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Full paper

Allicin sensitizes hepatocellular cancer cells to anti-tumor activity of 5-fluorouracil through ROS-mediated mitochondrial pathway

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ABSTRACT

Drug resistance and hepatic dysfunction are the two major factors that limit the application of chemotherapy for hepatocellular carcinoma (HCC). It has been reported that allicin has the hepatic protective effect and antitumor activity. Hence allicin may be an ideal enhancer to chemotherapy regimen of HCC. In the present study, we demonstrated that allicin enhanced 5-fluorouracil (5-FU) inducing cytotoxicity in HCC cells. *In vivo* experiment, combined treatment group with allicin (5 mg/kg/d; every two days for 3 weeks) and 5-FU (20 mg/kg/d; 5 consecutive days) showed a dramatic inhibitory effect on the growth of HCC xenograft tumors in nude mice. The co-treatment group showed highly apoptotic level compared with 5-FU treated alone. Cells combined treatment with allicin and 5-FU increased intracellular reactive oxygen species (ROS) level, reduced mitochondrial membrane potential ($\Delta\Psi m$), activated caspase-3 and PARP, and down-regulated Bcl-2 compared with DMSO, allicin and 5-FU treated alone. Moreover, the increase of activated caspase-3 and PARP was blocked by the ROS inhibitor antioxidant N-acetyl cysteine (NAC). In conclusion, this is the first study to demonstrate that allicin sensitized HCC cells to 5-FU induced apoptosis through ROS-mediated mitochondrial pathway. These results provided evidences for the combination used of allicin and 5-FU as a novel chemotherapy regimen in HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common human cancer and the second most common cause of cancer-related deaths in the world (1). The therapy approaches of HCC have been improved in the recent years, including liver transplantation, surgical resection, radiofrequency ablation (RFA), intervention, chemotherapy, radiotherapy, molecular targeted therapy, and biotherapy (2,3). Surgery remains the potentially curative therapies for early HCC patients, and non-surgical treatments play an important role in the therapy of advanced HCC. The use of systemic chemotherapy is limited because of the chemo-resistant in HCC cells, instinct or extrinsic (4,5). Hence, to find combination agents which can enhance the cytotoxicity of traditional chemotherapeutic regimens is of great importance.

Allicin (diallyl thiosulfinate), rapidly converted from allin by allinase, is the major component present in freshly crushed garlic, and one of the most biologically active compounds of garlic (6). Allicin has been used in the clinic for several decades with its effect of anti-inflammatory, anti-microbial, cardiovascular protection, and immunity boost (7–9). Previous investigations have shown that allicin exerts antitumor activity in multiple tumors such as gastric carcinoma, breast cancer, glioblastoma and HCC, by inhibiting cell proliferation and inducing cell apoptosis (10,11). Notably, allicin exhibits the chemosensitive effect of CPT-11 on the colorectal cancer cells *in vitro* (12). However, *in vivo* efficacy of its chemosensitivity and potential molecular mechanism in HCC is still unknown.

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In the present study, we explored whether sub-cytotoxic allicin could enhance the antitumor effect of traditional chemotherapy drug 5-fluorouracil (5-FU) in HCC cells, both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Cells and reagents

Human hepatocellular carcinoma (HCC) cell lines, SK-Hep-1 and BEL-7402, were obtained from Cell Bank of Type Culture Collection (CBTCC, Chinese Academy of Sciences, Shanghai, China). The cells were maintained in Dulbecco's Modified Eagle Medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Gibco) at 37 °C in a humidified incubator containing 5% CO_2. Allicin was purchased from Guangzhou Institute For Drug Control (Guangzhou, China). 5-Fluorouracil, N-acetyl cysteine (NAC) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Cell viability assays

Cells were plated at 1×10^4 cells per well in 96-well. After overnight incubation, cells were treated with allicin, 5-FU alone or a combination at the indicated concentrations. After 48 h, cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma) assay. Viability rate was calculated as follow formula: viability (%) = (average OD value of drug-treated sample/average OD value of control sample) × 100%. Agents' concentrations resulting in cell growth inhibition of 50% (IC_{50}) were calculated by Probit Regression Analysis.

2.3. Combined effect evaluation

Using the method proposed by Chou et al. (13), the interaction between allicin and 5-FU was quantified on the basis of the multiple drug-effect equation and determined by combination index (CI) value. The combination index was evaluated by CompuSyn software (ComboSyn, Inc., Paramus, NJ) which is available at http://www.combosyn.com/feature.html. The CI value <1, =1, and >1 indicate synergism, additive effect and antagonism, respectively.

2.4. In vivo tumor assays

Male athymic nude mice (4–6-week) were injected subcutaneously into left flank with 1×10^7 GFP-SK-Hep-1 cells. When the tumors had reached a size of 5 × 5 mm^2, mice were randomly divided into 4 groups (n = 5 in each group). Tumor-bearing animals were treated (i.p) either with DMSO alone (as control), allicin alone (5 mg/kg/d; every two days for 3 weeks), 5-FU (20 mg/kg/d; 5 consecutive days) alone or with a combination of allicin and 5-FU. The tumor mass was measured every 2 days throughout the study. Tumor volumes were determined by a caliper and calculated according to the formula ($width^2 \times length$)/2. Animals were sacrificed and tumors were collected 3 h after the last injection. The fluorescence emitted by the tumor cells was imaged by a whole-body GFP imaging system (Imagestation 2000MM; Kodak, Rochester, Rochester, NY) to visualize the formation of the tumor. All the *in vivo* experiments were performed according to our institutions' guidelines for the use of laboratory animals and approved by the institutional Animal Care and Use Committee of Nanfang Hospital.

