RESEARCH COMMUNICATION

# β-Elemene Induces Apoptosis in Human Renal-cell Carcinoma 786-0 Cells through Inhibition of MAPK/ERK and PI3K/Akt/mTOR Signalling Pathways

**Yun-Hong Zhan[1], Jing Liu[2], Xiu-Juan Qu[2], Ke-Zuo Hou[2], Ke-Feng Wang[1], Yun-Peng Liu[2]*, Bin Wu[1]***

## Abstract

**Background: Renal-cell carcinoma (RCC) is resistant to almost all chemotherapeutics and radiation therapy. β-Elemene, a promising anticancer drug extracted from a traditional Chinese medicine, has been shown to be effective against various tumors. In the present study, anti-tumor effects on RCC cells and the involved mechanisms were investigated. Methods: Human RCC 786-0 cells were treated with different concentrations of β-elemene, and cell viability and apoptosis were measured by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay and flow cytometry, respectively. Protein expression was assayed by western blotting. Autophagy was evaluated by transmission electron microscopy. Results: β-Elemene inhibited the viability of 786-0 cells in a dose- and time-dependent manner. The anti-tumor effect was associated with induction of apoptosis. Further study showed that β-elemene inhibited the MAPK/ERK as well as PI3K/Akt/mTOR signalling pathways. Moreover, robust autophagy was observed in cells treated with β-elemene. Combined treatment of β-elemene with autophagy inhibitors 3-methyladenine or chlorochine significantly enhanced the anti-tumor effects. Conclusions: Our data provide first evidence that β-elemene can inhibit the proliferation of RCC 786-0 cells by inducing apoptosis as well as protective autophagy. The anti-tumor effect was associated with the inhibition of MAPK/ERK and PI3K/Akt/mTOR signalling pathway. Inhibition of autophagy might be a useful way to enhance the anti-tumor effect of β-elemene on 786-0 cells.**

**Keywords:** Renal cell carcinoma - apoptosis - autophagy - mTOR

*Asian Pacific J Cancer Prev,* **13**, 2739-2744

## Introduction

Renal-cell carcinoma (RCC) is the most common type of kidney cancer. About 30% of RCC patients are diagnosed as the late phase diseases, missing the opportunity of taking curative surgical excision. RCC is resistant to almost all the chemotherapeutics and radiation therapy. Immunotherapy is the major treatment for the late phase disease, but the response rate is less than 20% (McDermott, 2009). Recently, some new biological agents such as sorafenib and sunitinib have shown better response in metastatic RCC, but the overall survival is still very poor (Escudier et al., 2007; Motzer et al., 2007). Thus, new anti-tumor agents with high efficiency are urgently needed.

Elemene (1-methyl-1-vinyl-2, 4-diisopropenyl-cyclohexane) is a novel anticancer agent extracted from the traditional Chinese medicine Rhizoma zedoariae (Li et al., 2009). In China, elemene emulsion has been approved by the State Food and Drug Administration of China to treat malignant effusions and some solid tumors. This indicates that elemene is a promising agent for the treatment of tumors. β-Elemene, the major active component of elemene, has been shown to be effective against various tumors such as lung cancer, prostate cancer and glioblastoma (Wang et al., 2005; Yao et al., 2008; Li et al., 2010). However, the molecular mechanisms associated with the anti-tumor effect of β-elemene are still not clear. Some studies showed that β-elemene inhibited cell proliferation by inducing apoptosis as well as cell cycle arrest (Wang et al., 2005; Yao et al., 2008; Li et al., 2009). The apoptosis triggered by β-elemene was reported through the mitochondrial-mediated pathway, as it was accompanied by the reduction of some anti-apoptosis proteins (Li et al., 2010). Yet the exact mechanisms, especially how up-stream signalling pathways regulate the apoptosis induced by β-elemene still need to be further elucidated.

