Am J Transl Res 2017;9(12):5567-5575
www.ajtr.org /ISSN:1943-8141/AJTR0053485

*Original Article*

# Curcumin combined with glycyrrhetinic acid inhibits the development of hepatocellular carcinoma cells by down-regulating the PTEN/PI3K/AKT signalling pathway

Mingxiang Chang[1,2,3*], Meimei Wu[2,3*], Hanmin Li[2,3]

*[1]First Clinical Medical School, Hubei University of Chinese Medicine, Wuhan 430061, P.R. China; [2]Hubei Provincial Hospital of Traditional Chinese Medicine, Wuhan 430061, P.R. China; [3]Hubei Province Academy of Traditional Chinese Medicine, Wuhan 430074, P.R. China. [*]Equal contributors.*

Received March 22, 2017; Accepted October 31, 2017; Epub December 15, 2017; Published December 30, 2017

**Abstract:** Curcumin is an active component of turmeric, which is derived from the rhizomes of Curcuma longa. Glycyrrhetinic acid (GA) is a natural compound extracted from liquorice. Both curcumin and GA are widely used as anticancer agents for treating many human cancers. In this study, curcumin and GA were used separately and in combination to treat human hepatocellular carcinoma (HCC) HepG2 cells. MTT assays were used to evaluate cell proliferation. Flow cytometry was carried out to measure cell apoptosis and determine cell cycle progression. Western blot analyses were applied to determine the expression levels of B-cell lymphoma-2 (Bcl-2), B-cell associated X protein (Bax), phosphatase and tensin homolog (PTEN), phosphorylated phosphoinositide 3-kinase (PI3K) and AKT serine/threonine kinase 1 (Akt). The results showed that combined treatment with curcumin and GA resulted in a significant reduction in proliferation and an increase in apoptosis and G1 cell cycle arrest in HepG-2 cells. A xenograft tumour model showed that curcumin and GA suppressed HCC development in vivo. Moreover, by knocking down the expression of PTEN, we confirmed that curcumin and GA exert their anticancer effects by inhibiting the PTEN/PI3K/Akt signalling pathway. Collectively, these results indicate that the combination of curcumin and GA could effectively inhibit the development of HepG2 cells by inhibiting PTEN/PI3K/Akt signalling and could be a promising treatment strategy for patients with HCC.

**Keywords:** Curcumin, GA, HCC, development, PTEN/PI3K/Akt

## Introduction

Hepatocellular carcinoma (HCC) is the sixth most prevalent type of malignant tumour and the third leading cause of cancer-related mortality worldwide [1]. Currently, liver transplantation and surgical resection remain the most effective therapeutic approaches for treating HCC [2-4]. Due to uncontrolled tumour metastasis and frequent intrahepatic spread, the prognosis of HCC patients is still unsatisfactory [5]. Therefore, the identification of effective and safe drug treatments for HCC is urgently needed.

Traditional Chinese medicine (TCM) has been used to treat malignant tumours for a long time in China. TCM has attracted attention in recent years because greater than two-thirds of novel anti-cancer drugs are natural [6, 7]. Curcumin is also called diferuloylmethane and is obtained from Curcuma longa. Previous studies have demonstrated that curcumin has anti-inflammatory, antioxidant and anti-infective properties [8]. Recently, studies have found that curcumin serves as a potent anticancer drug for treating various types of human cancers [9-11].

Glycyrrhetinic acid (GA) can be extracted from Glycyrrhiza glabra and is a well-known TCM that has been frequently used for treating various diseases. GA is extensively reported to have many pharmacological activities, including anti-ulcer, anti-inflammatory, immunomodulatory and anti-tumourigenic properties [12]. GA also has antiviral activity against HIV, hepatitis B

**Table 1.** Primers used for RT-qPCR

| Gene | Forward (5'-3') | Reverse (5'-3') |
|---|---|---|
| PTEN | GTGCAGATAATGACAAG | GATTTGACGGCTCCTCT |
| PI3K | GAATCCAATGGGAACTGT | GGGAGGGTAATAATAAGGT |
| AKT | TGCAGCATCGCTTCTTTG | TCTGGGCCGTGAACTCCT |
| GAPDH | CAAGGTCATCCATGACAA | GTCCACCACCCTGTTGCTG |

virus, and SARS (severe acute respiratory syndrome)-associated coronavirus [13]. Due to these properties, combining GA with other drugs could increase their pharmacological activities. In particular, when delivered with curcumin, GA was found to increase the anticancer effects of curcumin on prostate carcinoma and lung cancer. However, the effects of curcumin and GA treatment on HCC have not been elucidated.

Here, we investigated the effects of curcumin and GA on cell proliferation, cell cycle progression, and apoptosis in HCC HepG2 cells in vitro and in vivo. We presented evidence that curcumin combined with GA inhibited the development of hepatocellular carcinoma cells by blocking the PTEN/PI3K/Akt signalling pathway. Our study may provide a basis for using curcumin and GA for the clinical treatment of HCC.

