

RETRACTED ARTICLE: Tanshinol inhibits the growth, migration and invasion of hepatocellular carcinoma cells via regulating the PI3K-AKT signaling pathway

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Background: Tanshinol is an active constituent of *Salvia miltiorrhiza* and possess anti-inflammatory, antioxidant, and anti-bacterial activity. Herein, we explored the role of tanshinol on the growth and aggressiveness of hepatocellular carcinoma (HCC) cells in vitro and in vivo.

Materials and methods: The proliferation of a panel of HCC cell lines was measured using MTT assay. The expressions of phosphatidylinositol 3 kinase (PI3K) and protein kinase B (AKT) were detected by immunofluorescence staining and immunohistochemical assay. The levels of Bcl-2 and Bax were determined using immunoblotting assay. The secretions of matrix metalloproteinase-2 (MMP-2) and MMP-9 were detected by ELISA. The migration and invasion abilities of HepG2 cell were determined using wound healing and Transwell invasion assays. The apoptosis of HepG2 cell induced by tanshinol was analyzed by Annexin V/propidium iodide staining. A xenograft model was constructed to investigate the inhibitory effect of tanshinol on HepG2 cell growth in vivo. To further investigate the role of tanshinol on the metastasis of HepG2 cell in vivo, an experimental metastasis assay was performed.

Results: Tanshinol inhibited the growth and colony formation of HCC cell in vitro. Tanshinol also induced the apoptosis of HepG2 cell and inhibited the migration and invasion of HepG2 cells. In vivo experiments, tanshinol suppressed the tumor growth and metastasis of HepG2 cell. Furthermore, the phosphorylation of PI3K and AKT was decreased by tanshinol in vitro and in vivo.

Conclusion: Tanshinol exerts its anti-cancer effects via regulating the PI3K-AKT signaling pathway in HCC.

Keywords: tanshinol, HCC, migration, invasion, apoptosis

Introduction

Hepatocellular carcinoma (HCC) is one of the common malignant tumors and remains the leading cause of cancer-related death worldwide.^{1,2} Multiple genes influence the tumorigenesis and progression of HCC and accumulation of multiple molecular changes are involved in the progression of HCC.^{3,4} Surgery, radiotherapy, and chemotherapy are the major therapeutic options available for patients with HCC.⁵ Unfortunately, the clinical outcomes of patients with HCC are still poor owing to the limited therapeutic strategies. Hence, it is urgent to find a novel therapeutic treatment for HCC.

With a history of thousands of years of clinical practice, traditional Chinese medicine plays important roles in maintaining the health of Asian people and is being increasingly applied all over the world.⁶⁻¹⁰ *Salvia miltiorrhiza* has a long history of use for medicinal purposes in China. Currently, it is frequently used in herbal medicine for its anti-inflammatory activity, anti-arthritis properties, wound and burn healing capabilities,

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and anti-bacterial/ anti-cancer properties.^{11–15} There are several biologically active constituents in *S. miltiorrhiza*, including tanshinol.^{16–18} Previous investigation has demonstrated that tanshinol regulates adhesion molecule expression of tumor necrosis factor alpha (TNF- α)-induced endothelial cells by blocking activation of nuclear factor kappa B (NF- κ B).¹⁹ Additionally, tanshinol inhibits the growth of vascular smooth muscle cell (VSMC) and protective effect on the VSMC damage induced by hydrogen peroxide by increasing the expression of nitric oxide (NO).²⁰ Furthermore, combination of tanshinol and paeonol inhibits the isoproterenol-induced myocardial infarction in myocardial infarcted rats through inhibition of nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (Nrf2/HO-1) and PI3K/AKT signaling pathway.²¹ Nevertheless, the potential roles of tanshinol in anti-HCC are not well investigated.

In the current study, we investigated the suppressive roles of tanshinol on the growth and metastasis of HCC cell. The results indicated that tanshinol remarkably inhibited the proliferation, migration, and invasion of HCC HepG2 cell in vitro. We further demonstrated that tanshinol induced the apoptosis of HepG2 cell and suppressed the activation of the PI3K-AKT signaling cascade. In vivo, tanshinol suppressed the growth and metastasis of HCC cell. All these results indicated the promising preclinical activity of tanshinol in the treatment of HCC.

Materials and methods

Cell culture

Tanshinol was purchased from Pure One Bio-Technology (P0907, Purity $\geq 98\%$ Shanghai, China). The HCC cell lines, including SNU-423, PLC/PRF/5, SNU-449, and HepG2, were purchased from the Guangzhou Jennio Biotech Co., Ltd (Guangzhou, Guangdong, China) and were maintained in 1,100 medium or DMEM (Invitrogen, Carlsbad, CA, USA) and supplemented with 10% FBS (Invitrogen), 100 U/mL penicillin/streptomycin (Sigma-Aldrich, St Louis, MO, USA).

MTT assay

Briefly, HepG2 cells (1×10^4) were seeded in 96-well plates and then treated with tanshinol (0, 1, 5, 10, 20, and 30 μ M) for 48 hours. HepG2 cells were treated with 5 μ M tanshinol for 24, 48, and 72 hours. A total of 10 μ L of MTT (5 mg/mL; Sigma-Aldrich) was added into each well and then incubated for 4 hours at 37°C. Next, 200 μ L of dimethyl sulfoxide (DMSO) was added to the 96-well plates. Finally, the absorbance was measured at 490 nm on a microplate reader. The overall experiments were repeated at least three times.

Trypan blue exclusion test

HepG2 cells (5×10^5 cells/mL) were incubated with various concentrations of tanshinol (1, 5, and 10 μ M) for 48 hours in 96-well plates. HepG2 cells (5×10^5 cells/mL) were incubated with 5 μ M tanshinol for 24, 48, and 72 hours. After culture, cell numbers were evaluated by staining with trypan blue and counting using light microscopy.

