the higher the positive expression.

TUNEL staining

The samples were sliced into 5μm sections as described above. The samples were digested with 0.01M TBS freshly diluted Proteinase K (1:200) at 37°C and treated with TBS and DIG-d-UTP. After washing with TBS, the sections were blocked and incubated with biotinylated anti-digoxigenin antibody (1:100). After incubated with SABC for 30min at 37°C, DAB was used for color development, followed by mild counterstaining with hematoxylin and then sealed. The sections were observed with a microscope. Those with brown-yellow particles in the nucleus are positive cells, i.e, apoptotic cells.

STATISTICAL ANALYSIS

The statistical analysis was performed with the statistical software SPSS 19.0 (IBM, Chicago, IL, US). The measurement data was expressed as mean ± standard deviation (SD). If the data is in a normal distribution, single factor analysis of variance (ANOVA) was used for the comparison. If the data does not conform to the normal distribution, the rank sum test method was used for comparison. A $P<0.05$ was considered as statistically significant.

RESULTS

The expression of mTOR pathway genes FAK

To demonstrate the effect of β-carboline alkaloid on the expression of mTOR pathway genes, qRT-PCR and Western blot were performed. The results showed that the expression of FAK, PI3K, AKT, mTOR and BCL-2 proteins and mRNAs in the SGC-7901 cells of the β carboline alkaloid group was significantly lower than that in the blank control group ($P<0.05$) (fig. 1A and 1B). The levels of Bax protein and mRNA was significantly increased ($P<0.05$) (fig. 1A and 1B). Consistently, *in vivo* experiments showed that the FAK, PI3K, AKT, mTOR and BCL-2 mRNAs and proteins in the β-carboline alkaloid group and the fluorouracil group were significantly lower than the control group ($P<0.05$) (fig. 1C and 1D) whereas that Bax levels were significantly higher. There were no significant differences between fluorouracil group and low/medium dose of β-carboline alkaloid group. This indicates that β carboline alkaloids can reduce the expression of FAK, PI3K, AKT, mTOR proteins and mRNAs in human gastric cancer SGC-7901 cells, and they may be targets for β-carboline alkaloids in the FAK/PI3K/AKT/mTOR pathway.

Inhibition of tumor growth

To demonstrate the tumor-inhibitive effect of β-carboline alkaloids in vivo, tumor mice model was established. In the animal experiment, there was only one mouse died in the β-carboline alkaloids high dose group, but no death in other groups. There was no obvious antifeedant and diarrhea in each group of animals during the experiment. The tumor weight of each group was shown in fig. 2A. There was significant difference between the Fluorouracil group and the control group ($P<0.05$), and between the β-carboline alkaloids high dose group and the control group ($P<0.05$). There was no statistically significant difference among the other groups ($P>0.05$). This indicates that β carboline alkaloids can inhibit tumor growth *in vivo*.

HE staining of tumor sections

To demonstrate the morphological changes of tumor tissues after treated by β carboline alkaloids, HE staining of the tissue sections was performed. For the control group, the tumor cells varied in size and the density was high (arranged densely) (fig. 2B). The cells were deeply stained, were with obvious atypia and grew invasively. A large number of mitotic cells were observed (>10/HP). Peripheral tumor cells were grown actively and the rate of coagulative necrosis and apoptosis accounted for about 25-30%. The inflammation was light (-). For the Fluorouracil group, the tumor cells varied in size, densely packed and diffusively arranged. The cells showed obvious atypia and had a large number of mitotic cells (5-8/HP). The rate of coagulative necrosis and apoptosis accounted for about 40-50%. The inflammatory reaction was light and the cells at edge were grown actively. For the low dose group, the tumor cells arranged in a nest pattern and some interstitial inflammation was obvious. The rate of coagulative necrosis and apoptosis accounted for about 40-50%. The tumor cells were degenerated and necrotic, the staining was light and mitosis was visible (3-5/HP). For the medium dose group, the tumor cells arranged in a nest pattern and the interstitial inflammation was light. The rate of coagulative necrosis and apoptosis accounted for about 40-50%. The tumor cells were partially heterogeneous, degenerated and necrotic, and nuclear division was visible (5-8/HP). For the high dose group, the tumor cells arranged in a nest pattern and the interstitial inflammation was obvious (lymphocyte aggregation infiltration). The rate of coagulative necrosis and apoptosis accounted for >50%. The tumor cells were obviously heterogeneous, partially degenerated and necrotic and nuclear division was visible (2-5/HP). The cells at the edge were grown actively. This indicates that apoptosis occurs in tumor tissues of after treated with β carboline alkaloids.