2.5. Annexin V-fluorescein isothiocyanate (FITC)–propidium iodide (PI) assays

Cells were incubated with allicin and 5-FU alone or in combination for 48 h, collected and suspended in 1× binding buffer. Apoptotic cells were evaluated by Annexin V-FITC and PI Apoptosis Detection Kit (Apoptosis Detection Kit, KeyGEN, Nanjing, China) according to the manufacturer's protocol. Stained cells were analyzed by FACSCanto II Flow Cytometer (BD Biosciences, San Jose, CA). Cells that were Annexin V positive and PI negative were considered as apoptotic cells.

2.6. Western blot analysis

Cells were lysed with RIPA buffer (Cell Signaling Technology, Boston, MA) containing protease inhibitor (Roche, Indianapolis, IN). Equal amounts of protein were separated by electrophoresis on 10% SDS-PAGE gels and transferred onto polyvinylidene difluoride membranes (PVDF; Millipore, Bedford, MA). After blocking with 5% nonfat dry milk in Tris-buffered saline (TBST, 100 mM NaCl, 50 mM Tris, 0.1% Tween-20; pH 7.5), membranes were incubated with primary antibody for caspase-3 (Abcam, Cambridge, UK), Poly (ADP-ribose) polymerase (PARP; Abcam), Bcl-2 (Abcam), and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4 °C. After washing with TBST, the membranes were incubated with corresponding secondary antibodies conjugated to horseradish peroxidase. The signals were detected by enhanced chemiluminescence (ECL; Pierce, Rockford, IL).

2.7. Cellular reactive oxygen species (ROS) detection

The level of intracellular ROS was determined using Dichlorodihydro-fluorescein diacetate assay (DCFH-DA, Sigma–Aldrich). Briefly, after treatment, cells were incubated with DCFH-DA (5 mg/mL) at 37 °C for 30 min. Samples were then analyzed with Cyflogic v.1.2.1 software using FITC-A band pass filter on flow cytometer (BD Bioscience).

2.8. Mitochondrial membrane potential

To detect the changes in mitochondrial transmembrane potentials of cells in different treated groups, JC-1 staining was used. After treatment, cells were trypsinized, washed twice in PBS and suspended in mitochondrial incubation buffer (KeyGEN Biotech, China). After adding JC-1 dye, cells were incubated at 37 °C in the dark for 15 min. Cells were washed twice in PBS and the pellet was re-suspended in mitochondrial incubation buffer. Mitochondrial membrane potential was determined by flow cytometry (BD Bioscience) using FL1-H (green fluorescence, Ex = 488 nm) and FL2-H (red fluorescence, Ex = 530 nm) band pass filters.

2.9. TUNEL assay

Tumor xenografts were embedded in OCT (Sakura Finetek, Torrance, CA), and cut into 6 μm cryopreserved sections. Tumor tissues were stained by terminal deoxy-nucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL; In Situ Cell Death Detection Kit, Roche). In brief, tissue sections were fixed in 4% paraformaldehyde, added permeabilisation solution, and incubated with TUNEL reaction mixture. A minimum of three randomly selected high-power fields containing representative sections of each tumor were imaged. At least five tumors were examined per treatment group. TUNEL positive cells and total cells were counted and percent apoptotic cells calculated.

3. Statistical analysis

Statistics were analyzed with the SPSS 16.0 software (Abbott Laboratories, Chicago, IL). The quantitative data were shown as means ± SD from three independent experiments. In all experiments, the significance of the difference between groups was calculated using one-way analysis of variance (ANOVA). A value of $P < 0.05$ indicated a significant difference.