Colony formation assay

HepG2 cells (1×10^3) were cultured into 6-well plates and were allowed to grow for 2 weeks with added tanshinol (1, 5, and 10 μ M). Then, colonies were stained using 1% crystal violet. The cell colonies were photographed and the number of colonies was counted.

Hoechst 33342 staining

Cells (1×10^3) were treated with tanshinol (1, 5, and 10 μ M) for 48 hours. Cells were washed three times with PBS and cell nucleus were stained using 5 μ g/mL Hoechst 33342 (Beyotime, Nanjing, Jiangsu, China) for 10 minutes. Then, images were taken using the ZEN 2011 imaging software on a Zeiss inverted microscope (Carl Zeiss, Hallbergmoos, Germany).^{22,23}

Apoptosis analysis

Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (Beyotime) was used to detect the apoptosis of tanshinol-treated HepG2 cells according to the manufacturer's instructions. HepG2 cells were seeded into 6 well plates with a density of 1×10^5 cells/well for 24 hours and incubated with the tanshinol (1, 5, and 10 μ M) for 48 hours. After incubating with tanshinol, HepG2 cells were harvested, washed three times with PBS, suspended in Annexin V binding buffer, and incubated with FITC-labeled Annexin V and PI for 5 minutes at room temperature in the dark. Then, the samples were immediately analyzed by Flow cytometry (BD Biosciences, San Jose, CA, USA).

Migration assay

Cells were cultured into 6-well plates overnight. When the degree of fusion reached 95% or more, a 100 μ L sterile pipette tip was applied to create a wound in each well. After washing with PBS, cells were treated with medium containing tanshinol (1, 5, and 10 μ M) for 24 hours. The wounds were observed and photographed at 0 hour or 24 hours under a Nikon microscope. The rate of scratch healing was calculated. Scratch healing rate = (0 hour width of scratch – 24 hours width of scratch)/0 hour width of scratch $\times 100\%$.²⁴

Invasion assay

Transwell chambers (Corning Incorporated, Corning, NY, USA) with polycarbonate filters (8 μ m pore size) were pre-coated with 50 μ L Matrigel (BD Biosciences). HepG2 cells were pre-treated with tanshinol for 24 hours, and then cells were collected and re-suspended in FBS-free DMEM. A total of 100 μ L cell suspensions (1×10^4) were plated into the upper chamber. After this, 600 μ L DMEM medium containing 20% FBS was added into the lower chamber. After 12 hours, membranes were stained using 1% crystal violet and the number of the invasive cells was manually counted from five random fields by bright field microscopy.²⁵ Each experiment was repeated thrice.

Immunocytochemistry staining

HepG2 cells (1×10^3) were grown on the glass slide and treated with tanshinol (1, 5, and 10 μ M) for 24 hours. Then, cells permeabilized using 1% TritonX-100 for 10 minutes. Then, the cells were incubated with primary antibody Bcl-2 or Bax (1:100) (Cell Signaling Technology, Danvers, MA, USA) for overnight. After being washed with PBS for three times, cells were incubated with the biotin labeled secondary antibody for 30 minutes and stained with the chain mildew avidin peroxidase (Beyotime). Finally, 3,3'-diaminobenzidine was applied to color reaction.

ELISA

HepG2 cells (1×10^3) were cultured into 96 well plates for overnight and treated by tanshinol (1, 5, and 10 μ M) for 24 hours. Then, the culture media were harvested and stored at -20°C . Commercially available human matrix metalloproteinase (MMP)-2/MMP-9 ELISA kits (R&D Systems, Minneapolis, MN, USA) were used to measure the levels of MMP-2 and MMP-9, according to the manufacturer's instructions.

Western blot assay

Proteins were extracted from HepG2 cells using RIPA lysis (Beyotime) according to manufacturer's protocol and were quantified using a bicinchoninic acid kit (Beyotime). A total of 30 μ g proteins were loaded to 10% SDS-PAGE gel and transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). After blocking with 5% skim milk in Tris-buffered saline with Tween-20 (TBST), PVDF membrane was incubated with primary antibodies (phospho-PI3 Kinase p85^{Tyr458}/p55^{Tyr199}, phospho-AKT^{Ser473}, total-PI3K, total-AKT, Bcl-2, Bax, and glyceraldehyde-3-phosphate dehydrogenase) (Cell Signaling Technology) overnight. After that, the membrane was washed with

TBST three times and incubated with a secondary antibody (1:5,000; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) for 2 hours at 37°C . Target proteins were detected using the enhanced chemiluminescence system (Millipore) and visualized with the ChemiDoc XRS system (Bio-Rad, Hercules, CA, USA).

Mouse xenograft and metastasis model

A total of 100 μ L HepG2 cells (2×10^6) were injected into 6-week-old athymic nude mice. Then, mice were randomly assigned to four groups: control group, vehicle-treated group, 10 mg/kg tanshinol-treated group, and 30 mg/kg tanshinol-treated group. Tanshinol was dissolved in DMSO. Mice in the experimental groups were administered tanshinol intragastrically daily for 5 weeks. Control mice in vehicle-treated group received the same dose of vehicle. Tumor width (W) and length (L) were measured each week. The tumor volume was calculated using the following formula: $L \times W^2/2$. After 5 weeks, mice were sacrificed. In lung metastasis model, nude mice were injected with 100 μ L HepG2 cells (1×10^6) into the lateral tail vein. Mice in the experimental groups were administered tanshinol intragastrically daily. Tanshinol was dissolved in DMSO. Control mice in vehicle-treated group received the same dose of vehicle only. After 1 month, mice were euthanized and the lungs were removed for H&E staining. Animal experiments were approved by the Ethics Committee of Yangzhou University. Our animal experiments were conducted in accordance with Institutional Guidelines and the Guide for the Care and Use of Laboratory Animals (NIH publication no 85-23, revised 1996).