## Materials and methods

### *Chemicals and antibodies*

Curcumin and GA were purchased from Sigma (St. Louis, MO, USA). Curcumin and GA were dissolved in dimethylsulfoxide (DMSO) and maintained as a stock solution at -20°C. The final concentration of DMSO was kept below 0.2% (v/v) throughout the study. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO, USA). Primary antibodies against PTEN, PI3K, Akt, Bcl-2, Bax and GAPDH were obtained from Cell Signaling Technology (Beverly, MA, USA).

### *Cell culture and transfection*

HCC cells (HepG2) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA, USA) supplemented with 10% foetal bovine serum (Invitrogen, Carlsbad, CA, USA). All cells were maintained at 37°C in a humidified chamber with 95% air and 5% $CO_2$. PTEN siRNA (siPTEN) and control siRNA were obtained from GenePharma (Shanghai, China). HepG2 cells were transfected by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

### *RNA isolation and quantitative real-time RT-PCR (qRT-PCR)*

Total RNA from cells and tissues was extracted using TRIzol reagent (Invitrogen, CA, USA). cDNA was synthesized by using Oligo-dT or specific microRNA stem loop RT primers with 1 μg of total RNA according to the manufacturer's instructions. qRT-PCR was performed by using the SYBR® Premix EX TaqTMIIPCR Kit (Takara, Dalian, China) according to the manufacturer's instructions and a Roche LightCycler® 480 II Real-Time PCR System. Human GAPDH was used as an internal control. The primers are shown in **Table 1**. The PCR reactions were performed under the following conditions: 98°C for 2 min, 98°C for 10 s, 55°C for 30 s, and 72°C for 30 s for 40 cycles, followed by 72°C for 5 min. The results were analysed using the Applied Biosystems Comparative CT Method [14].

### *Protein extraction and Western blot analysis*

Total protein from cells and tissues was extracted using RIPA reagents (Beyotime Biotech, Shanghai, China) according to the manufacturer's instructions. Cell lysates were washed with PBS three times and incubated in cold RIPA buffer for 30 min. Then, the cell lysates were centrifuged at 13,000 g for 15 min at 4°C. After that, 10% SDS-PAGE (Beyotime Biotech, Shanghai, China) was used to separate equal amounts of total protein, and the proteins were transferred to PVDF membranes (Millipore, MA, USA). After blocking with 5% non-fat milk for 1 h, the membranes were incubated with anti-Bcl-2 (1:1,500), Bax (1:1,000), PTEN (1:500), PI3K (1:1,000), Akt (1:1,000), p-Akt (1:2,000) and GAPDH (1:1,000) antibodies overnight at 4°C. After that, the membranes were incubated with the corresponding HRP-linked secondary antibodies (Santa Cruz Biotechnology, USA) for 2 h at room temperature. Positive bands were detected using an ECL kit (Thermo Scientific, Waltham, MA, USA).

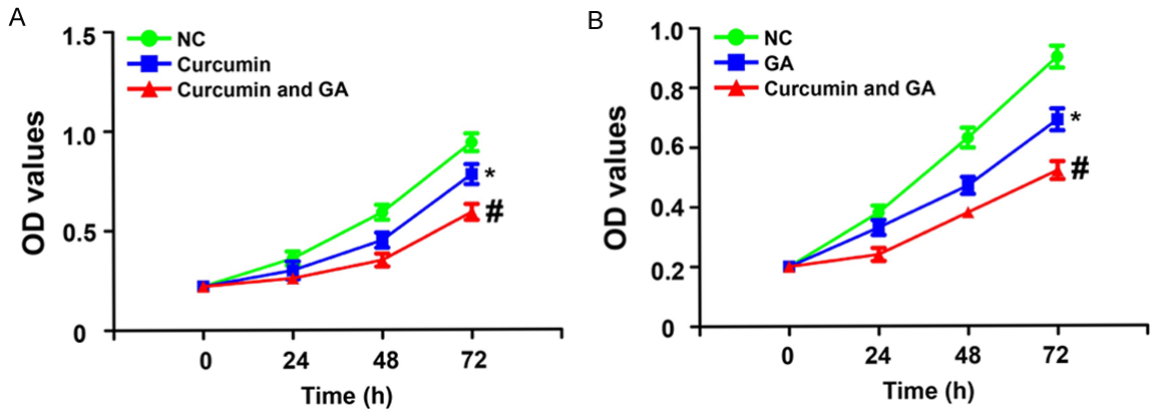


**Figure 1.** Curcumin combined with GA significantly inhibited the growth of HepG2 cells. HepG2 cells were grown in 96-well plates and treated with curcumin (A) and/or GA (B) for different time periods (0 h, 24 h, 48 h and 72 h) as indicated. Cell growth was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-biphenyl tetrazolium bromide (MTT) assays. GA: Glycyrrhetinic acid; NC: Negative control. *p<0.05 vs NC, #p<0.05 vs GA- or curcumin-treated group.