Mitogen-activated protein kinases (MAPKs) are a group of serine/threonine protein kinases that respond

[1]*Department of Urology, the Shengjing Hospital,* [2]*Department of Medical Oncology, the First Hospital, China Medical University, Shenyang, China *For correspondence: cmuwubin@yahoo.cn, cmuliuyunpeng@yahoo.cn*

to extracellular stimuli and regulate various cellular activities, such as differentiation, proliferation, cell survival and apoptosis (Weston et al., 2002). There are several members in MAPKs family, among which the extracellular-signal-regulated kinases (ERK) and c-Jun N-terminal kinases (JNK) are the most important ones in regulating cell death and survival. It has been reported that some anti-cancer drugs could kill RCC cells by altering the activities of ERK and JNK (Li et al., 2008; Takano et al., 2010). ERK and JNK thus become important molecular targets for the treatment of RCC.

PI3K/Akt/mTOR is another important intracellular signalling pathway in regulating cell survival and death. The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase which works down stream of protein kinase B (Akt). Once activated, mTOR triggers the phosphorylation of the downstream target p70S6K1, enhances the transcription of certain mRNA, and increases the expression of proteins associated with proliferation (Borders et al., 2010). It has been reported that mTOR signalling pathway is over-activated in most renal clear cell carcinoma (Robb et al., 2007). mTOR inhibitors have been recommended by the National Comprehensive Cancer Network (NCCN) for the treatment of metastatic RCC (Anandappa et al., 2010; Bullock et al., 2010). These indicate mTOR might be the major target for the treatment of RCC.

Other than playing important role in regulating cell proliferation and apoptosis, mTOR is also a key regulator of autophagy (Jung et al., 2010). Autophagy is an intracellular degradation system that delivers cytoplasmic constituents to the lysosome (Mizushima, 2008). Under normal conditions, autophagy is a mechanism for the turnover of proteins and elimination of damaged organelles to maintain cell homeostasis; under pathological conditions it functions as an adaptive cell response, allowing the cell to survive bioenergetic stress (Mizushima, 2008). However, extensive or persistent autophagy also results in cell death (Thorburn, 2008). Thus, autophagy is a decisive factor in cell death and survival. Recent studies have shown that some chemotherapeutics known to activate apoptosis also induce autophagy; inhibition of autophagy by pharmacological inhibitors can enhance the anti-tumor activity of certain agents (Amaravadi et al., 2007; Li et al., 2010; Sasaki et al., 2010; Zhu et al., 2010). The protective autophagy induced by anti-cancer agents has now been thought to be a new mechanism responsible for the drug-resistance.

In the present study, the anti-tumor effect of β-elemene on RCC 786-0 cells was evaluated, and the impacts on MAPKs and PI3K/Akt/mTOR signalling pathways were further investigated. More importantly, a protective autophagy was observed under the treatment of β-elemene, which contributed negatively to the anti-tumor effect of β-elemene.

## Materials and Methods

*Chemicals and Reagents*

β-Elemene was obtained from Yuanda Pharmaceuticals (Dalian, China). Propidium iodide (PI), RNase A, 3-methyladenine (3MA) and chlorochine (CQ) were purchased from Sigma-Aldrich (St. Louis, Mo. USA). Anti-Bcl-2, anti-Bax, anti-Survivin, anti-Actin, anti-phospho-ERK, anti-ERK and anti-Akt antibodies were purchased from Santa Cruz Biotechnology (USA). Anti-Poly (ADP-ribose) polymerase (PARP), Anti-phospho-Akt (Ser-473), anti-phospho-mTOR, anti-mTOR, anti-phospho-p70S6K6, and anti-p70S6K1 antibodies were from Cell Signaling Technology (USA). Anti-LC3 antibodies were from Novus Biological (Littleton, CO, USA).

*Cell culture*

The human RCC 786-0 cells were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in RPMI-1640 medium (Gibco) containing 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 mg/mL) at 37 ˚C under an atmosphere of 95% air and 5% $CO_2$.