Immunohistochemical (IHC) staining

Briefly, formalin-fixed paraffin-embedded tumor tissues were prepared in 4- μ m sections. After performing dewaxing, rehydration, blocking endogenous peroxidase activity, and antigen retrieval steps, sections were blocked with 10% normal goat serum at room temperature for 15 minutes and incubated with primary antibodies against Bcl-2, Bax, p-PI3K, and p-AKT at 4°C overnight. The sections were subsequently treated with horseradish peroxidase-conjugated secondary antibody (Nichirei, Tokyo, Japan), and the reactions were visualized with diaminobenzidine. The number of immunopositive cells was counted using the BZ-9000 analysis software programmer (Keyence, Osaka, Japan).

Statistical analysis

Statistical analysis was conducted using SPSS (Statistical Package for the Social Sciences) 13.0 (SPSS Inc., Chicago,

IL, USA). All data were expressed as mean $\pm$ SD. Differences were evaluated using either two-tailed Student's *t*-test or one-way ANOVA followed by *post hoc* Dunnett's test. $P < 0.05$ was considered statistically significant.

Results

Tanshinol inhibits the growth of HCC cell in vitro

To evaluate the inhibitory effect of tanshinol on HCC cells, four human HCC cell lines were treated with different concentrations of tanshinol (0, 1, 5, 10, 20, and 30 μ M) or 5 μ M tanshinol for different times (24, 48, and 72 hours). As shown in Figure 1A and B, the MTT assay suggested that tanshinol suppressed the growth of HCC cells in a dose- dependent and time-responsive manner. We also found that the HepG2 cell was most sensitive to tanshinol. Consistent with the results of MTT test, the trypan blue exclusion assay demonstrated that tanshinol effectively inhibited the proliferation of HCC cell in a dose- dependent and time-responsive manner (Figure S1A and B). Hence, HepG2 cell was selected as the optimal cell model for the following examinations. We adopted the

doses 0, 1, 5, and 10 μ M of tanshinol and performed colony formation assay. Quantitative analysis revealed that the number of cell colonies was reduced by tanshinol (Figure 1C). All these data indicate that tanshinol suppresses the proliferation and colony formation of HCC cell in vitro.

Tanshinol induces the apoptosis of HepG2 cell

We further examined the mechanism behind impairment of HCC cell viability by tanshinol, the Hoechst 33342 nuclear staining assays were conducted to observe the nuclear morphology of HepG2 cell after treating with tanshinol. As shown in Figure 2A, the nuclei of control cells were stained weakly and homogeneously blue, whereas in the cells that were treated with tanshinol, the emission was much brighter than in the control cells. Then, the apoptosis of HepG2 cell induced by tanshinol was further confirmed using flow cytometry analysis. As shown in Figure 2B, the apoptosis of HepG2 cell treated with tanshinol was significantly increased compared with control group. Furthermore, the protein expression of pro- and anti-apoptotic mitochondria

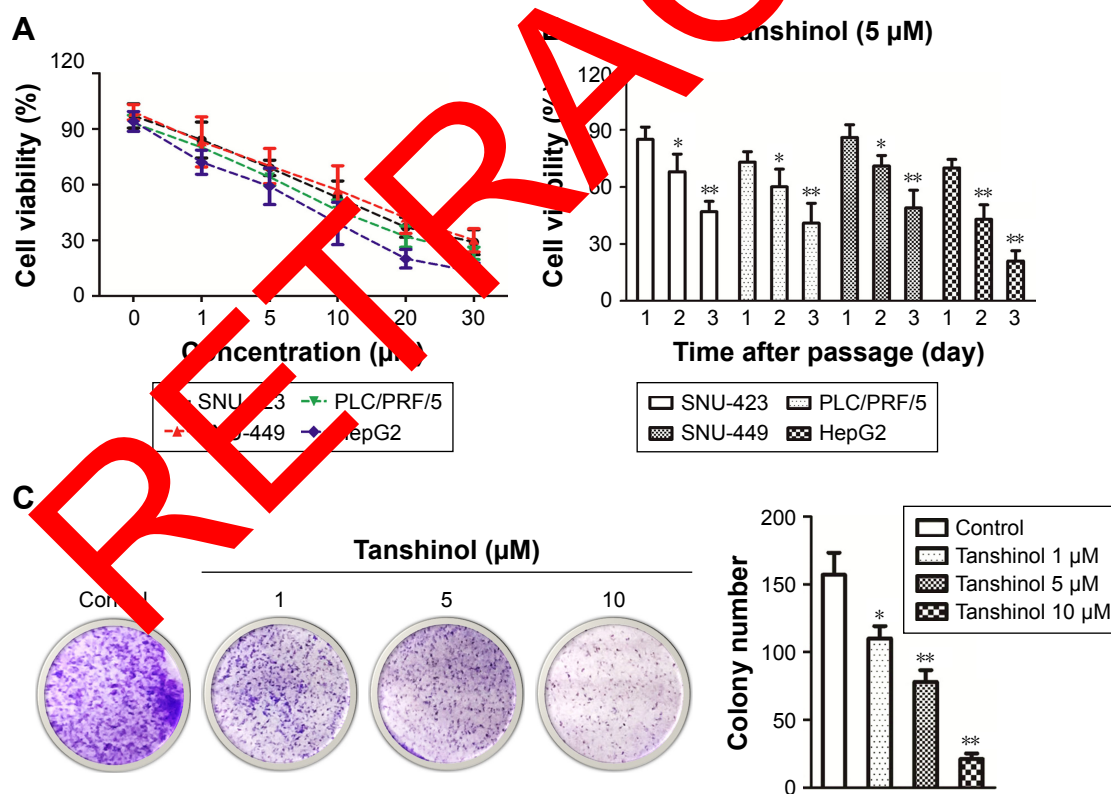


Figure 1 Tanshinol inhibits HepG2 cell growth in a dose- and time-dependent manner.