*Cell proliferation assay*

Cell growth was determined using MTT assays performed according to the manufacturer's instructions. Cells (approximately $1\times10^4$ cells/well) were seeded into 96-well culture plates 24 h prior to treatment. After 0, 24, 48 or 72 h of treatment, 20 μl of MTT (5 mg/ml) was added to each plate and incubated for 4 h at 37°C. Then, 150 μl of dimethyl sulfoxide (DMSO) was added to each plate and agitated for 10 min at 37°C to solubilize the crystals. The number of cells per plate was measured by using the absorbance (480 nm) at the indicated time points.

*Cell apoptosis assay*

HepG2 cells were seeded in 6-well plates (approximately $2\times10^6$ cells/well) after treatment. Cell apoptosis was detected by using a annexin V-fluorescein isothiocyanate (FITC)/PI detection kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. In brief, cells were collected and stained with 5 μl of FITC-Annexin V and 3 μl of propidium iodide (PI). Finally, flow cytometry (EPICS, XI-4, Beckman, CA, USA) was used to evaluate the percentage of apoptotic cells after incubation for 15 min at 37°C.

*Cell cycle assay*

Treated HepG2 cells in the log phase of growth were collected and fixed in 75% ethanol at -20°C for 16 h. For cell cycle analysis, treated cells were stained with propidium iodide and examined with a fluorescence-activated cell sorting (FACS) flow cytometer (BD Biosciences, CA, USA); DNA histograms were analysed with modified software. Each test was repeated in triplicate.

*Nude mouse tumour xenograft model*

All experimental procedures involving the use of animals were in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of the Affiliated Hospital of Hubei University of Chinese Medicine (Wuhan, China). Nude mice (6 weeks old, n = 12) received 200-μl subcutaneous injections of $4\times10^6$ HepG2 cells in their left flank area. Once palpable tumours developed, the volumes of the tumours were measured with a calliper every 4 days, and the following formula was used: volume = (length × width$^2$)/2. When the tumour volume reached an average of 75 to 100 mm$^3$, the mice were divided randomly into two groups (six mice per group). The mice were then intratumourally injected with 100 μl of siPTEN or control siRNA three times per week for 2 weeks.

*Statistical analysis*

All statistical analyses were conducted using SPSS 17.0 software (SPSS Statistics Inc., IL, USA). The data are presented as the mean ± standard deviation (mean ± SD). The differences between groups were analysed by using Student's t-test or one-way ANOVA. $P<0.05$ was considered statistically significant. All of the experiments were conducted at least three times.

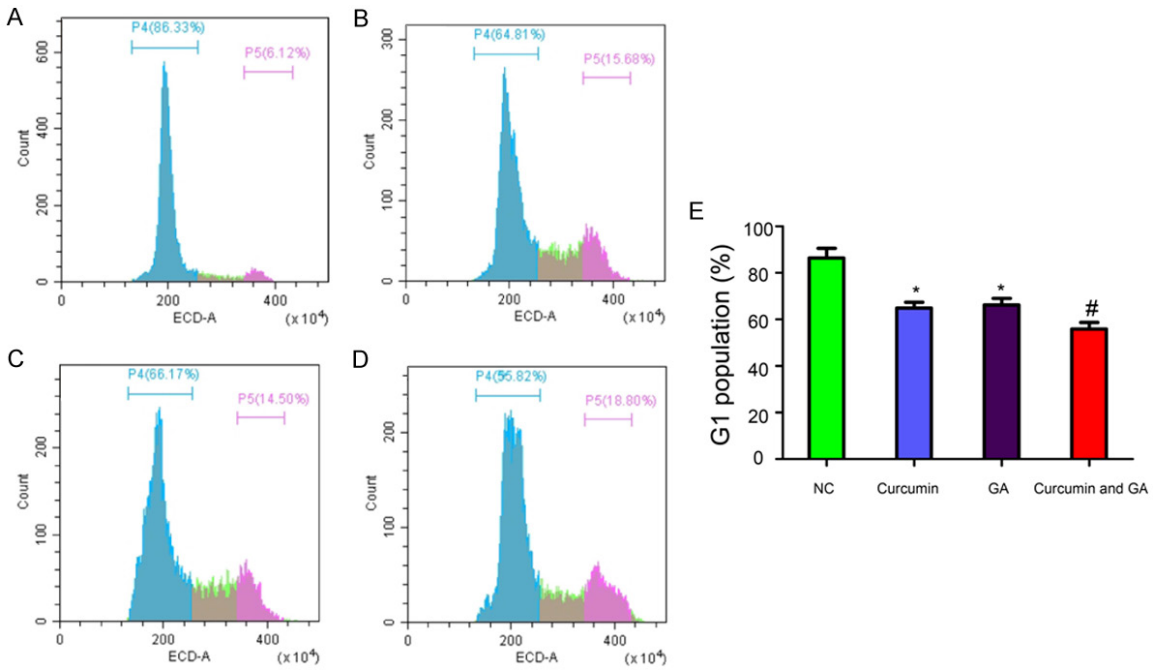


**Figure 2.** Curcumin combined with GA induced G1-phase cell cycle arrest in HepG2 cells. Cell cycle analysis of HepG2 cells by fluorescence-activated cell sorting (FACS) after treatment with GA and/or curcumin. (A) HepG2 cells treated with DMSO were used as the negative control (NC) group. Curcumin (B)- or GA (C)-treated HepG2 cells had a significant increases in cells in the G1-phase population compared with those in the NC group. (D) Curcumin- and GA-treated HepG2 cells had markedly increased numbers of cells in the G1 phase compared with those in the curcumin or GA treated group. (E) G1 population of cells in the four groups. *p<0.05 vs NC, #p<0.05 vs GA- or curcumin-treated group.