*Cell viability assay*

Cell viability was measured using a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The cells were seeded at 5x104 cells/well in 96-well plates, incubated overnight and then exposed to the indicated concentrations of β-elemene for the indicated times. Thereafter, 20 μl of MTT solution (5 mg/mL) was added to each well, and the cells were incubated for another 4 h at 37 ˚C. After removal of the culture medium, the cells were lysed in 200 μl of dimethylsulfoxide (DMSO), and the optical density (OD) was measured at 570 nm with a microplate reader (Model 550; Bio-Rad Laboratories, USA). The following formula was used: cell viability = (OD of the experimental sample/OD of the control group) × 100%.

*Analysis of apoptosis*

Cells were seeded at $3 \times 10^5$ cells/well in 6-well plates, incubated overnight and then exposed to the indicated concentrations of β-elemene for the indicated times. The cells were then collected and washed twice with phosphate-buffered saline (PBS). After being fixed with ice-cold 70% ethanol for 12 h, the samples were washed twice with PBS and then incubated with 20 μg/mL RNase A at 37 ˚C and 10 μg/mL PI for 30 min in the dark. Finally, the samples were evaluated by flow cytometry, and the data were analyzed by WinMDI software.

*Transmission electron microscopy*

Cells were treated and collected by trypsinization, then fixed with 2.5% phosphate-buffered gluteraldehyde, postfixed in 1% phosphate-buffered osmium tetroxide. Cells were then embedded, sectioned, double stained with uranyl acetate and lead citrate, and analyzed using a JEM-1200EX transmission electron microscope (JEOL, Japan).

*Western blotting*

Cells were washed twice with ice-cold PBS and solubilized in 1% Triton lysis buffer on ice, then quantified

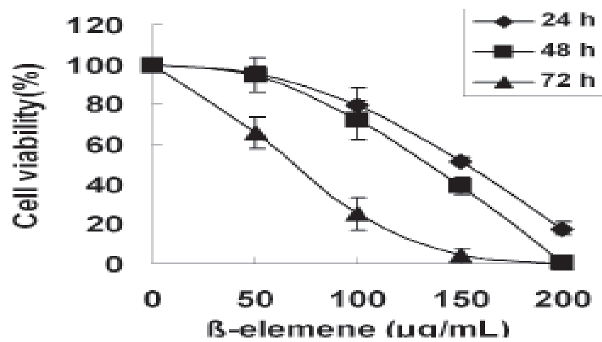


**Figure 1. Effect of β-Elemene on the Viability of 786-0 Cells.** 786-0 cells were treated with 50, 100, 150, or 200 μg/mL β-elemene for 24, 48 or 72 h, and the cell viability was determined by MTT assay. Dots: mean of three independent experiments; bars: SD

using the Lowry method. Cell lysate proteins (40 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose membranes (Immoblin-P; Millipore, USA). The membranes were blocked with 5% skim milk in TBST buffer at room temperature for 1 h and incubated overnight at 4 ˚C with the indicated primary antibodies. After the membranes were washed with TBST buffer, they were reacted with the appropriate horseradish peroxidase-conjugated secondary antibodies for 30 min at room temperature. After extensive washing with TBST buffer, the proteins were visualized with enhanced chemiluminescence reagent (SuperSignal Western Pico Chemiluminescent Substrate; Pierce, Rockford, IL, USA). The images were analyzed using NIH Image J software.

*Statistical analysis*

The experiments were repeated at least three times. Data are expressed as the means ± SD. Differences in the results for two groups were evaluated by the Student's t-test. $P<0.05$ was considered to be statistically significant.

## Results

*β-Elemene inhibited the viability of 786-0 cells*

Human RCC 786-0 cells were treated with different concentrations of β-elemene for 24, 48 or 72 h. Cell viability assay showed that β-elemene inhibited the proliferation of 786-0 cells in a dose- and time-dependent manner (Figure 1). The IC50 values at 24, 48 and 72 h were 145.60 μg/mL, 126.90 μg/mL and 63.84 μg/mL, respectively. These data indicated that β-elemene has significantly anti-tumor effect on 786-0 cells.