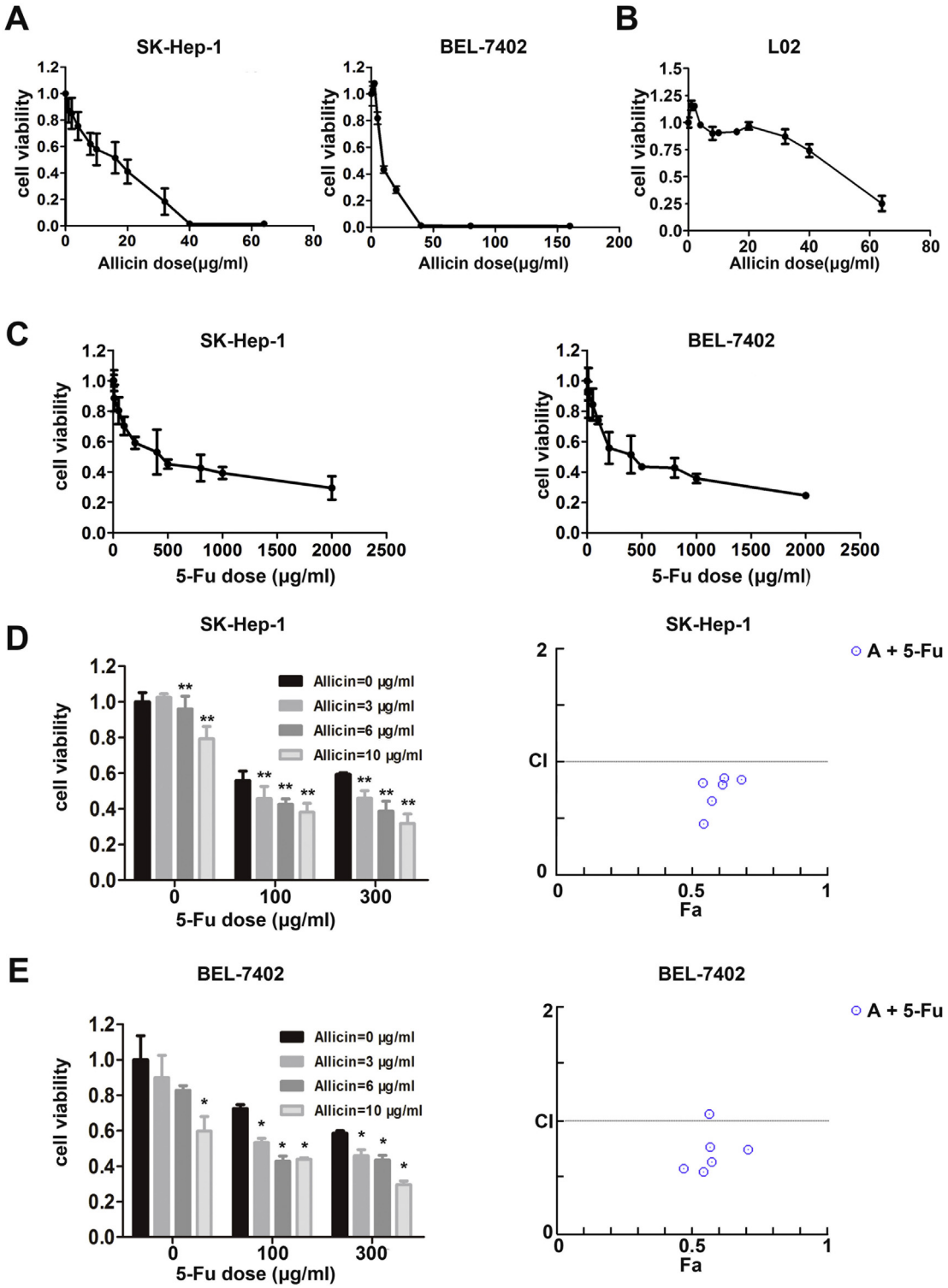

Fig. 1. Allicin synergistically sensitized hepatocellular cancer cells to 5-fluorouracil *in vitro*. (A) SK-Hep-1 and BEL-7402 cells were incubated with various concentrations of allicin (0, 1, 2, 4, 8, 10, 16, 20, 32, 40, 64 μg/ml for SK-Hep-1 and 0, 1.25, 2.5, 5, 10, 20, 40, 80, 160 μg/ml for BEL-7402) for 48 h, and cell viability was detected by MTT assay. (B) L02 cells were treated with different concentrations of allicin (0, 1, 2, 4, 8, 10, 16, 20, 32, 40, 64 μg/ml), and cell viability was evaluated by MTT assay. (C) SK-Hep-1 and BEL-7402 cells were treated with various concentrations of 5-FU (0, 5, 10, 50, 100, 200, 400, 500, 800, 1000, 2000 μg/ml) for 48 h, and cell viability was evaluated by MTT assay. (D and E) SK-Hep-1 and BEL-7402 were co-treated with allicin and 5-FU with various concentrations. Cell viability was assessed using MTT assay and the combination index of each combination treatment was calculated by CompuSyn software (Right). Points below the dotted line indicate synergy. (*$P < 0.05$, **$P < 0.01$).

4. Results

4.1. Allicin synergistically sensitizes hepatocellular cancer cells to 5-fluorouracil in vitro

To explore the anti-proliferative effect of allicin and 5-FU, the viability of HCC cells treated with allicin or 5-FU alone was assessed by MTT assay. As presented in Fig. 1A, allicin inhibited the growth of SK-Hep-1 and BEL-7402 cells in a dose-dependent manner. The IC_{50} value of allicin was 10.389 μg/ml and 10.004 μg/ml for SK-Hep-1 and BEL-7402 cells, respectively. Allicin at the concentration under 20 μg/ml had no significant inhibiting effect on normal hepatocytes indicating its safety as a chemotherapy regimen in HCC (Fig. 1B). As showed in Fig. 1C, 5-FU inhibited the growth of SK-Hep-1 and BEL-7402 cells in a dose-dependent manner. The IC_{50} value of 5-FU was 438.945 μg/ml and 410.336 μg/ml for SK-Hep-1 and BEL-7402 cells, respectively. To determine the synergistic effect of allicin and 5-FU, HCC cells were exposed to different concentration of allicin (0, 3, 6, 10 μg/ml) and 5-FU (0, 100, 300 μg/ml) alone or in combination, and cell viability was detected. The anti-proliferative effect of the combined treatment was significantly greater than that in single agent treatment, even in subcytotoxic dose ($<IC_{50}$) allicin (Fig. 1D and E). In addition, combination index (CI) of each combined treatment was analyzed and showed in the Fa-Combination Index Plot. CI value <1 at different drug combination doses (allicin 3/6/10 μg/ml with 5-Fu 100/300 μg/ml in SK-Hep-1 cells; allicin 3/6 μg/ml with 5-Fu 100/300 μg/ml and allicin 10 μg/ml with 5-Fu 300 μg/ml in BEL-7402 cells), indicated a synergistic interaction between allicin and 5-FU both in SK-Hep-1 and BEL-7402 cells (Table .1).