## Results

*Curcumin combined with GA significantly inhibited the growth of HepG2 cells*

The effects of curcumin (5 μM) and/or GA on HepG2 cells were assessed by MTT assays. HepG2 cells treated with DMSO were used as the negative control (NC) group. As shown in **Figure 1**, treating the HepG2 cells for 24, 48 or 72 h with curcumin or GA obviously inhibited cell viability compared with that of the NC group ($p<0.05$). Cell growth was decreased significantly when HepG2 cells were treated with GA and curcumin compared with that in the groups treated with only curcumin or GA ($p<0.05$). These findings suggest that curcumin combined with GA markedly inhibits cell growth in HepG2 cells.

*Curcumin combined with GA induced G1-phase cell cycle arrest in HepG2 cells*

To investigate the role of curcumin and GA in cell cycle progression, we treated HepG2 cells with curcumin and/or GA. Cell cycle assays showed that curcumin- or GA-treated HepG2 cells had a significantly increased proportion of cells in the G1 phase compared with that in the NC treatment group (**Figure 2**, $p<0.05$). Additionally, we examined the effects of curcumin combined with GA on cell cycle progression and found that the proportion of HepG2 cells in the G1 phase was significantly increased in the curcumin- and GA-treated groups compared with that in the curcumin or GA treated group (**Figure 2**, $p<0.05$). These findings indicate that curcumin combined with GA significantly induces G1-phase cell cycle arrest in HepG2 cells.

*Curcumin combined with GA markedly induced apoptosis in HepG2 cells*

Prior to treatment, the apoptosis rate in curcumin- or GA-treated (5 μM) HepG2 cells was also measured. Following treatment with curcumin or GA for 48 h, the apoptosis rate markedly increased compared with that in the NC group (**Figure 3A**, $p<0.05$). Cell apoptosis was