*β-Elemene induced apoptosis in 786-0 cells*

To explore if the anti-tumor effect of β-elemene on 786-0 cells was associated with apoptosis induction, the cells were treated with different concentrations of β-elemene, and the apoptosis was evaluated by flow cytometry following PI staining. In the cells treated with β-elemene, the apoptotic cells were significantly increased (Figure 2A). The apoptosis was further confirmed by the cleavage of PARP to its active form measured with western blotting (Figure 2B).To understand the molecular mechanisms involved in the apoptosis induced by

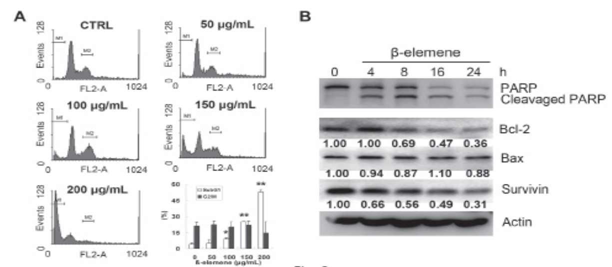


**Figure 2. Effect of β-Elemene on Apoptosis of 786-0 Cells.** (A) 786-0 cells were treated with 50, 100, 150, or 200 μg/mL β-elemene for 24 h, and apoptosis was evaluated by flow cytometry followed by PI staining. Columns: mean of three independent experiments; bars: SD. * $P<0.05$, ** $P<0.01$ vs. cells treated with β-elemene alone. (B) Cells were treated with 100 μg/mL β-elemene for indicated times, and the cleavage of PARP and the expression of Bcl-2, Bax and Survivin was detected by western blotting. The results were representatives of three independent experiments. Actin was used as loading control. The numbers indicated the relative levels of proteins corrected for actin

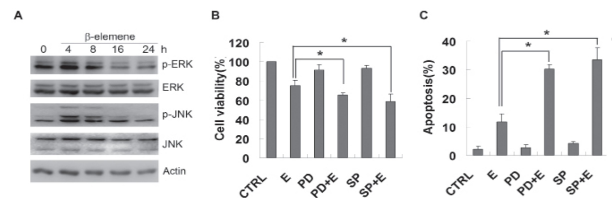


**Figure 3. Effect of β-Elemene on MAPKs/ERKs and JNKs Signalling Pathways.** (A) 786-0 cells were treated with 100 μg/mL β-elemene for 0-24 h, and the levels of p-ERK and p-JNK were detected by western blotting. (B) Cells were treated with 100 μg/mL β-elemene alone for 24 h, or pre-treated with 20 umol/L PD98059 or 20 umol/L SP600125 for 1 h, followed by exposure to 100 μg/mL β-elemene for another 24 h, and the cell viability was determined by MTT assay. (C) Cells were treated as described above, and apoptosis was analyzed by flow cytometry. CTRL: untreated control cells; E: β-elemene; PD: PD98059; SP: SP600125. Columns: mean of three independent experiments; bars: SD. * $P<0.05$ vs. cells treated with β-elemene alone

β-elemene, the effect of β-elemene on the expression of apoptosis-associated proteins was next investigated. As shown in Figure 2B, β-elemene treatment significantly down-regulated the levels of Bcl-2 and Survivin, but had little effect on Bax expression. These data indicated that the anti-tumor effect of β-elemene on 786-0 cells was associated with apoptosis induction.

*Effects of β-elemene on MAPKs/ERK and JNK signalling pathways*

To evaluate the effects of β-elemene on the activities of MAPK/ERK and JNK signalling pathways, the cells were treated with 100 μg/mL β-elemene, and the levels of phospho-ERK and phospho-JNK were measured by western blotting. As shown in Figure 3A, the levels of phospho-ERK and phospho-JNK were transiently up-regulated at 4 h and 8 h. Prolonged exposure to 16 h and 24 h decreased significantly the phosphorylation of ERK, whereas the level of phospho-JNK was as same as that in the untreated control cells. To understand the changes of ERKs and JNKs activity contributed anything to the anti-tumor effect of β-elemene, the cells were pre-treated with ERK inhibitor PD98059 or JNK inhibitor SP600125