Notes: (A) Four HCC cell lines were treated with tanshinol (ranging from 1 to 30 μ M). Cell treated with 0 μ M tanshinol was used as control. After culturing for 48 hours, the cell viability was detected using MTT assay. (B) Tanshinol 5 μ M inhibited the growth of HCC cell in a time-dependent manner. (C) Colony formation assay was performed using HepG2 cell treated with tanshinol (1, 5, or 10 μ M). The clones were stained with crystal violet and the number of cell colonies was counted. * $P < 0.05$, ** $P < 0.01$ compared with control.

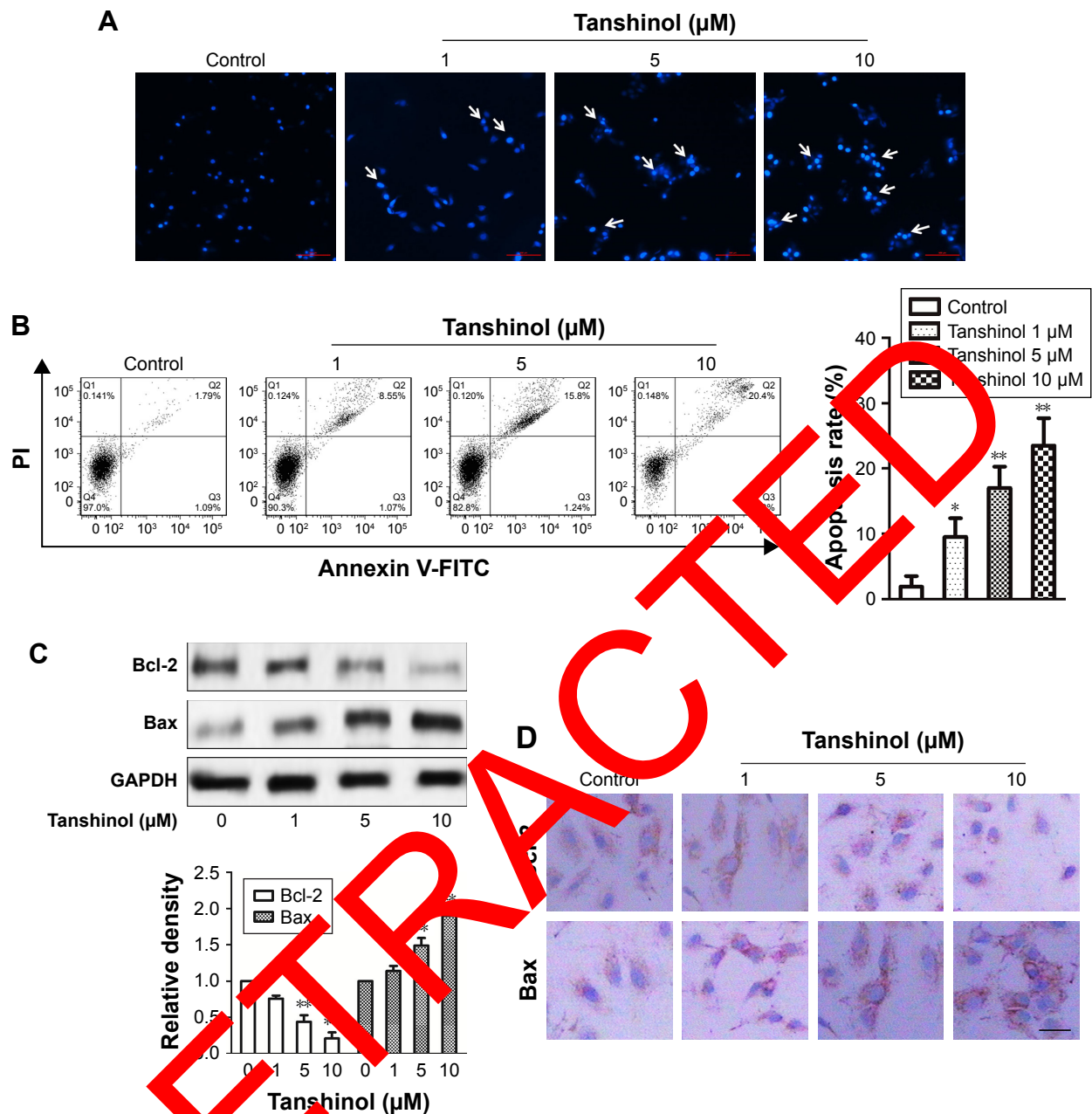


Figure 2 Tanshinol induces the apoptosis of HepG2 cell.

Notes: (A) Representative images of fluorescence staining of cells treated with tanshinol. Arrows: apoptotic nuclear changes. Scale bar: 500 μm . (B) The apoptosis of HepG2 cell was analyzed by flow cytometry with Annexin V-FITC/PI staining. (C) The expression of Bax and Bcl-2 were detected using Western blotting assay. GAPDH was used as loading control. (D) The expression of Bcl-2 and Bax in HepG2 cell was analyzed using immunohistochemical staining. * $P < 0.05$, ** $P < 0.01$ compared with control. Scale bar: 50 μm .

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FITC, fluorescein isothiocyanate; PI, propidium iodide.

protein Bax and Bcl-2 in HepG2 cell were analyzed by immunoblotting assay. As shown in Figure 2C, tanshinol increased the level of Bax and conversely reduced the protein expression of Bcl-2 in HepG2 cell. The levels of Bax and Bcl-2 in HepG2 cell were also confirmed by IHC staining (Figure 2D). These findings suggest that the inhibitory impact of tanshinol on the growth of HCC cell is closely correlated with it inducing apoptosis.