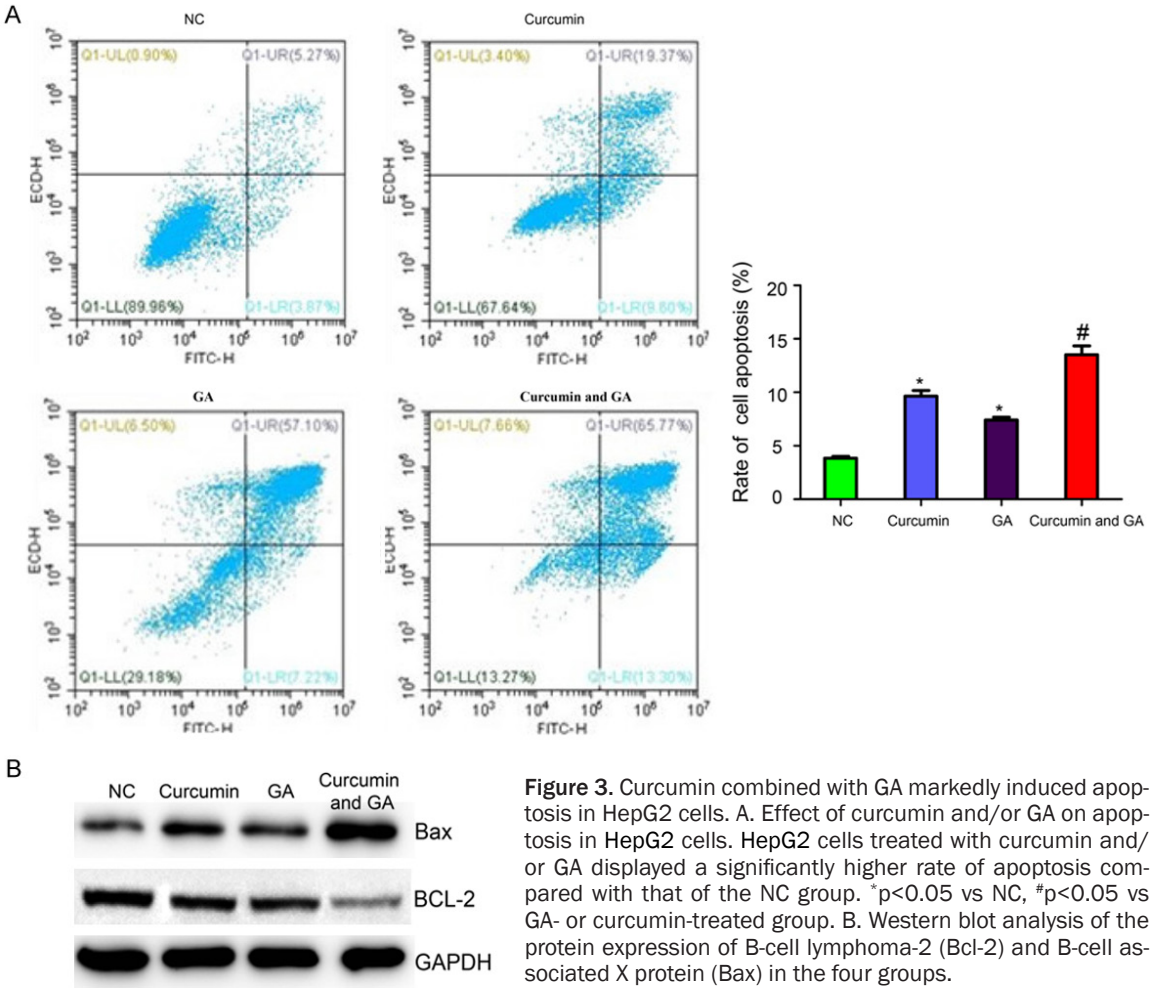


**Figure 3.** Curcumin combined with GA markedly induced apoptosis in HepG2 cells. A. Effect of curcumin and/or GA on apoptosis in HepG2 cells. HepG2 cells treated with curcumin and/or GA displayed a significantly higher rate of apoptosis compared with that of the NC group. *p<0.05 vs NC, #p<0.05 vs GA- or curcumin-treated group. B. Western blot analysis of the protein expression of B-cell lymphoma-2 (Bcl-2) and B-cell associated X protein (Bax) in the four groups.

increased significantly when HepG2 cells were treated with the combination of GA and curcumin compared with that of cells treated with only curcumin or GA (**Figure 3A**, p<0.05).

Furthermore, the expression of B-cell lymphoma-2 (Bcl-2) and B-cell associated X protein (Bax) was also measured. As shown in **Figure 3B**, we found that Bcl-2 expression was downregulated after treatment with curcumin and/or GA for 48 h, whereas Bax expression was upregulated. These data indicate that curcumin combined with GA markedly induced apoptosis in HepG2 cells.

*Curcumin combined with GA inhibited the PTEN/PI3K/AKT signalling pathway in HepG2 cells*

To determine whether curcumin and/or GA influenced PTEN/PI3K/AKT signalling in HCC cells, we treated HepG2 cells with curcumin and/or GA. **Figure 4A** shows that treatment with curcumin or GA decreased PI3K and AKT mRNA expression and upregulated PTEN mRNA expression in HepG2 cells compared with that in the NC cells (p<0.05). The mRNA and protein levels of PI3K, AKT and p-AKT were decreased significantly when HepG2 cells were treated with the combination of GA and curcumin compared with those in the groups treated with curcumin or GA only, and the protein expression of PTEN was upregulated markedly in HepG2 cells (**Figure 4B**).

*Curcumin combined with GA remarkably suppressed HCC development via the PTEN/PI3K/AKT signalling pathway in vivo*

To investigate whether curcumin and/or GA inhibited HCC development in vivo, we used a xenograft tumour model to examine the biological effects. As shown in **Figure 5A** and **5B**, the tumours formed by curcumin- and/or GA-trea-

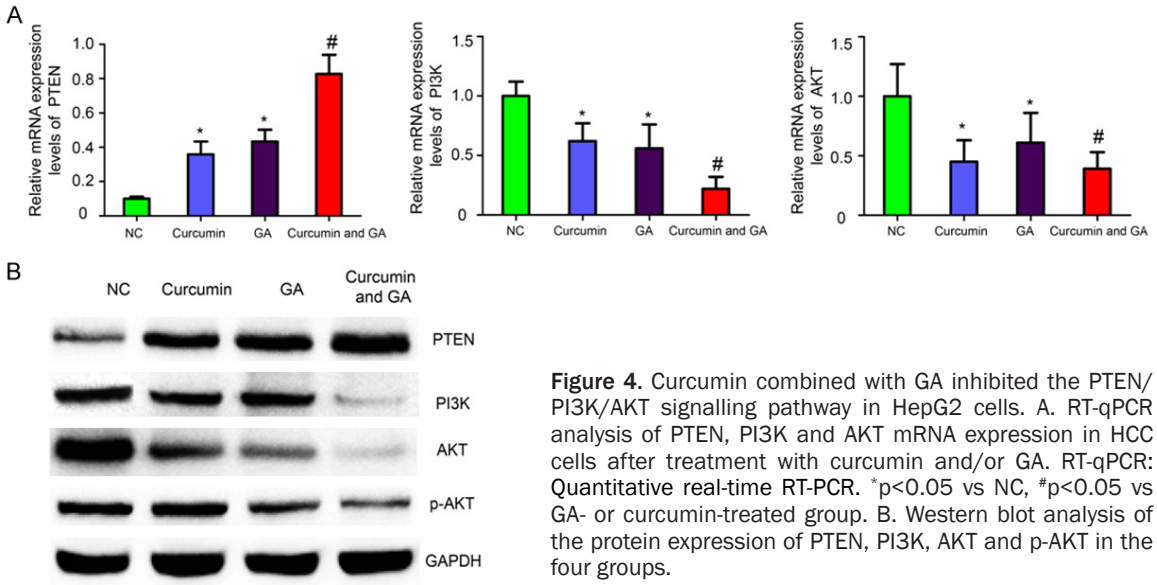


**Figure 4.** Curcumin combined with GA inhibited the PTEN/PI3K/AKT signalling pathway in HepG2 cells. A. RT-qPCR analysis of PTEN, PI3K and AKT mRNA expression in HCC cells after treatment with curcumin and/or GA. RT-qPCR: Quantitative real-time RT-PCR. *p<0.05 vs NC, #p<0.05 vs GA- or curcumin-treated group. B. Western blot analysis of the protein expression of PTEN, PI3K, AKT and p-AKT in the four groups.