Immunohistochemical staining of tumor sections

To demonstrate the *in vivo* expression of mTOR pathway genes after the treated with β carboline alkaloids, immunohistochemical staining was performed. According to the microscopic observation, the expressions of BCL-2, FAK, PI3K, AKT and m-TOR in the control group were the strongest, but that of the β carboline alkaloids group was not obviously different from the control group. The fluorouracil group and the high dose β carboline alkaloids group showed higher expression of BAX compared with the control group (fig. 3). This indicates that the expression of BCL-2, FAK, PI3K, AKT and mTOR in the tumor tissues after β carboline alkaloids treatment is reduced while that of BAX is increased.

TUNEL staining

To detect whether the tumor cells were apoptosis after β carboline alkaloids treatment, TUNEL staining was performed. The control group had the least apoptotic tumor cells, and the fluorouracil group, the middle and low dose β carboline alkaloids groups all had apoptotic cells and the high dose group had the highest apoptosis rate (fig. 4). This indicates that β carboline alkaloids can induce cell apoptosis of tumor tissues.

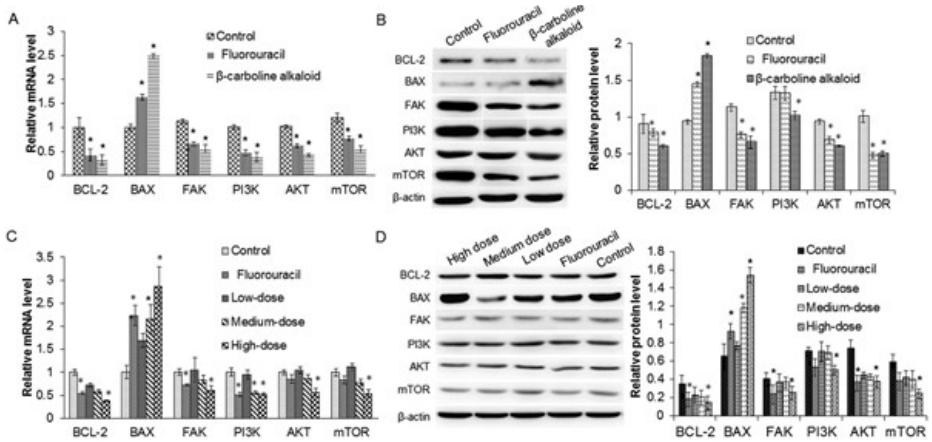

Fig. 1: The mRNA and protein expression of mTOR signaling pathway. (A) The changes in mRNA expression of human gastric cancer cell SGC-7901 after treated with β-carboline alkaloids; (B) The changes in protein expression of human gastric cancer cell SGC-7901 after treated with β-carboline alkaloids; (C) The changes in mRNA expression of transplanted tumor in the mouse model after treated with β-carboline alkaloids; (D) The changes in protein expression of transplanted tumor in the mouse model after treated with β-carboline alkaloids. *P<0.05, compared with control group.

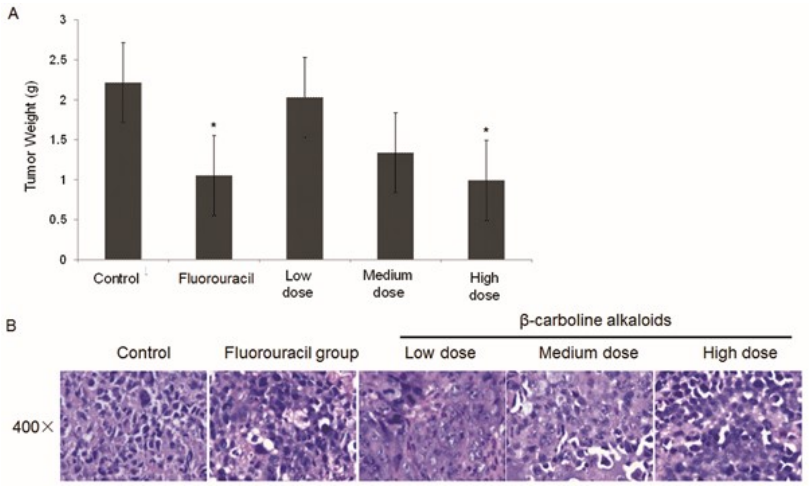

Fig. 2: Tumor weight and morphological changes of tumor tissues. (A) The tumor weight of different groups of mice; *P<0.05, compared with control group. (B) The HE staining of the tumor sections of each group.