4.2. Allicin enhances the chemosensitivity of 5-FU in vivo

To verify the chemotherapy sensitizing activity of allicin, a subcutaneous xenograft tumor model was established by transplanting SK-Hep-1-GFP cells into nude mice. The combined treatment group exhibited a reduced gain of tumor sizes compared with DMSO group, allicin group, and 5-FU group (Fig. 2A). The tumor volume and weight were also found significantly reduced in co-treated group as compared with three other groups ($P < 0.05$; Fig. 2B, C). The apoptotic level of xenograft tumors was much higher in co-treatment group, compared with those in DMSO, allicin, or 5-FU treated group, as determined by TUNEL assay ($P < 0.05$; Fig. 2D, E).

Table 1
Fa value and combination index of combined treatments.

Cell	Drug (μg/ml)		Fa value	Combination index
	Allicin	5-Fu		
SK-Hep-1	3	100	0.54 ± 0.07	0.46
	6	100	0.58 ± 0.03	0.66
	10	100	0.62 ± 0.05	0.86
	3	300	0.54 ± 0.04	0.82
	6	300	0.61 ± 0.06	0.80
	10	300	0.68 ± 0.05	0.84
BEL-7402	3	100	0.47 ± 0.02	0.58
	6	100	0.57 ± 0.03	0.64
	10	100	0.56 ± 0.01	1.06
	3	300	0.54 ± 0.03	0.56
	6	300	0.57 ± 0.03	0.77
	10	300	0.70 ± 0.02	0.75

Fa value is presented as the mean ± SD of three independent tests. Combination index was calculated by using the average value of Fa derived from three independent experiments.

4.3. Subcytotoxic dose allicin enhances 5-FU induced apoptosis in HCC cells

To investigate cellular apoptosis caused by combined treatment, SK-Hep-1 and BEL-7402 cells treated with allicin and 5-FU alone or in combination for 48 h and stained with Annexin V/PI followed by Flow cytometry (FCM) analysis. As showed in Fig. 3A and B, allicin did not increase the percentage of Annexin V-positive cells whereas it significantly increased 5-FU induced apoptosis in both SK-Hep-1 and BEL-7402 cells ($P < 0.01$). Consistent with FCM data, the expressions of apoptotic related proteins, cleaved caspase-3 and cleaved PARP, were remarkably increased, and Bcl-2 was decreased in combined treatment compared with 5-FU treated alone, as determined by western blotting (Fig. 3B, C). Collectively, allicin significantly increased sensitivity of HCC cells to 5-FU by inducing cellular apoptosis.

4.4. Allicin enhances 5-FU induced apoptosis through ROS-mediated mitochondrial pathway

Previous studies have shown that allicin is involved in oxidative stress. We speculated that allicin enhanced 5-FU-induced apoptosis via increasing reactive oxygen species (ROS). To validate this hypothesis, the fluorochrome DCFH-DA was used to determine ROS level by FCM analysis. The results showed that ROS generation was more rapidly increased in cells co-treated by allicin and 5-FU compared with cells treated by allicin or 5-FU alone ($P < 0.001$ for both SK-Hep-1 and BEL-7402 cells; Fig. 4A and B).

Because excessive ROS accumulation may cause oxidative stress and mitochondrial dysfunction, we evaluated mitochondrial function using JC-1 dye, an indicator of mitochondrial membrane potential, by FCM analysis. The results showed that the mitochondrial membrane potential ($\Delta\Psi m$) in cells co-treated by allicin and 5-FU was more significantly decreased compared with cells treated by allicin or 5-FU alone ($P < 0.001$ for both SK-Hep-1 and BEL-7402 cells; Fig. 4C and D). Moreover, using ROS inhibitor NAC reversed the increased sensitivity of HCC cells to 5-FU induced by allicin (Fig. 4E). Taken together, these results indicated that allicin increase the sensitivity of HCC cells to 5-FU-induced apoptosis through ROS-mediated mitochondrial pathway.

5. Discussion

In this study, we investigate the role of allicin in sensitization of HCC cells to 5-FU-inducing cell death *in vitro* and *in vivo*. Our results showed the synergistic effect of subcytotoxic allicin and 5-FU on inducing HCC cells death through ROS-mediated mitochondrial pathway, which proposes the therapeutic value of allicin in HCC chemotherapy.

Palliative chemotherapy has not been used routinely for patients with advanced HCC. Drug resistance and hepatic dysfunction are the two major factors that limit the application of chemotherapy for HCC (14). Compared with other monotherapy, 5-FU has been more acceptable because adequate doses can usually be administered in the setting of hepatic dysfunction or jaundice (15). Although response rate with 5-FU monotherapy has been low, given in combination with other agents, response rates as high as 28% had been reported (15). And recently, FOLFOX regimen (5-FU, oxaliplatin, and leucovorin) was found to have a higher objective response rate, higher disease control rate and better median survival in HCC patients (16). Thus, treatments combined 5-FU with other agents may play a role in therapies of advanced HCC.