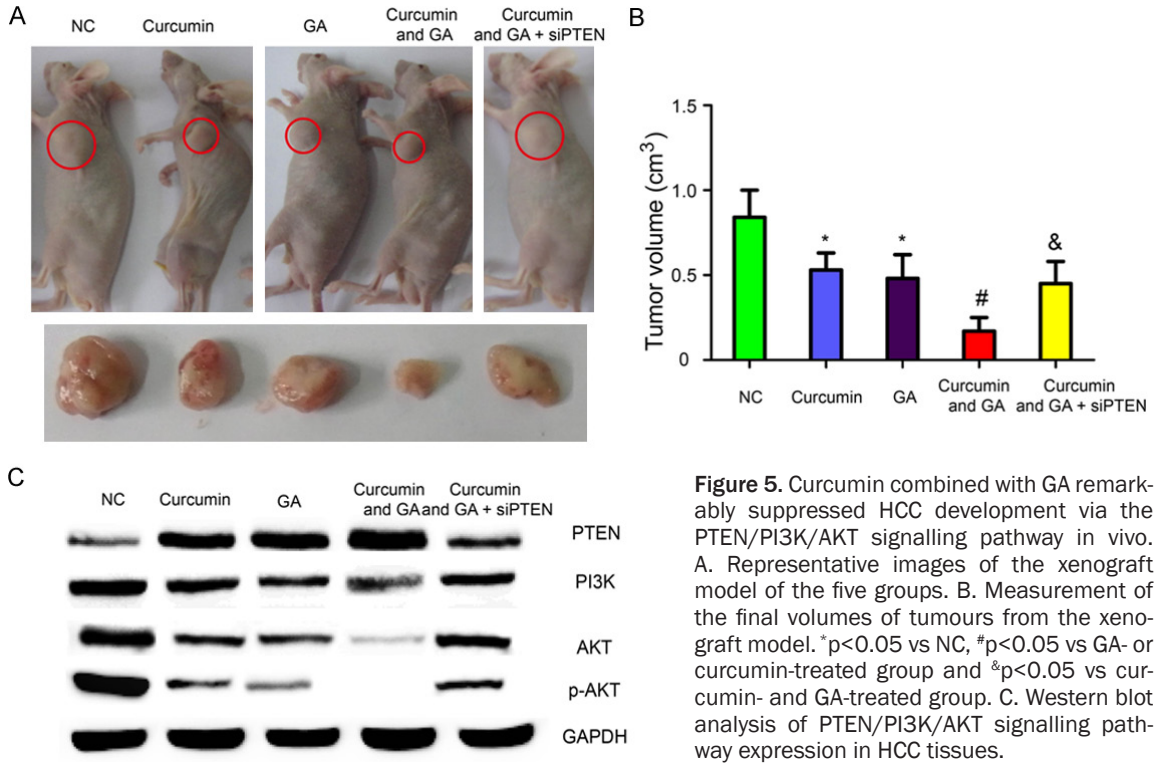


**Figure 5.** Curcumin combined with GA remarkably suppressed HCC development via the PTEN/PI3K/AKT signalling pathway in vivo. A. Representative images of the xenograft model of the five groups. B. Measurement of the final volumes of tumours from the xenograft model. *p<0.05 vs NC, #p<0.05 vs GA- or curcumin-treated group and &p<0.05 vs curcumin- and GA-treated group. C. Western blot analysis of PTEN/PI3K/AKT signalling pathway expression in HCC tissues.

ted HepG2 cells were smaller in size and weight than the tumours formed by NC-treated HepG2 cells. Conversely, the tumours formed by siPTEN-transfected HepG2 cells were larger than those of the curcumin and GA treated group ($p<0.05$). Furthermore, Western blot assays revealed that curcumin and GA suppressed PI3K, AKT and p-AKT protein expression in HCC tissues and promoted PTEN levels, while the knockdown of PTEN partly reversed this effect. (**Figure 5C**). Collectively, these results strongly demonstrate that curcumin combined with GA remarkably inhibits the development of HepG2 cells by down-regulating the PTEN/PI3K/AKT signalling pathway.