Table 1: Sequences of the primers used in this study

Primer	Sequence
BCL2 -F	ATGCCTTTGTGGAACTATATGGC
BCL2 -R	GGTATGCACCCAGAGTGATGC
Bax -F	TGAAGACAGGGGCCTTTTTG
Bax -R	AATTCGCCGGAGACACTCG
PI3K -F	GTCAGTGAAGTGCCAGAGTGA
PI3K -R	CCAAAAAGGTCCCGTCTGCT
AKT -F	CCGCCTGATCAAGTTCTCCT
AKT -R	TTCAGATGATCCATGCGGGG
FAK -F	CCGGTCTCTGGGCTACAATG
FAK -R	ACTCAGAAGGCAGCAGTGAC
mTOR -F	CAGTGGAATTAAAAAGGGTGGCA
mTOR -R	TTGGCGATGGCTTCCATTCT
β-actin-F	TGACGTGGACATCCGCAAAG
β-actin-R	CTGGAAGGTGGACAGCGAGG

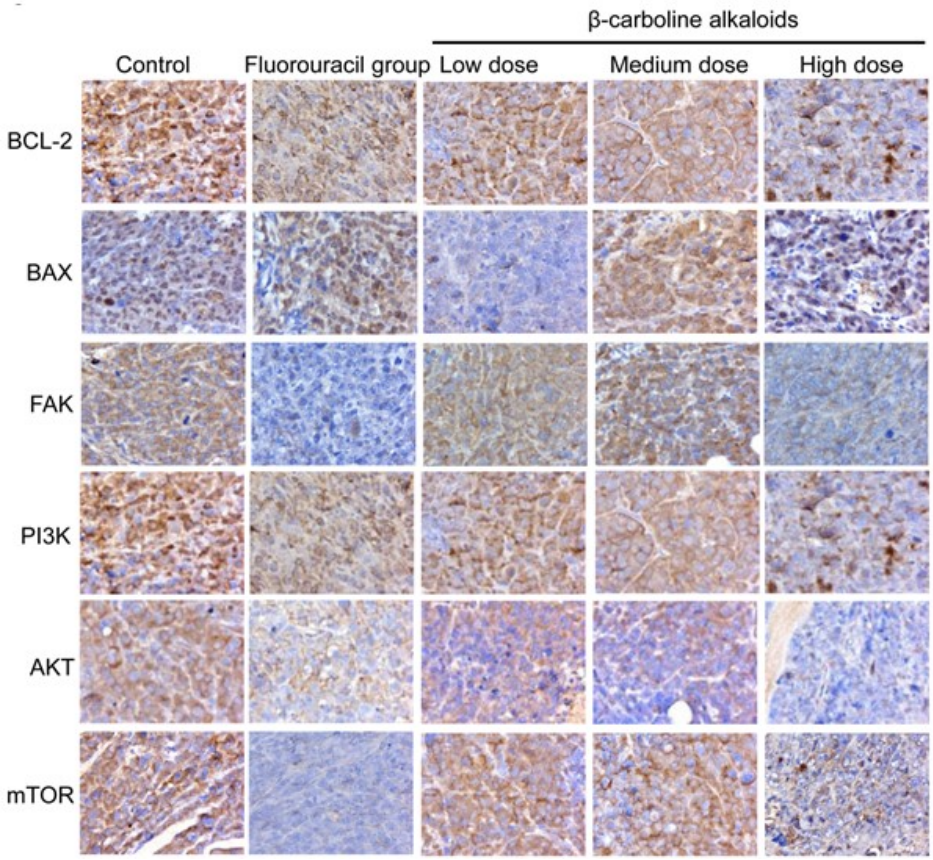

Fig. 3: The immunohistochemical staining of the tumor sections of each group to detect the expression of mTOR signaling pathway *in vivo*.

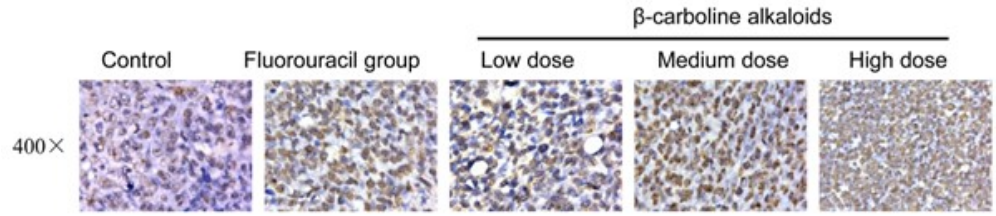

Fig. 4: The TUNEL staining of the tumor sections of each group to detect the cell apoptosis of tumor tissues.