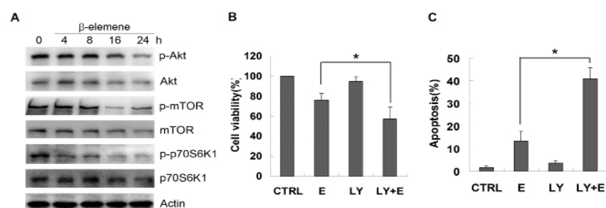


**Figure 4. Effect of β-Elemene on PI3K/Akt/mTOR Signalling Pathway.** (A) 786-0 cells were treated with100 μg/mL β-elemene for 0-24 h, and the levels of p-Akt , p-mTOR and p-p70S6K1 were detected by western blotting. (B) Cells were treated with 100 μg/mL β-elemene alone for 24 h, or pre-treated with 25 umol/L LY294002 for 1 h, followed by exposure to 100 μg/mL β-elemene for another 24 h. The cell viability was determined by MTT assay. (C) Cells were treated as described above, and the apoptosis was analyzed by flow cytometry. CTRL: untreated control cells; E: β-elemene; LY: LY294002. Columns: mean of three independent experiments; bars: SD. * $P<0.05$ vs. cells treated with β-elemene alone

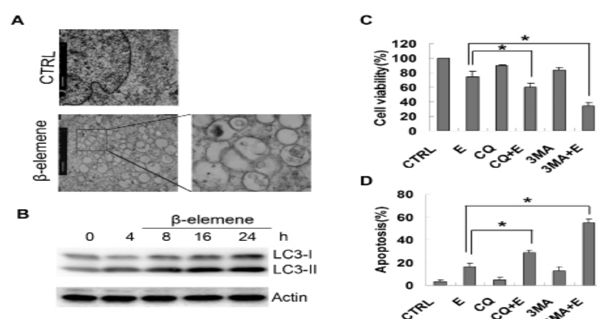


**Figure 5. Effect of β-Elemene on Autophagy.** (A) 786-0 cells were treated with 100 μg/mL β-elemene for 24 h, then harvested and subjected to transmission electron microscopy as described in Materials and Methods. (B) After the cells were exposed to 100 μg/mL β-elemene for 0-24 h, cell lysates were subjected to western blotting with an anti-LC3 antibody. The results were representatives of three independent experiments. Actin was used as loading control. (C) Cells were exposed to either 100 μg/mL β-elemene, 5 mmol/L 3MA, 10 μmol/L CQ, or a combined treatment of β-elemene and 3MA or CQ for 24 h, and cell viability was measured by MTT assay. (D) Cells were treated as described above, and apoptosis was analyzed by flow cytometry. CTRL: untreated control cells; E: β-elemene; CQ: chlorochine; 3MA: 3-methyladenine. Columns: mean of three independent experiments; bars: SD. * $P<0.05$ vs. cells treated with β-elemene alone

for 1 h, then were exposed to 100 μg/mL β-elemene for another 24 h. Compared with the cells treated with β-elemene alone, pre-treatment of ERK or JNK inhibitor followed by β-elemene reduced significantly the viability of 786-0 cells and enhanced apoptosis (Figure 3B and 3C). These data indicated that the transient activation of JNK might contribute negatively to the anti-tumor effect of β-elemene; β-elemene induced apoptosis at least partly by inhibiting ERK activity.

*Effects of β-elemene on PI3K/Akt/mTOR activity*

It has been reported that PI3K/Akt/mTOR signalling plays important role in cell proliferation and apoptosis. The impact of β-elemene on PI3K/Akt/mTOR activity was next investigated. As shown in figure 4A, β-elemene significantly down-regulated the levels of phospho-Akt, phospho-mTOR, and the down-stream protein phospho-p70S6K1 at 16 h and 24 h. Combination of β-elemene with LY294002, the specific inhibitor of PI3K/Akt, inhibited the cell viability and enhanced the apoptosis significantly, compared with the cells treated with β-elemene alone (Figure 4B and 4C). These data indicated that β-elemene induced apoptosis in 786-0 cells at least partly by inhibition of PI3K/Akt/mTOR activity.