## Discussion

With the improvement of modern medical technology and cancer prevention, the incidence

of HCC has decreased remarkably in the past few years. However, HCC remains the sixth leading cause of mortality in malignant diseases. Alternative therapies, including chemotherapy, radiotherapy, and radiochemotherapy, though effective, are not curative [15, 16]. In recent decades, several natural products originating from medicinal herbs have broadened our understanding of cancer treatment because of their extensive biological activities. Drugs such as emodin, curcumin, and GA have been demonstrated to have anti-cancer effects by inhibiting the proliferation, invasion, and metastasis of multiple malignant cancers [17-19]. Although many studies have revealed their pharmacological mechanisms, much research is still needed.

In the present study, the effects of curcumin combined with GA on proliferation, cell cycle progression and apoptosis in HCC cells and the possible mechanisms were investigated. The results indicated that curcumin combined with GA significantly inhibited cell growth in HepG2 cells and induced G2-phase cell cycle arrest. Cell apoptosis was increased significantly when HepG2 cells were treated with the combination of GA and curcumin. Furthermore, we demonstrated that curcumin combined with GA inhibited the development of HCC in vivo.

Numerous studies have shown that activation of the PI3K/Akt signalling pathway is essential for the development and progression of HCC and can modulate the malignant behaviour of HCC, such as cell proliferation, invasiveness, angiogenesis and metastasis [20-23]. Of note, PTEN, which acts as an inhibitor of the PI3K/Akt pathway, has been found to be inhibited in HCC due to gene deletions or mutation during oncogenesis [24, 25]. PTEN is an essential regulator of cell proliferation, differentiation, growth and apoptosis, and its deficiency is closely associated with HCC development and progression [26]. In addition, PTEN can inhibit tumour cell growth and invasion by suppressing the PI3K/Akt pathway [27].

Xu et al have demonstrated that curcumin inhibits the invasion and migration of FTC133 cells via downregulating the PI3K/Akt signalling pathway in thyroid cancer cells [28]. Xu et al suggested that curcumin inhibits tumour proliferation in lung cancer through the PI3K/Akt pathway [29]. Schee et al revealed that miR-22-3p, miR-143-3p and miR-192-5p regulated and were involved in the APC, TGFβ and PI3K pathways in colorectal cancer cells [30]. Herein, we discovered that GA combined with curcumin inhibited PI3K/Akt signalling through activating PTEN, suggesting that GA and curcumin may represent potential therapeutic drugs for HCC treatment.

In conclusion, to the best of our knowledge, this study is the first to focus on the effects of GA combined with curcumin against HepG2 cells. The data demonstrate that GA combined with curcumin significantly inhibits the development of HepG2 cells through suppressing the PTEN/PI3K/AKT signalling pathway. The conclusions of the present study indicate that GA and curcumin are potential drugs for treating HCC. However, the present study reveals some limitations as we have not examined the detailed relationship between GA and curcumin and PTEN/PI3K/AKT signalling in HCC cells. Additional clinical studies and large-scale statistical analyses should be performed to verify the results of the present study.

## Acknowledgements

This study was supported by the National Natural Science Foundation of China (NO. 1274147) and the Natural Science Foundation of Hubei province (2012FFC04601).

## Disclosure of conflict of interest

None.

**Address correspondence to:** Hanmin Li, Institute of Liver Diseases of Hubei Provincial Hospital of Traditional Chinese Medicine (Affiliated Hospital of Hubei University of Chinese Medicine, Hubei Province Academy of Traditional Chinese Medicine), Huayuanshan 4, Yanzhi Road, Wuhan 430061, Hubei Province, P.R. China. Tel: +86-027-88929180; Fax: +86-027-88929180; E-mail: lihanmin@hbhtcm.com

## References

[1] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.

[2] Meguro M, Mizuguchi T, Kawamoto M and Hirata K. The molecular pathogenesis and clinical implications of hepatocellular carcinoma. Int J Hepatol 2011; 2011: 818672.

[3] Bosch FX, Ribes J, Diaz M and Cleries R. Primary liver cancer: worldwide incidence and trends. Gastroenterology 2004; 127: S5-S16.