Tanshinol inhibits the migration and invasion of HepG2 cell

The effects of tanshinol on HepG2 cell metastasis were subsequently assessed. As shown in Figure 3A, tanshinol obviously inhibited the migration of HepG2 cell compared with control as shown in the wound healing assay. In Transwell assay, the number of invasive HepG2 cells was markedly decreased in tanshinol-treated group (Figure 3B). Besides,

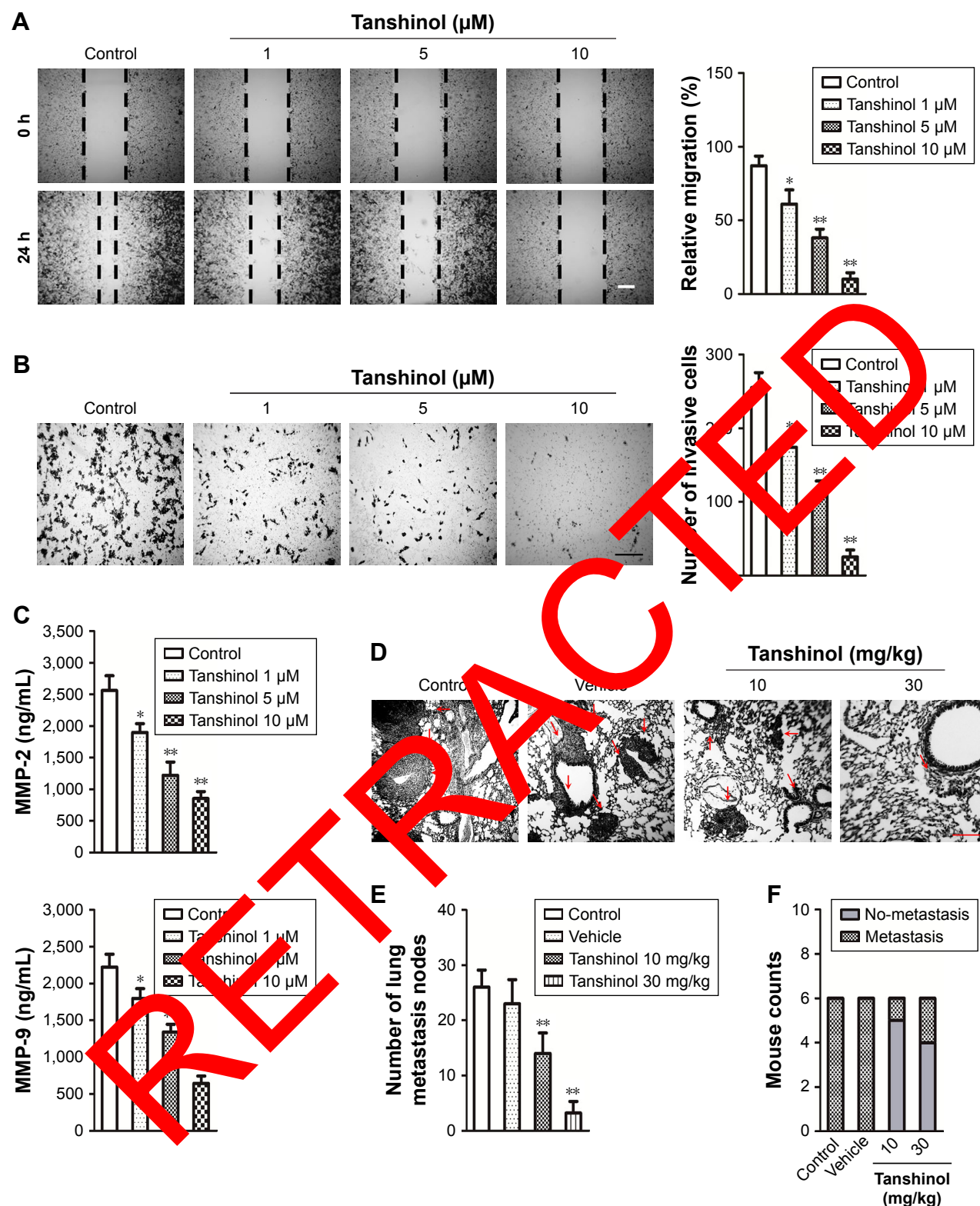


Figure 3 Tanshinol inhibits the migration and invasion of HepG2 cell.

Notes: (A) The wound healing assay was performed to observe the effect of tanshinol on the migration ability of HepG2 cell. Scale bar: 100 μm . (B) Transwell invasion assay was performed to observe the effect of tanshinol on the invasive ability of HepG2 cell. Scale bar: 100 μm . (C) The level of MMP-2 and MMP-9 in the supernatant, which was collected from control or tanshinol-treated HepG2 cell, was determined by ELISA assay. (D) Representative photos of histological H&E staining of lung metastasis were shown for each group. Red arrows indicates the metastasis foci. (E) A bar graph summarizing the number of lung metastasis nodes in the four groups. (F) A bar graph summarized the incidence of lung metastasis in the four groups. * $P < 0.05$, ** $P < 0.01$ compared with control.

Abbreviation: MMP, matrix metalloproteinase.

the secretions of MMP-2 and MMP-9 were significantly reduced by tanshinol (Figure 3C). In order to assess the impact of tanshinol on the metastasis of HepG2 cell in vivo, the animal model of experimental pulmonary metastasis was constructed. The number of metastatic colonies in the lung from the mice that were treated with tanshinol was significantly less than that in the control group (Figure 3D and E). Furthermore, there was a higher incidence of tumor nodules in the lung from mice without tanshinol treatment compared with those mice treated with tanshinol (Figure 3F). Our data suggest that tanshinol inhibits the metastasis of HCC cell in vivo and in vitro.

Tanshinol inhibits tumor growth of HepG2 cell in vivo

A xenograft model of HCC was constructed to further investigate the impact of tanshinol on the growth of HepG2 cell in vivo. Mice in experimental groups were treated with 10 mg/kg or 30 mg/kg of tanshinol daily for 5 weeks. The tumor volume of the control group was significantly larger than those of the experimental groups (Figure 4A and B). Consistently, the tumor weight was largest in control mice and was smaller in the tanshinol-treated mice (Figure 4C). Moreover, in tanshinol-treated xenograft tumors, the levels of Bax and Bcl-2 were detected using IHC staining assay (Figure 4D). Consistently, the protein expression of Bcl-2 was significantly inhibited by tanshinol compared with control group whereas the expression of Bax was remarkably increased by tanshinol treatment (Figure 4E). These data suggest that tanshinol inhibits HepG2 cell growth in vivo.