*β-Elemene induced autophagy and apoptosis*

It has been well documented that inhibition of mTOR activity might lead to activation of autophagy, we then tested if there was any autophagy induced by β-elemene. In the cells treated with 100 μg/mL β-elemene, many double-membraned vesicles containing engulfed organelles were observed in the cytoplasm under transmission electron microscopy (Figure 5A). These vesicles indicated the formation of autophagosome. Meanwhile, a conversion of LC3-I to LC3-II was also observed in the cells treated with β-elemene, which further confirmed the induction of autophagy (Figure 5B). To understand how autophagy contributed to the anti-tumor effect of β-elemene, the cells were exposed to either β-elemene alone, or were treated with β-elemene combined with autophagy inhibitor, 3MA or CQ. Compared with the cells treated with β-elemene alone, combined treatment with β-elemene and 3MA or CQ significantly reduced the cell viability and enhanced the apoptosis. These data indicated that autophagy induced by β-elemene was protective and prevented the cells from undergoing apoptosis.

## Discussion

Elemene is a nature traditional Chinese medicinal herb with promising anti-tumor effects. In China elemene emulsion injection has been used to treat some solid tumors in clinic and yielded mild side effect (Wang et al., 1994; Tan et al., 2000). The high efficiency towards tumors and the low toxicity to normal tissues make elemene a potential therapeutic for malignant diseases. The extract of elemene is a mixture of β-, γ- and δ-elemene, with β-elemene as the main component (Wang et al., 2005). It has been reported that β-elemene could inhibit the proliferation of some chemotherapy-resistant tumors, such as prostate cancer, melanoma and glioblastoma (Li et al., 2010; Chen et al., 2011; Zhu et al., 2011). RCC is the most refractory tumor to chemotherapy. In the present study, we reported that β-elemene had obvious anti-tumor effect on RCC 786-0 cells. The IC50 values of β-elemene were close to those in other types of cancers. This indicated that β-elemene might become a potential therapeutics for RCC.

Although the anti-tumor effects of β-elemene have been observed in various types of tumors, the exact mechanism is so far not clear. It has been reported that β-elemene could induce apoptosis in prostate cancer cells and other types of solid tumour cells by down-regulating some anti-apoptotic proteins (Li et al., 2009; Li et al., 2010). Others reported that β-elemene induced cell cycle arrest at G0/G1 or G2/M phase (Li et al., 2005; Yao et al., 2008; Zhu et al., 2011). In the present study, β-elemene induced apoptosis accompanied with the down-regulation of Bcl-2 and Survivin, whereas the cell cycle distribution was not affected significantly. These may due to the

different regulating mechanism of cell cycle progress in different types of cells.

MAPKs family members play important role in cell proliferation and apoptosis regulation. It has been reported that the anti-tumor effect of β-elemene on glioblastoma cells depends on MKK3/6 and p38 MAPK activation (Yao et al., 2008; Zhu et al., 2011). Yet little is known about how β-elemene affects ERK and JNK pathways. In the present study, a short time exposure of 786-0 cells to β-elemene resulted in transient activation of ERK and JNK pathway. Combination of β-elemene with inhibitors of ERK and JNK enhanced significantly the anti-tumor effect of β-elemene. These data indicated that the transient activation of ERK and JNK might be a rapid survival response towards stress, and tried to stop the cells from apoptosis. ERK and JNK pathway contributed negatively to the anti-tumor effect of β-elemene. Prolonged exposure of the cells to β-elemene to 24 h reduced significantly the ERK activity but not JNK activity, suggested β-elemene inhibited the proliferation of 786-0 cells through inhibition of ERK but not JNK pathway.

The PI3K/Akt/mTOR is another important signalling pathway in regulating cell proliferation and apoptosis. It has been reported that Akt, mTOR and p70S6K1 are constitutively activated in RCC cells compared to normal renal tissue (Lin et al., 2006; Robb et al., 2007; Abou et al., 2011). mTOR is so that becoming an important target for the treatment of RCC. Our data showed that β-elemene inhibited significantly the activity of PI3K/Akt/mTOR pathway, and a combination of β-elemene with PI3K inhibitor further enhanced the anti-tumor effect of β-elemene. These indicated that β-elemene induced apoptosis at least partly by inhibiting PI3K/Akt/mTOR activity.