Allicin has been reported that have anti-tumor effect in some cancers through growth inhibition and apoptosis induction. In HCC, allicin caused p53-mediated autophagic cell death (17). In

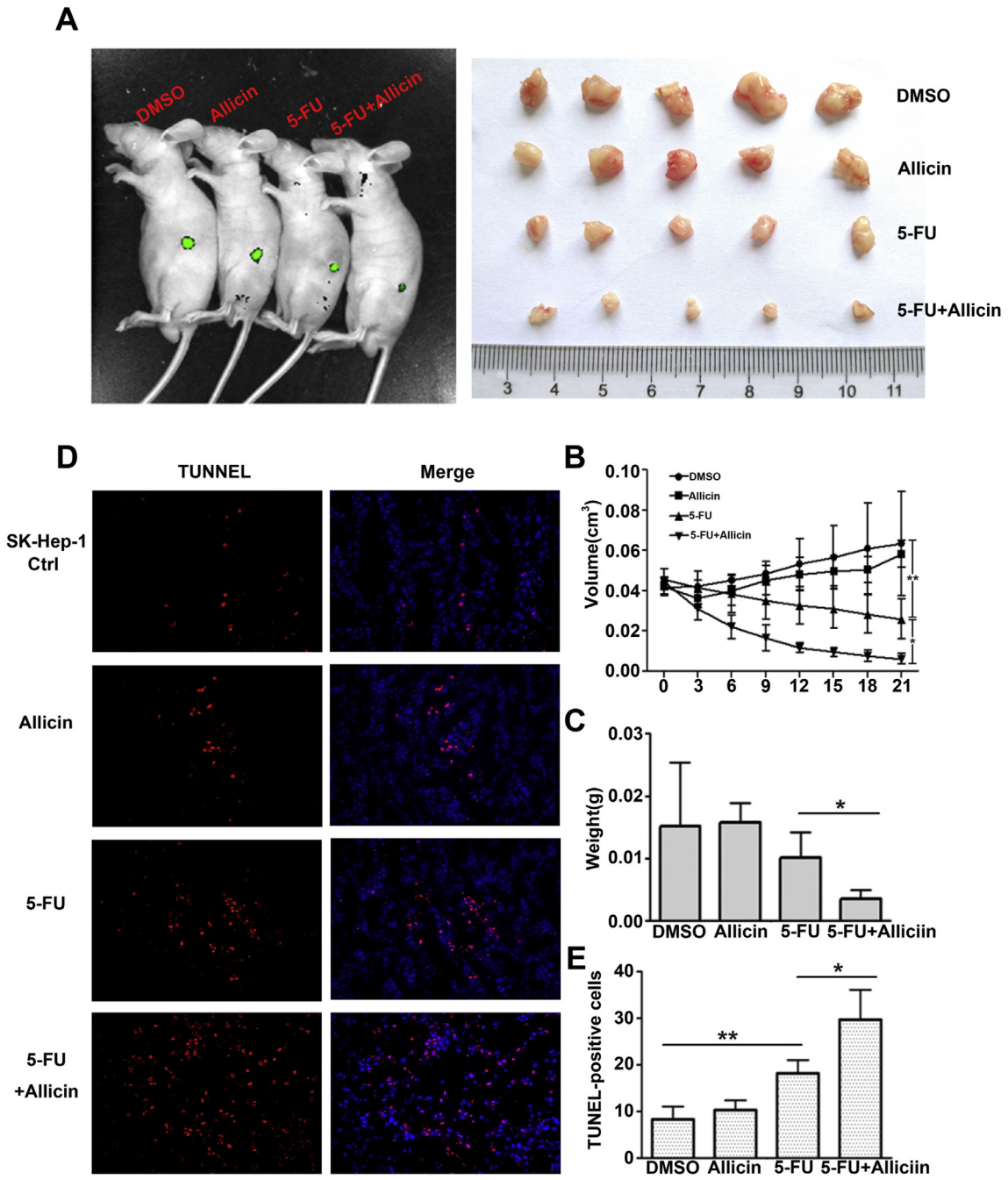

Fig. 2. Co-treatment of allicin and 5-FU significantly inhibited HCC tumor growth *in vivo*. (A) Treatments with DMSO (as control), allicin, 5-FU or with a combination of allicin and 5-FU were performed on twenty nude mice which were subcutaneously injected with SK-Hep-1 cells (1×10^7 per mouse) stably expressing GFP, and were randomly divided into four groups (n = 5 per group). Representative images of vivo xenograft tumors expressed GFP and tumors isolated from each group on day 21. (B) Continuous quantification of tumor volumes of each group during the experiment. (*$P < 0.05$, **$P < 0.01$) (C) The weight of xenograft tumors were showed as the mean ± SD of five tumors excised from each group. (*$P < 0.05$). (D) TUNEL assay was used to evaluate DNA fragmentation of xenograft tumors after different administrations. (E) Quantitative analysis of TUNEL-positive cells (red) of each group. Each value is presented as the mean ± SD of six random fields. (*$P < 0.05$, **$P < 0.01$).

glioblastoma, allicin induced apoptosis through mitochondrial pathway of Bcl-2/Bax, the MAPK/ERK signaling pathway, and antioxidant enzyme systems (18). In colon cancer, allicin enhanced hypodiploid DNA content and increased capability of releasing cytochrome C from mitochondria to cytosol, which resulted in apoptotic cell death (19). In gastric cancer, allicin inhibited proliferation and induced apoptosis through the p38 mitogen-activated protein kinase/caspase-3 signaling pathway (20). In our study, we also found that allicin had anti-proliferative effect in HCC cells.