Tanshinol inhibits the activities of the PI3K and AKT

After treatment of HepG2 cell with tanshinol, the phosphorylation of PI3K-AKT family member (PI3K and AKT), which regulate a wide variety of cellular functions was detected using Western blot assay. As shown in Figure 5A, the phosphorylation of PI3K and AKT in HepG2 cell was remarkably decreased by tanshinol. Furthermore, tanshinol significantly suppressed the phosphorylation of PI3K and AKT in vivo as demonstrated by IHC staining assay (Figure 5B). Consistently, the Western blotting assay indicated that the phosphorylation of PI3K and AKT in tumor tissue was significantly inhibited by tanshinol compared with control group (Figure S3). These data suggest that the PI3K/AKT signaling pathway is inactivated by tanshinol.

Discussion

HCC is still one of the leading causes of cancer-related death worldwide. During the past few years, the clinical diagnosis and treatment for patients with HCC have made great progress.²⁶ However, a large proportion of patients with HCC are diagnosed at the late stage of disease, and recurrence or metastasis in HCC are closely associated to poor overall survival. Tanshinol is an active constituent of the Chinese medicinal herb *S. miltiorrhiza* and has been shown to exert anti-inflammatory and anti-fibrotic effects, and has anti-bacterial properties.^{27–32} Previous investigation indicates that tanshinol has anti-tumor activities in melanoma cell through inhibiting angiogenesis and cell metastasis.³³ In addition, tanshinol promotes the radioresponse of Lewis lung carcinoma in mice model by elevating tumor cell hypoxia.³⁴ Recently, investigation has proved that tanshinol has protective effects on the carbon tetrachloride (CCl₄)-induced liver fibrosis by suppressing oxidative stress and inflammation via regulating Nrf2/HO-1 signaling pathway.¹⁶ Nevertheless, the potential anti-cancer role of tanshinol in HCC should be further explored. In the present study, we mainly investigated the possible therapeutic values of tanshinol against HCC.

First, we found that tanshinol treatment inhibited the proliferation of HCC cell in both dose- and time-dependent manner. In colony formation assay, the clonogenic potential of HepG2 cell was remarkably inhibited by tanshinol, which further confirmed that tanshinol suppressed the growth of HepG2 cell in vitro. In addition, a marked increase of apoptosis in HepG2 cell was observed following tanshinol treatment. Importantly, tanshinol inhibited tumor growth of HepG2 cell in vivo. In all, our data suggest that tanshinol has a potential anti-cancer effect in HCC. Cancer metastasis requires several crucial interrelated events, such as cancer cell migration and invasion. In this study, we also investigated the migration and invasion of HepG2 cell that was treated with tanshinol. As expected, the migration and invasion abilities were remarkably reduced by tanshinol. Dissemination of cancer cells from the primary tumor is another crucial event in the process of cancer invasion and metastasis. This process involves degradation of the extracellular matrix by many proteases of which MMP-2 and MMP-9 appear to play a key role.³⁵ Indeed, the up-regulation of the expression of MMPs correlates with the increased metastatic potential of cancer cells.³⁶ Therefore, inhibiting the level of MMP-2/9 is an important approach in the fight against cancer metastasis. Here, we clearly demonstrated that the MMP-2 and MMP-9, which are promoters