DISCUSSION

Peganum harmala is a local medicinal herb used for the treatment of cough, rheumatism and inflammation in traditional Chinese medicine. Its active ingredient, total alkaloids, has a significant inhibitory effect on the growth of digestive tract tumor cells (Zhang *et al*, 2016; Wang *et al*, 2015; Wang *et al*, 2016). Our preliminary experiments found that the β-carboline alkaloids from *Peganum harmala* showed certain anti-tumor effects, mainly through the induction of apoptosis and affecting cell proliferation (Chen *et al*, 2015; Fan *et al*, 2015). The occurrence and development of tumors are closely related to the "Angiogenesis Theory" (Zhao *et al*, 2015; Christopher *et al*, 2016). However, the effect of β-carboline alkaloids on the angiogenesis-related FAK/PI3K/AKT/mTOR pathway rarely reported.

The Bcl-2 gene family is an important regulatory gene in the process of apoptosis. It can be divided into three classes according to its functions. One is Bcl-2 with BHL-4 structure that has anti-apoptotic effect, including Bcl-xl, Bcl-w, Mcl-1, etc. The second type contains BHL-3 and has a pro-apoptotic effect, such as Bax, Bak, Bad, Bid and Bim. The third type includes only BH3, which promotes apoptosis by modulating the above proteins (Smith *et al*, 2015; Kim *et al*, 2017; Wei *et al*, 2018). In tumor cells, over expressed Bcl-2 forms a heterodimer with Bax and exerts an inhibitory effect on apoptosis. This study found that Bax was highly expressed in the cells and tumors after β-carboline alkaloids treatment, while Bcl-2 expression was decreased. Therefore, it is speculated that β-carboline alkaloids may the formation of homodimers of Bax protein by regulating the expressions of Bcl-2 and Bax, thereby inducing apoptosis of cells (Wei *et al*, 2018).

This may be one of the mechanisms of action of β-carboline alkaloids against gastric cancer.

FAK is a cytoplasmic tyrosine kinase. High level of FAK-induced metastasis is associated with poor prognosis in gastric cancer (Chen *et al*, 2016; Haemmerle *et al*, 2016). Tyrosine kinase inhibitors can inhibit the migration of tumor cells by inhibiting the phosphorylation of FAK (Zhang *et al*, 2017). This study observed that FAK declined in cells and tumor tissues after treatment by β-carboline alkaloids, and the expression of apoptosis-related protein BCL-2 decreased, while that of BAX increased. This suggests that FAK is involved in the induction of cell apoptosis. Meanwhile, FAK, as the upstream protein of PI3K and AKT, also showed good consistency in this experiment, that is, the expression of FAK decreased, and the expression of PI3K and AKT also decreased. It is suggested that β-carboline alkaloids may inhibit tumor growth through the FAK/PI3K/AKT/mTOR signaling pathway. Phosphoinositide 3 kinase/protein kinase B (PI3K/Akt) signaling pathway regulates malignant cell growth, invasion and metastasis, and angiogenesis, and is closely related to the occurrence and development of various tumors (Costa *et al*, 2018). PI3K is a heterodimer consisting of a catalytic subunit (p110) and a regulatory subunit (PI3KR1). PI3K is responsible for the conversion of phosphorylation of 4,5-diphosphophosphatidylinositol to 3,4,5-triphosphatidylinositol, which is a key activation step of extra cellular growth factor stimulation. PI3K activates the pathway via up regulation of serine/threonie kinase (AKT) phosphorylation (p-AKT). AKT regulates many downstream signaling proteins involved in cell cycle regulation, initiation of apoptosis, angiogenesis, enhancement of telomerase activity and promotion of cell invasion, which play an important role in the occurrence and development of tumors (Golob-Schwarzl *et al*, 2017). Different signaling pathways have different effects on angiogenesis. Activated AKT can regulate vascular endothelial growth factor (VEGF) to promote angiogenesis and also induce the expression of hypoxia factor. Because hypoxia factor can cause hypoxia of the internal environment, it can stimulate the over expression of VEGF, thereby activating PI3K, forming feedback and stimulating angiogenesis. Tumor neovascularization is an important mechanism to promote tumorigenesis and development (Zhu *et al*, 2018), which is the target of many anti-tumor drugs.