Meanwhile, allicin has hepatic protective effect. In experimental animal studies, allicin showed the beneficial effects against liver damage caused by chemical poisons such as bromobenzene, doxorubicin, and tamoxifen (6,21). Also allicin may contribute to protection against liver injury induced by ethanol (22). Given its hepatic beneficial effect and antitumor activity, allicin is an ideal enhancer to 5-FU-based chemotherapy regimen in HCC. In our data, we provide the first evidence about sensitive activation of allicin in HCC cells, both *in vitro* and *in vivo*. Compared with the control,

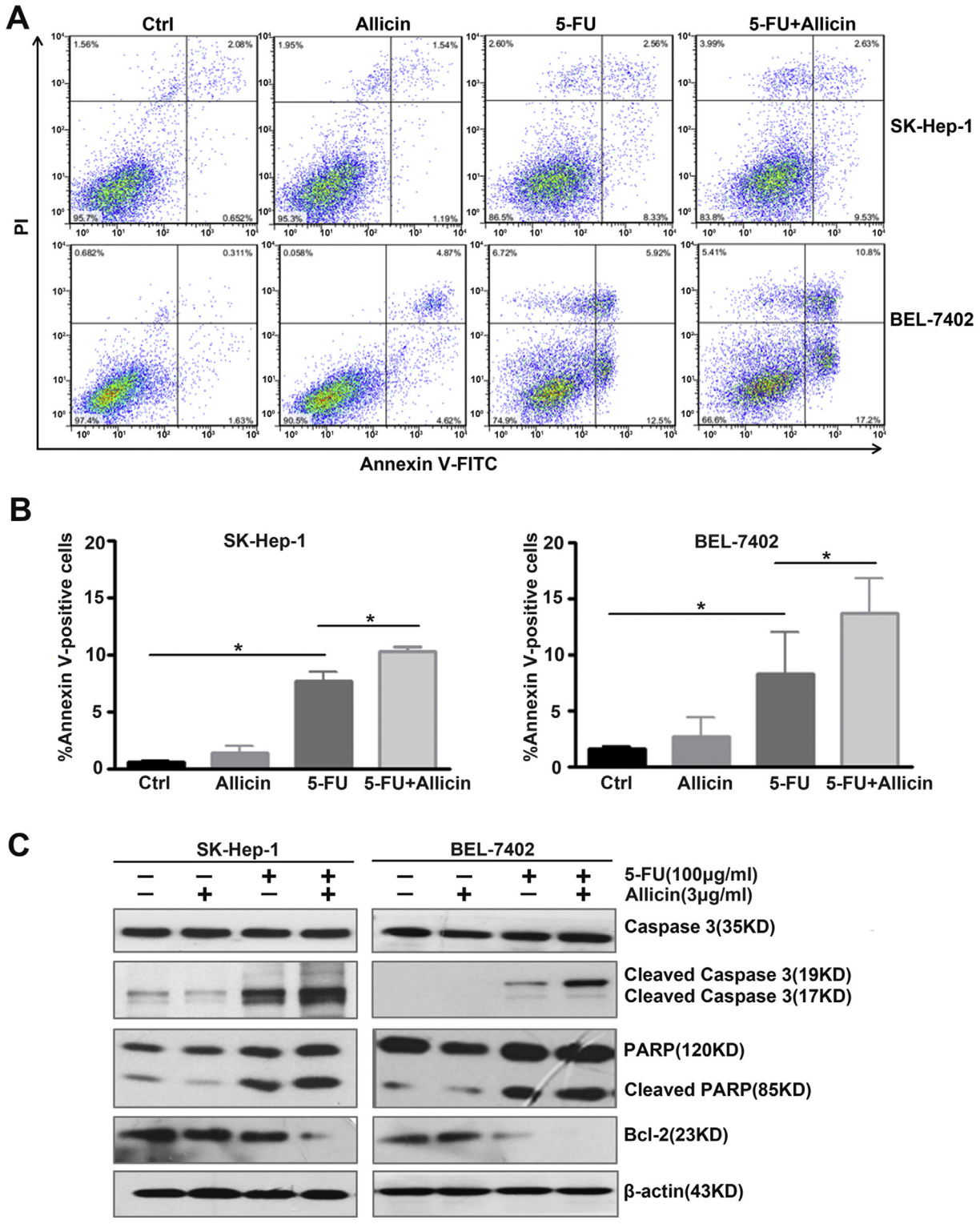

Fig. 3. Apoptosis induced by allicin and 5-FU in HCC cells. (A) Flow cytometric analysis of cell apoptosis mediated by allicin and 5-FU in SK-Hep-1 and BEL-7402 cells. Cells were treated with allicin (3 μg/ml), 5-FU (100 μg/ml) or combination for 48 h and were stained with Annexin V/PI. (B) Histogram showed the early apoptotic rate of cells in different groups of these two cell lines from three independent experiments (*$P < 0.05$). (C) Cells were treated as described in A. Expression of proteins related in cell apoptosis was examined by western blotting analysis. β-actin was used as a loading control.

allicin, or 5-FU alone treatment groups, combined treatment with allicin and 5-FU inhibited cell proliferation and induced cellular apoptosis. Using CI equation, which allows for the evaluation of two or more drugs at different concentrations and effect levels, we demonstrated that allicin and 5-FU had the synergistic effect. These results provided the evidence for the combination used of allicin and 5-FU in clinical chemotherapy in the future.