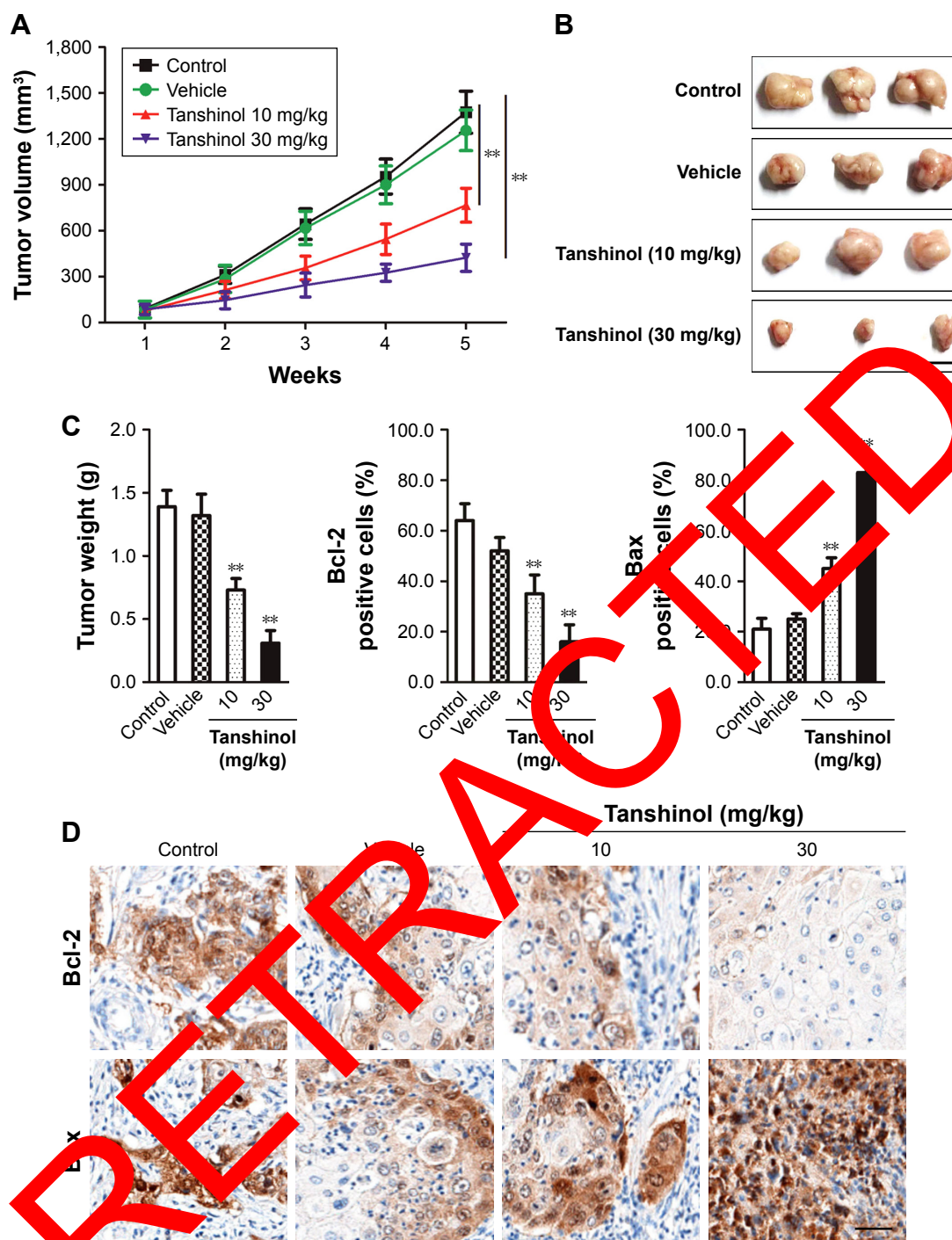


Figure 4 Tanshinol inhibits the growth of HepG2 cell in a xenograft model.

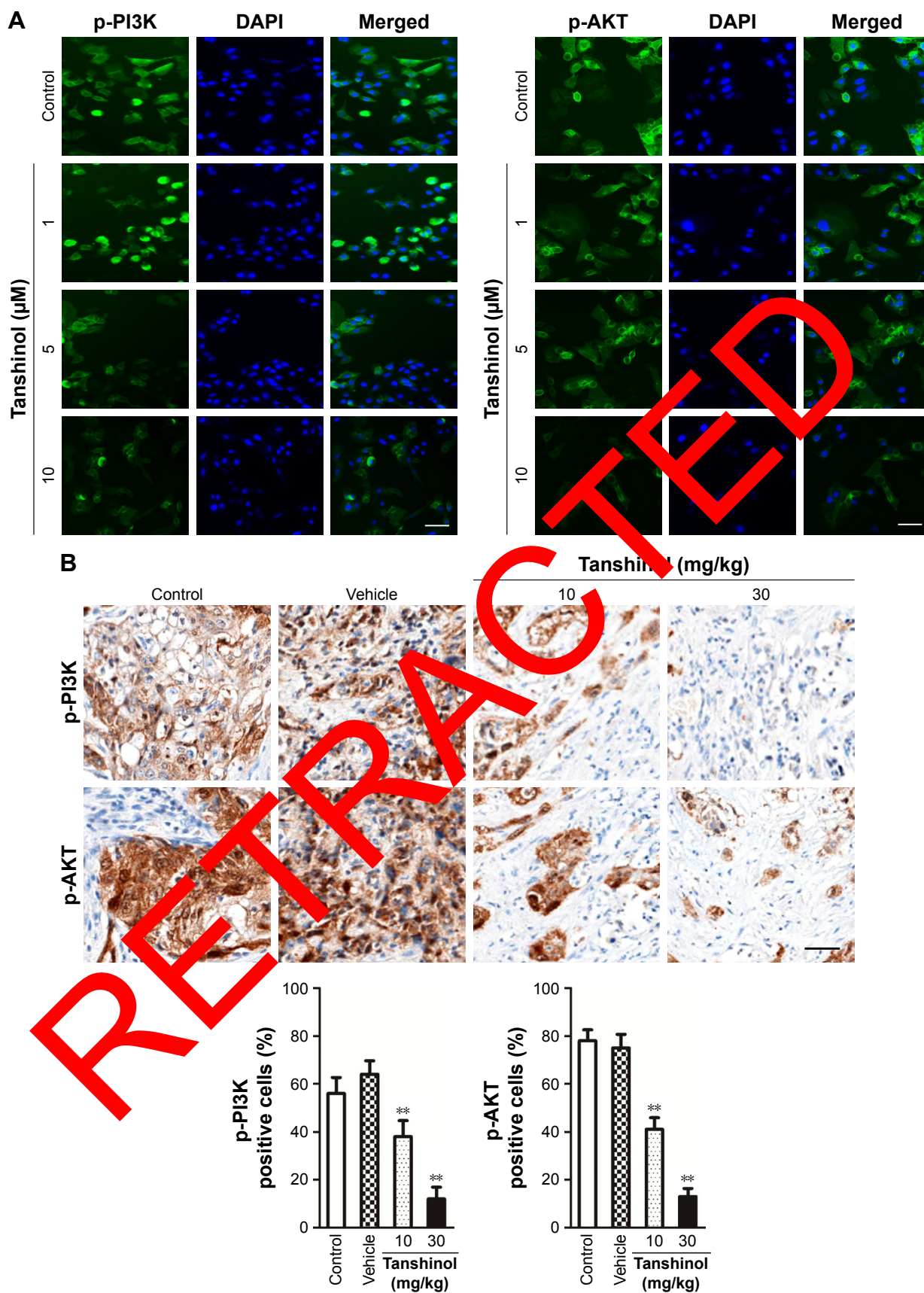
Notes: (A) A xenograft model of HCC was established using HepG2 cell. Mice in the experimental groups were administrated tanshinol intragastrically daily for 5 weeks. Control mice in vehicle-treated group received the same dose of vehicle only. Tumor volumes in each group were calculated. (B) Five weeks after inoculation, tumor tissues in each group were dissected from mice. (C) Dissected tumors were weighed. The average weight of tumors from each group of mice was calculated. (D) The expression of Bcl-2 and Bax was detected in xenografts by IHC. Scale bar: 100 μ m. $^{**}P < 0.01$ compared with control.