Meanwhile, mTOR is a key regulator of autophagy; inhibition of mTOR leads to induction of autophagy (Yang et al., 2010). In our study β-elemene treatment induced an accumulation of double membrane enclosed vesicles in the cytoplasm and increased the level of LC3-II, a conjugated form of LC3 with phosphatidylethanolamine (PE) located on the double membrane of autophagosome. These data indicated that inhibition of mTOR by β-elemene resulted in the activation of autophagy. Since autophagy results in both survival and cell death, we further investigated what autophagy contributed to the anti-tumor effect of β-elemene. We found that inhibition of autophagy with specific inhibitor 3MA or CQ enhanced significantly the anti-tumor effect of β-elemene, suggested that autophagy induced by β-elemene was a protective response and prevented the cells from apoptosis. Our data indicated that inhibition of PI3K/Akt/mTOR signalling by β-elemene resulted in two opposite consequences: on the one hand, it inhibited cell proliferation and induced apoptosis; on the other hand, it induced a protective autophagy and prevented the cells from apoptosis. The balance of apoptosis and protective autophagy finally decided the fate of cells under the treatment of β-elemene.

Taken together, our data provided the first evidence that β-elemene could inhibit the proliferation of RCC 786-0 cells by inducing apoptosis. The anti-tumor effect was associated with the inhibition of MAPK/ERK and PI3K/Akt/mTOR activity. More importantly, a protective autophagy was induced by β-elemene and prevented the cells from apoptosis. Inhibition of autophagy might be a useful way to enhance the anti-tumor effect of β-elemene towards 786-0 cells.

## Acknowledgements

This work was supported by Chinese National Foundation of National Sciences grants (No. 30770993, No. 81101746) and the Fund for Scientific Research of The First Hospital of China Medical University (No. fsfh1002). The authors declare that there is no conflict of interest with this work.

## References

Abou Youssif T, Fahmy MA, Koumakpayi IH, et al (2011). The mammalian target of rapamycin pathway is widely activated without PTEN deletion in renal cell carcinoma metastases. *Cancer*, **117**, 290-300.

Amaravadi RK, Yu D, Lum JJ, et al (2007). Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. *J Clin Invest*, **117**, 326-36.

Anandappa G, Hollingdale A, Eisen T (2010). Everolimus - a new approach in the treatment of renal cell carcinoma. *Cancer Manag Res*, **2**, 61-70.

Borders EB, Bivona C, Medina PJ (2010). Mammalian target of rapamycin: biological function and target for novel anticancer agents. *Am J Health Syst Pharm*, **67**, 2095-106.

Bullock A, McDermott DF, Atkins MB (2010). Management of metastatic renal cell carcinoma in patients with poor prognosis. *Cancer Manag Res*, **2**, 123-32.

Chen W, Lu Y, Wu J, et al (2011). Beta-elemene inhibits melanoma growth and metastasis via suppressing vascular endothelial growth factor-mediated angiogenesis. *Cancer Chemother Pharmacol*, **67**, 799-808.

Escudier B, Eisen T, Stadler WM, et al (2007). TARGET Study Group: Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med*, **356**, 125-34.

Jung CH, Ro SH, Cao J, Otto NM, Kim DH (2010). mTOR regulation of autophagy. *FEBS Lett*, **584**, 1287-95.

Li L, Gao Y, Zhang L, et al (2008). Silibinin inhibits cell growth and induces apoptosis by caspase activation, down-regulating survivin and blocking EGFR-ERK activation in renal cell carcinoma. *Cancer Lett*, **272**, 61-9.

Lin F, Zhang PL, Yang XJ, et al (2006). Morphoproteomic and molecular concomitants of an overexpressed and activated mTOR pathway in renal cell carcinomas. *Ann Clin Lab Sci*, **36**, 283-93. Li QQ, Wang G, Huang F, Banda M, Reed E (2010). Antineoplastic effect of beta-elemene on prostate cancer cells and other types of solid tumour cells. *J Pharm Pharmacol*, **62**, 1018-27.