The chemotherapy drug induced cell apoptosis is a complicated process, which is mediated through various pathway and regulated by lots of apoptotic related proteins (23). In our study, we looked

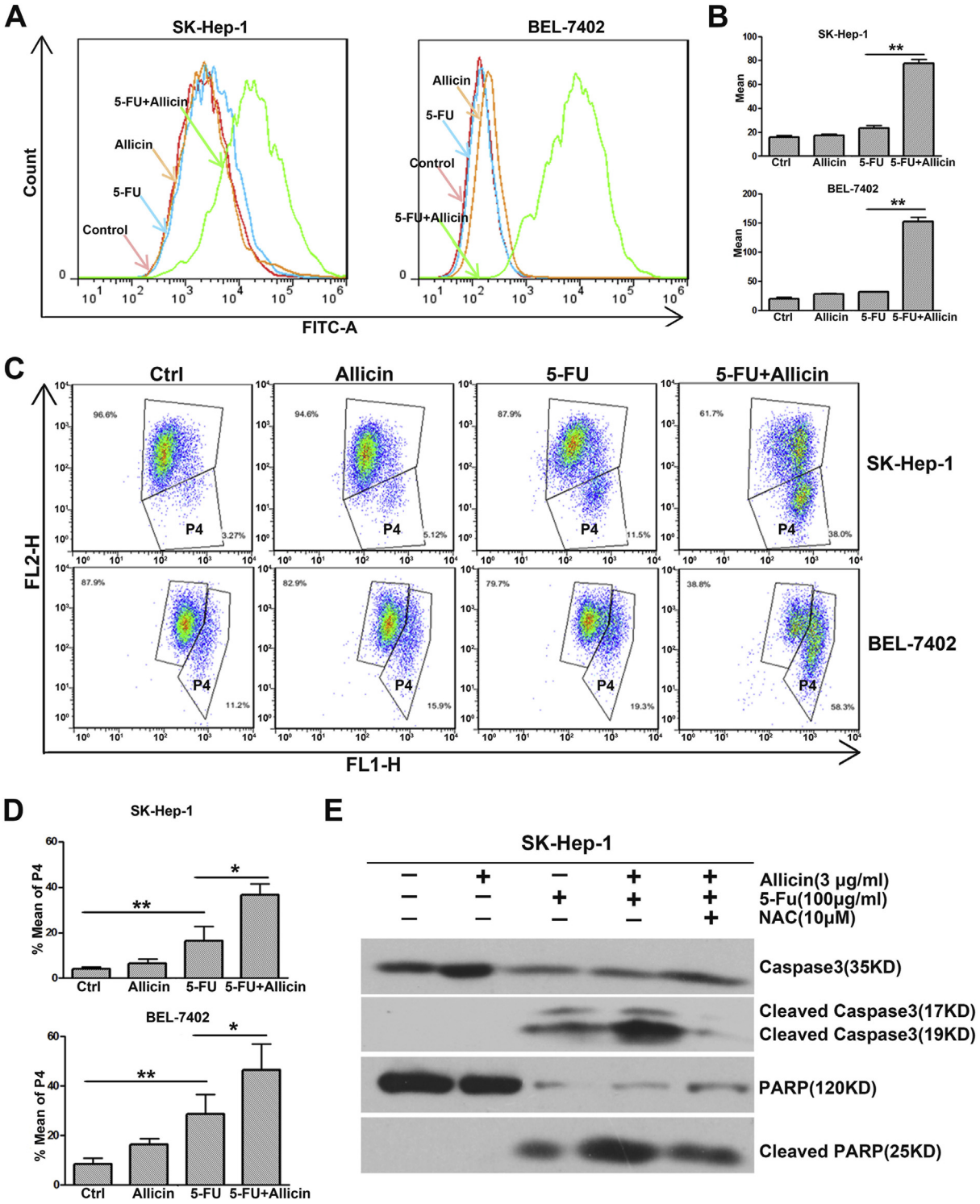

Fig. 4. Co-treatment of allicin and 5-FU affected the ROS-mediated mitochondrial pathway in HCC cells. (A) Dichloro-dihydro-fluorescein diacetate assay was used to measure the level of intracellular ROS. SK-Hep-1 and BEL-7402 cells treated with Allicin (3 μg/ml) and 5-FU (100 μg/ml), or combination for 48 h were prepared for detection. (B) Histogram showed the mean of DCF fluorescence intensity in each group. (**$P < 0.01$). (C) Mitochondrial transmembrane potential of cells treated with Allicin (3 μg/ml) and 5-FU (100 μg/ml), or combination for 48 h was detected by Flow cytometry using JC-1 staining. (D) Histogram presented the percentage mean of P4 in each group from triplicate studies (*$P < 0.05$, **$P < 0.01$). (E) SK-Hep-1 cells were treated with allicin (3 μg/ml), 5-FU (100 μg/ml), combination of allicin (3 μg/ml) and 5-FU (100 μg/ml), and combination of allicin (3 μg/ml), 5-FU (100 μg/ml) and NAC (10 μM) for 48 h. Cell apoptosis was examined by western blotting analysis.

insight into the preliminary mechanism of the sensitive effect of allicin in HCC cells. We suggested that allicin sensitized HCC cells to 5-FU-induced apoptosis through ROS-mediated mitochondrial pathway. As we know, chemotherapy agents induce oxidative stress, and cause ROS generation (24). The accumulation of ROS in the mitochondria could inhibit the mitochondrial respiration chain, which resulted in mitochondrial membrane rupture and apoptotic cell death (25,26). In our study, we found that there was a moderate

increase ROS level both in allicin and 5-FU single-agent groups. However, when HCC cells were treated in combination, the ROS production was dramatically increased. These results indicated the synergistic effect of ROS generation in combined treatment.

Taken together, our study has demonstrated the growth inhibition of allicin combined with 5-FU in HCC cells by inducing apoptosis both *in vitro* and *in vivo*, and the analysis of enhanced efficacy indicates synergism. Combined treatment induced cellular ROS production and decreased mitochondrial membrane potential, followed by cellular apoptosis. These results provide the evidence for the combination used of allicin and 5-FU as a novel chemotherapy regimen in HCC. Further clinical investigations are needed to provide evidence on the efficacy in HCC patients.