Abbreviations: HCC, hepatocellular carcinoma; IHC, immunohistochemistry.

of distant metastasis, were also decreased in the tanshinol-treatment group. Collectively, our data show that tanshinol significantly inhibits the growth and metastasis of HCC cell.

The PI3K/AKT signaling pathway is elevated in a significant portion of primary and metastatic cancer.^{37,38} Recent

studies have presented that PI3K/AKT pathway activation is a key character of the metastasis of several types of cancers.^{39,40} PI3K-AKT signaling pathway regulates various important cellular processes, including cancer cell proliferation, apoptosis survival, and adhesion.^{41,42} Hence, inhibition



of PI3K-AKT signaling pathway offers a promising strategy in targeting malignant cancer. In our current study, the levels of PI3K and AKT phosphorylation were decreased in the tanshinol-treated HepG2 cell, indicating that tanshinol could inhibit the activation of PI3K/AKT pathway. Consistently, tanshinol reduced the activities of PI3K and AKT in the xenograft model of HCC that was established using HepG2 cell. Increased levels of MMP-2 and MMP-9 are known to promote migration, invasion, and metastasis of various types of cancers.^{43–45} PI3K-AKT is the most important positive regulator of the expressions of MMP-2 and MMP-9.⁴⁶ As expected, tanshinol treatment significantly reduced the levels of MMP-2 and MMP-9 in HCC cell. Thus, we speculated that tanshinol inhibited the growth and metastasis of HCC via regulating the PI3K-AKT signaling pathway. Although, we found that tanshinol inhibited the growth, migration, invasion, and metastasis of HCC cell, the correlation between the anti-cancer effects of tanshinol and its suppression of the activity of PI3K-AKT has not been well investigated. In future studies, to confirm the anti-cancer effect of tanshinol on HCC, the specific PI3K-AKT inhibitor or siRNA interference could be applied.

Conclusion

In summary, the present study identified tanshinol as a novel therapeutic compound to inhibit the proliferation, migration, invasion, and metastasis of HCC cell. The anti-cancer activity of tanshinol might be through regulation of the PI3K-AKT signaling pathway.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

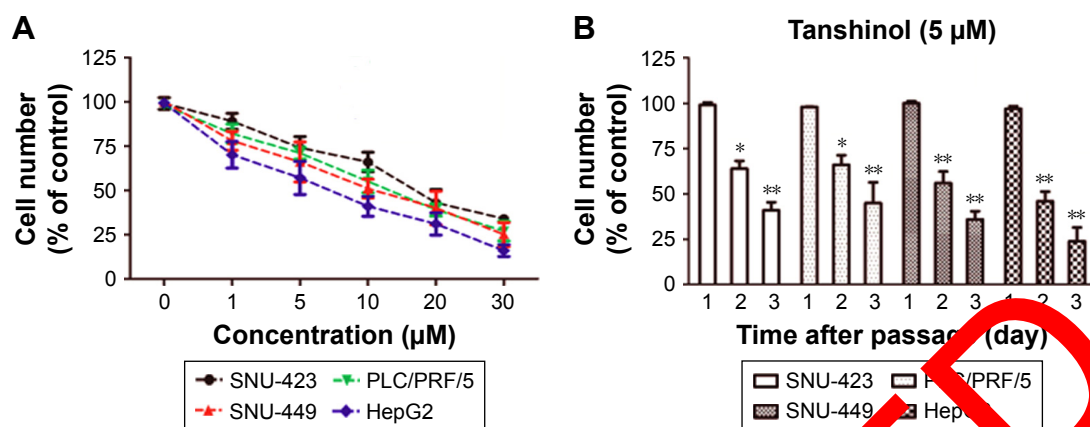


Figure S1 Trypan blue exclusion test.

Notes: (A) HepG2 cells were incubated with various concentrations of tanshinol (1, 5, and 10 μM) for 48 hours. After culture, cell numbers were evaluated by staining with trypan blue and counting using light microscopy. (B) HepG2 cells were incubated with 5 μM tanshinol for 24, 48, and 72 hours. After culture, cell numbers were evaluated by staining with trypan blue and counting using light microscopy. * $P < 0.05$, ** $P < 0.01$ compared to day 1.

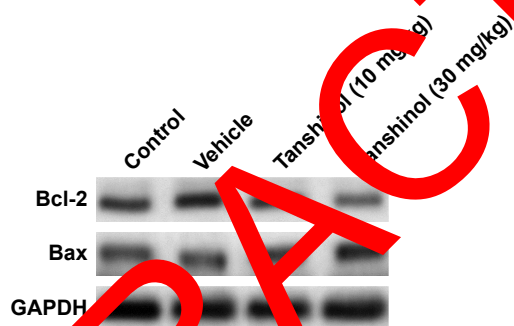


Figure S2 The levels of Bcl-2 and Bax in tumor tissues that were formed by HepG2 cells were detected by Western blotting assay.

Abbreviation: GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

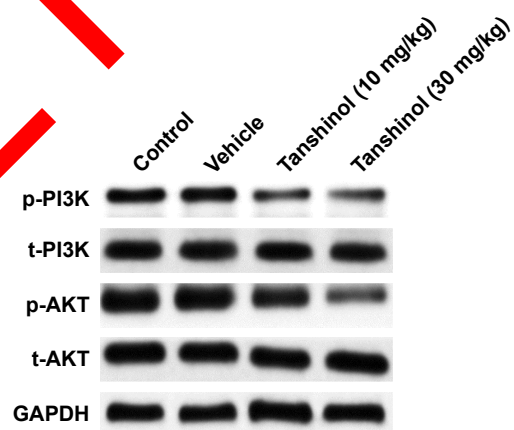


Figure S3 The levels of p-PI3K and p-AKT in tumor tissues that were formed by HepG2 cells were detected by Western blotting assay.

Abbreviations: AKT, protein kinase B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PI3K, phosphatidylinositol 3 kinase.

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