Li QQ, Wang G, Reed E, Huang L, Cuff CF (2010). Evaluation of Cisplatin in Combination with beta-Elemene as a Regimen for Prostate Cancer Chemotherapy. *Basic Clin Pharmacol Toxicol*, **107**, 868-76.

Li QQ, Wang G, Zhang M, et al (2009). beta-Elemene, a novel plant-derived antineoplastic agent, increases cisplatin chemosensitivity of lung tumor cells by triggering apoptosis. *Oncol Rep*, **22**, 161-70.

Li X, Fan Z (2010). The epidermal growth factor receptor antibody cetuximab induces autophagy in cancer cells by downregulating HIF-1{alpha} and Bcl-2 and activating the beclin 1/hVps34 complex. *Cancer Res*, **70**, 5942-52.

Li X, Wang G, Zhao J, et al (2005). Antiproliferative effect of beta-elemene in chemoresistant ovarian carcinoma cells is mediated through arrest of the cell cycle at the G2-M phase. *Cell Mol Life Sci*, **62**, 894-904.

McDermott DF (2009). Immunotherapy of metastatic renal cell carcinoma. *Cancer*, **115**, 2298-305.

Mizushima N (2008). Autophagy: process and function. *Genes Dev*, **21**, 2861-73.

Motzer RJ, Hutson TE, Tomczak P, et al (2007). Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med*, **356**, 115-24.

Robb VA, Karbowniczek M, Klein-Szanto AJ, Henske EP (2007). Activation of the mTOR signaling pathway in renal clear cell carcinoma. *J Urol*, **177**, 346-52.

Sasaki K, Tsuno NH, Sunami E, et al (2010). Chloroquine potentiates the anti-cancer effect of 5-fluorouracil on colon cancer cells. *BMC Cancer*, **10**, 370.

Takano Y, Iwata H, Yano Y, et al (2010). Up-regulation of connexin 32 gene by 5-aza-2'-deoxycytidine enhances vinblastine-induced cytotoxicity in human renal carcinoma cells via the activation of JNK signalling. *Biochem Pharmacol*, **80**, 463-70.

Tan P, Zhong W, Cai W, et al (2000). [Clinical study on treatment of 40 cases of malignant brain tumor by elemene emulsion injection]. *Zhongguo Zhong Xi Yi Jie He Za Zhi*, **20**, 645-8. [Article in Chinese]

Thorburn A (2008). Apoptosis and Autophagy: regulatory connections between two supposedly different processes. *Apoptosis*, **13**, 1-9.

Wang G, Li X, Huang F, et al (2005). Antitumor effect of beta-elemene in non-small-cell lung cancer cells is mediated via induction of cell cycle arrest and apoptotic cell death. *Cell Mol Life Sci*, **62**, 881-93.

Wang J, Zhang H, Sun Y, et al (1996). Phase III clinical trial of elemenum emulsion in the management of malignant pleural and peritoneal effusions. *Zhonghua Zhong Liu Za Zhi*, **18**, 464-7 (in Chinese).

Weston CR, Lambright DG, Davis RJ (2002). Signal transduction: MAP kinase signaling specificity. *Science*, **296**, 2345-7.

Yang Z, Klionsky DJ (2010). Mammalian autophagy: core molecular machinery and signaling regulation. *Curr Opin Cell Biol*, **22**, 124-31.

Yao YQ, Ding X, Jia YC, et al (2008). Anti-tumor effect of beta-elemene in glioblastoma cells depends on p38 MAPK activation. *Cancer Lett*, **264**, 127-34.

Zhu K, Dunner K Jr, McConkey DJ (2010). Proteasome inhibitors activate autophagy as a cytoprotective response in human prostate cancer cells. *Oncogene*, **29**, 451-62.

Zhu T, Zhao Y, Zhang J, et al (2011). ß-Elemene inhibits proliferation of human glioblastoma cells and causes cell-cycle G0/G1 arrest via mutually compensatory activation of MKK3 and MKK6. *Int J Oncol*, **38**, 419-26.