mTOR is a conserved serine-threonine kinase and is a major target of the downstream pathway of AKT. The key process of stimulating cells is to mediate the nutrient-dependent intracellular signaling of Ser/Thr protein kinases and cell growth, proliferation and differentiation. As a central regulator of cell growth, mTOR combines with two key regulators, raptor or rictor, to form different protein complexes, i.e. TORC1 (Mtorc1/raptor complex) and TORC2 (mTOR/ rictor complex). mTORC1 stimulates protein synthesis by phosphorylating ribosomal protein S6 kinase (p70S6K) and translating eukaryotic initiation factor 4E binding protein 1 (4E-BP-1) to support cell growth and proliferation, cell metabolism and angiogenesis. mTORC2 phosphorylates AKT and SGK1, which regulate cell survival, apoptosis and cytoskeletal organization. Under normal conditions, PI3KAKT-mediated inactivation of TSC2 leads to proteasomal degradation of the TSC1/TSC2 protein complex, thereby activating mTOR. Similarly, loss of PTEN function, mutation or amplification of PI3K, amplification of AKT, and inactivation or mutation of AKT-related mTOR regulatory protein can also activate TOR (Magrys *et al*, 2018; Meng *et al*, 2018; O'Donnell *et al*, 2018). In this study, mTOR, as the most downstream protein, decreased to varying degrees in the SGC-7901 cells and mice treated with β-carboline alkaloids, suggesting that β-carboline alkaloids may inhibit tumor growth through the FAK/PI3K/AKT/mTOR signaling pathway.

As shown in this study, the expressions of PI3K, p-PI3K, AKT, p-AKT, mTOR mRNAs and proteins in human gastric cancer SGC-7901 cells were down-regulated after treated with β-carboline alkaloids, compared with the Control group. In the animal experiment, the mRNA level of PI3K and AKT, p-AKT protein levels in the high dose group were statistically different from the Control group. The mTOR mRNA levels in the medium and high dose groups were statistically different from the Control group. *In vitro* and *in vivo* experiments showed that PI3K, p-PI3K, AKT, p-AKT and mTOR were all reduced in each group. The results of *in vitro* experiments showed that β-carboline alkaloids significantly inhibited the growth of transplanted tumors. In HE staining, the β carboline alkaloids group and the high-dose group had obvious apoptotic cells compared with the control group, suggesting that the β-carboline alkaloid has significant anti-tumor effect. In the TUNEL experiment, the β-carboline alkaloid high-dose group had the highest apoptosis rate, suggesting that high-dose β-carboline alkaloids have a better anti-tumor effect than the fluorouracil group. The mechanism may be related to the reduced expression of AKT, PI3K and mTOR by β-carboline alkaloids, and β-carboline alkaloids can induce apoptosis of tumor cells by regulating the expressions of Bcl-2 and Bax. In the immunohistochemistry experiment, the PI3K, p-PI3K, AKT, p-AKT and mTOR were widely expressed in the cytoplasm of the cells of the control group, showing a brownish yellow color, while the gray value of the high dose group was significantly increased, suggesting a decreased expression of these proteins. These results indicate that β-carboline alkaloids significantly inhibit the protein expression levels of PI3K, p-PI3K, AKT, p-AKT and mTOR. Although this is inconsistent with the RT-PCR and Western blot results of the animal experiments, the results of animal experiments

still suggest that β-carboline alkaloids have anti-tumor effects, which may be due to the complicated anti-tumor mechanism or the existence of other activated signaling pathway in mice. Taken together, it is suggested that β-carboline alkaloids can induce cell apoptosis and have certain anti-tumor effects *in vitro*. The mechanism may be through regulating the expression of Bcl-2 and Bax and down-regulating the expression of PI3K/AKT/mTOR, which may induce apoptosis and reduce the expression of VEGF, resulting in the inhibition of transplanted tumors growth. Clinical trials (Oudart *et al*, 2016; Ella *et al*, 2018) also indicated that FAK, PI3K, AKT and mTOR were over expressed in gastric cancer tissues, suggesting they are involved in the development of gastric cancer, which was inconsistent with the results of this study. The MAPK/ERK pathway and the downstream of PI3K/AKT associated with angiogenesis have not been studied, which should be further researched to investigate the molecular mechanism of anti-tumor angiogenesis of β-carboline alkaloids.

In conclusion, β-carboline alkaloids treatment can induce cell apoptosis of human gastric cancer cell line SGC-7901 and tumor tissues, which may be related with the increase of BAX and decrease of BCL-2, FAK, PI3K, AKT and mTOR. Our findings may have certain guiding roles for the development of new drugs for gastric cancer treatment.

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