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Conflicts of interest statement

The authors declare no conflicts of interest.

References

(1) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65:87–108.
(2) Wang CH, Wey KC, Mo LR, Chang KK, Lin RC, Kuo JJ. Current trends and recent advances in diagnosis, therapy, and prevention of hepatocellular carcinoma. Asian Pac J Cancer Prev. 2015;16:3595–3604.
(3) Kulik LM, Chokechanachaisakul A. Evaluation and management of hepatocellular carcinoma. Clin Liver Dis. 2015;19:23–43.
(4) Fitzmorris P, Shoreibah M, Anand BS, Singal AK. Management of hepatocellular carcinoma. J Cancer Res Clin Oncol. 2015;141:861–876.
(5) Zhong JH, Zhong QL, Li LQ, Li H. Adjuvant and chemopreventive therapies for resectable hepatocellular carcinoma: a literature review. Tumour Biol. 2014;35:9459–9468.
(6) Majewski M. *Allium sativum*: facts and myths regarding human health. Rocz Panstw Zakl Hig. 2014;65:1–8.
(7) Mikaili P, Maadirad S, Moloudizargari M, Aghajanshakeri S, Sarahroodi S. Therapeutic uses and pharmacological properties of garlic, shallot, and their biologically active compounds. Iran J Basic Med Sci. 2013;16:1031–1048.
(8) Chan JY, Yuen AC, Chan RY, Chan SW. A review of the cardiovascular benefits and antioxidant properties of allicin. Phytother Res. 2013;27:637–646.
(9) Kyung KH. Antimicrobial properties of allium species. Curr Opin Biotechnol. 2012;23:142–147.
(10) Rajput S, Mandal M. Antitumor promoting potential of selected phytochemicals derived from spices: a review. Eur J Cancer Prev. 2012;21:205–215.
(11) Tsubura A, Lai YC, Kuwata M, Uehara N, Yoshizawa K. Anticancer effects of garlic and garlic-derived compounds for breast cancer control. Anticancer Agents Med Chem. 2011;11:249–253.
(12) Gao Y, Liu Y, Cao W, Deng Z, Liu H, Xu L, et al. [Allicin enhances cytotoxicity of CPT-11 to colon cancer LoVo cell in vitro]. Zhongguo Zhong Yao Za Zhi. 2009;34:3092–3095.
(13) Chou TC. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev. 2006;58:621–681.
(14) Waghray A, Murali AR, Menon KN. Hepatocellular carcinoma: from diagnosis to treatment. World J Hepatol. 2015;7:1020–1029.
(15) Roderburg C, do ON, Fuchs R, Bubenzer J, Spannbauer M, Luedde T, et al. Safe use of FOLFOX in two patients with metastatic colorectal carcinoma and severe hepatic dysfunction. Clin Colorectal Cancer. 2011;10:E6–E9.
(16) Zhang Q, Chen H, Li Q, Zang Y, Chen X, Zou W, et al. Combination adjuvant chemotherapy with oxaliplatin, 5-fluorouracil and leucovorin after liver transplantation for hepatocellular carcinoma: a preliminary open-label study. Invest New Drugs. 2011;29:1360–1369.
(17) Chu YL, Ho CT, Chung JG, Raghu R, Lo YC, Sheen LY. Allicin induces anti-human liver cancer cells through the p53 gene modulating apoptosis and autophagy. J Agric Food Chem. 2013;61:9839–9848.
(18) Cha JH, Choi YJ, Cha SH, Choi CH, Cho WH. Allicin inhibits cell growth and induces apoptosis in U87MG human glioblastoma cells through an ERK-dependent pathway. Oncol Rep. 2012;28:41–48.
(19) Bat-Chen W, Golan T, Peri I, Ludmer Z, Schwartz B. Allicin purified from fresh garlic cloves induces apoptosis in colon cancer cells via Nrf2. Nutr Cancer. 2010;62:947–957.
(20) Zhang X, Zhu Y, Duan W, Feng C, He X. Allicin induces apoptosis of the MGC-803 human gastric carcinoma cell line through the p38 mitogen-activated protein kinase/caspase-3 signaling pathway. Mol Med Rep. 2015;11:2755–2760.
(21) Suddek GM. Allicin enhances chemotherapeutic response and ameliorates tamoxifen-induced liver injury in experimental animals. Pharm Biol. 2014;52:1009–1014.
(22) Zeng T, Zhang CL, Song FY, Zhao XL, Yu LH, Zhu ZP, et al. The activation of HO-1/Nrf-2 contributes to the protective effects of diallyl disulfide (DADS) against ethanol-induced oxidative stress. Biochim Biophys Acta. 2013;1830:4848–4859.
(23) Das T, Sa G, Saha B, Das K. Multifocal signal modulation therapy of cancer: ancient weapon, modern targets. Mol Cell Biochem. 2010;336:85–95.
(24) Victorino VJ, Pizzatti L, Michelletti P, Panis C. Oxidative stress, redox signaling and cancer chemoresistance: putting together the pieces of the puzzle. Curr Med Chem. 2014;21:3211–3226.
(25) Tsuchiya A, Kaku Y, Nakano T, Nishizaki T. Diarachidonoylphosphoethanolamine induces apoptosis of malignant pleural mesothelioma cells through a Trx/ASK1/p38 MAPK pathway. J Pharmacol Sci. 2015;129:160–168.
(26) Gogvadze V, Orrenius S, Zhivotovsky B. Mitochondria as targets for chemotherapy. Apoptosis. 2009;14:624–640.