[4] Block TM, Mehta AS, Fimmel CJ and Jordan R. Molecular viral oncology of hepatocellular carcinoma. Oncogene 2003; 22: 5093-5107.
[5] Villanueva A, Hernandez-Gea V and Llovet JM. Medical therapies for hepatocellular carcinoma: a critical view of the evidence. Nat Rev Gastroenterol Hepatol 2013; 10: 34-42.
[6] Hsiao WL and Liu L. The role of traditional Chinese herbal medicines in cancer therapy–from TCM theory to mechanistic insights. Planta Med 2010; 76: 1118-1131.
[7] Yin SY, Wei WC, Jian FY and Yang NS. Therapeutic applications of herbal medicines for cancer patients. Evid Based Complement Alternat Med 2013; 2013: 302426.
[8] Priyadarsini KI. Chemical and structural features influencing the biological activity of curcumin. Curr Pharm Des 2013; 19: 2093-2100.
[9] Yu LL, Wu JG, Dai N, Yu HG and Si JM. Curcumin reverses chemoresistance of human gastric cancer cells by downregulating the NF-kappaB transcription factor. Oncol Rep 2011; 26: 1197-1203.
[10] Lin SS, Lai KC, Hsu SC, Yang JS, Kuo CL, Lin JP, Ma YS, Wu CC and Chung JG. Curcumin inhibits the migration and invasion of human A549 lung cancer cells through the inhibition of matrix metalloproteinase-2 and -9 and Vascular Endothelial Growth Factor (VEGF). Cancer Lett 2009; 285: 127-133.
[11] Zhang YP, Li YQ, Lv YT and Wang JM. Effect of curcumin on the proliferation, apoptosis, migration, and invasion of human melanoma A375 cells. Genet Mol Res 2015; 14: 1056-1067.
[12] van Rossum TG, Vulto AG, de Man RA, Brouwer JT and Schalm SW. Review article: glycyrrhizin as a potential treatment for chronic hepatitis C. Aliment Pharmacol Ther 1998; 12: 199-205.
[13] Dai JH, Iwatani Y, Ishida T, Terunuma H, Kasai H, Iwakula Y, Fujiwara H and Ito M. Glycyrrhizin enhances interleukin-12 production in peritoneal macrophages. Immunology 2001; 103: 235-243.
[14] Schmittgen TD and Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 2008; 3: 1101-1108.
[15] Hao K, Luk JM, Lee NP, Mao M, Zhang C, Ferguson MD, Lamb J, Dai H, Ng IO, Sham PC and Poon RT. Predicting prognosis in hepatocellular carcinoma after curative surgery with common clinicopathologic parameters. BMC Cancer 2009; 9: 389.
[16] Boyault S, Rickman DS, de Reynies A, Balabaud C, Rebouissou S, Jeannot E, Herault A, Saric J, Belghiti J, Franco D, Bioulac-Sage P, Laurent-Puig P and Zucman-Rossi J. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology 2007; 45: 42-52.
[17] Bimonte S, Barbieri A, Palma G, Luciano A, Rea D and Arra C. Curcumin inhibits tumor growth and angiogenesis in an orthotopic mouse model of human pancreatic cancer. Biomed Res Int 2013; 2013: 810423.
[18] Liu X, Sun K, Song A, Zhang X and He X. Curcumin inhibits proliferation of gastric cancer cells by impairing ATP-sensitive potassium channel opening. World J Surg Oncol 2014; 12: 389.
[19] Sharma G, Kar S, Palit S and Das PK. 18beta-glycyrrhetinic acid induces apoptosis through modulation of Akt/FOXO3a/Bim pathway in human breast cancer MCF-7 cells. J Cell Physiol 2012; 227: 1923-1931.
[20] Courtney KD, Corcoran RB and Engelman JA. The PI3K pathway as drug target in human cancer. J Clin Oncol 2010; 28: 1075-1083.
[21] Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C and Gonzalez-Baron M. PI3K/Akt signalling pathway and cancer. Cancer Treat Rev 2004; 30: 193-204.
[22] Cheng GZ, Park S, Shu S, He L, Kong W, Zhang W, Yuan Z, Wang LH and Cheng JQ. Advances of AKT pathway in human oncogenesis and as a target for anti-cancer drug discovery. Curr Cancer Drug Targets 2008; 8: 2-6.
[23] Cappellini A, Tabellini G, Zweyer M, Bortul R, Tazzari PL, Billi AM, Fala F, Cocco L and Martelli AM. The phosphoinositide 3-kinase/Akt pathway regulates cell cycle progression of HL60 human leukemia cells through cytoplasmic relocalization of the cyclin-dependent kinase inhibitor p27(Kip1) and control of cyclin D1 expression. Leukemia 2003; 17: 2157-2167.
[24] Song MS, Salmena L and Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol 2012; 13: 283-296.
[25] Cantley LC and Neel BG. New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. Proc Natl Acad Sci U S A 1999; 96: 4240-4245.
[26] Rahman MA, Kyriazanos ID, Ono T, Yamanoi A, Kohno H, Tsuchiya M and Nagasue N. Impact of PTEN expression on the outcome of hepatitis C virus-positive cirrhotic hepatocellular carcinoma patients: possible relationship with COX II and inducible nitric oxide synthase. Int J Cancer 2002; 100: 152-157.
[27] Tamura M, Gu J, Tran H and Yamada KM. PTEN gene and integrin signaling in cancer. J Natl Cancer Inst 1999; 91: 1820-1828.
[28] Xu X, Qin J and Liu W. Curcumin inhibits the invasion of thyroid cancer cells via down-regu-

lation of PI3K/Akt signaling pathway. Gene 2014; 546: 226-232.

[29] Xu Y, Zhang J, Han J, Pan X, Cao Y, Guo H, Pan Y, An Y and Li X. Curcumin inhibits tumor proliferation induced by neutrophil elastase through the upregulation of alpha1-antitrypsin in lung cancer. Mol Oncol 2012; 6: 405-417.

[30] Schee K, Lorenz S, Worren MM, Gunther CC, Holden M, Hovig E, Fodstad O, Meza-Zepeda LA and Flatmark K. Deep sequencing the microRNA transcriptome in colorectal cancer. PLoS One 2013; 8: e66165.