

Paris polyphylla 26 triggers G2/M phase arrest and induces apoptosis in HepG2 cells via inhibition of the Akt signaling pathway

Journal of International Medical Research

2019, Vol. 47(4) 1685–1695

© The Author(s) 2019

Article reuse guidelines:

sagepub.com/journals-permissions

DOI: 10.1177/0300060519826823

journals.sagepub.com/home/imr



Qiang Li^{1,*}, Zifan He^{2,*}, Jiming Liu³,
Jianlong Wu¹, Guixiang Tan⁴, Jianwei Jiang²,
Zexuan Su⁵  and Mingrong Cao¹

Abstract

Objectives: *Paris polyphylla 26* (PP-26) is a monomer purified from *Paris polyphylla*, which has traditionally been used as an antimicrobial, hemostatic, and anticancer agent in China. The anti-proliferation effect and underlying molecular mechanism of PP-26 were investigated in vitro.

Methods: The effects of PP-26 on various tumor cells were detected by MTT assay. PP-26-affected cell cycle and cell cycle-related proteins in HepG2 cells were detected by flow cytometry and western blotting, respectively. Apoptosis in response to PP-26 was assessed by Hoechst 33258 staining and flow cytometry. PP-26-affected apoptosis-related proteins and Akt signaling were detected by western blotting. The inhibitory effect of PP-26 on HepG2 cells, when combined with 5-fluorouracil (5-FU), was also assessed.

Results: PP-26 inhibited proliferation of HepG2 cells in a dose-dependent manner by triggering G2/M-phase arrest. Moreover, PP-26 induced apoptosis of HepG2 cells. Expression levels of apoptosis proteins caspase 9, caspase 3, PARP, Bcl-2, Bcl-xL, and Mcl-1 were downregulated, while the expression level of apoptosis protein Bax was upregulated. Expression levels of p-Akt, p-GSK-3 β , and p-Foxo3 were downregulated. Combination with PP-26 enhanced 5-FU inhibition of HepG2 cell proliferation.

¹Department of General Surgery, The First Affiliated Hospital, Jinan University, Guangzhou, China

²Department of Biochemistry, Medical College, Jinan University, Guangzhou, China

³Department of General Surgery, The Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou, China

⁴School of Nursing, Guangdong Pharmaceutical University, Guangzhou, China

⁵Department of Urology, The First Affiliated Hospital, Jinan University, Guangzhou, China

*These authors contributed equally to this work.

Corresponding author:

Zexuan Su, Department of Urology, The First Affiliated Hospital, Jinan University, Avenue West 613, Guangzhou 510630, P.R. China.

Email: suz2008@126.com



Conclusions: PP-26 triggers G2/M-phase arrest and induces apoptosis in HepG2 cells via inhibition of the Akt signaling pathway.

Keywords

Hepatocellular cancer, PP-26, cell cycle, apoptosis, *Paris polyphylla*, Akt signaling, proliferation, caspase

Date received: 18 October 2018; accepted: 4 January 2019

Introduction

Liver cancer primarily comprises hepatocellular carcinoma (HCC), which is one of the most common and aggressive malignant tumors worldwide.¹ In men, HCC has the second highest lethality of all cancers worldwide and the sixth highest lethality in developed countries. Notably, an estimated 782,500 new liver cancer cases were diagnosed and 745,500 deaths occurred worldwide during 2012. In China, HCC morbidity and mortality contribute to approximately 50% of the total number of cases liver cancer and liver cancer-related deaths.² Current treatments for HCC primarily comprise surgery and chemotherapy, but these do not have considerable therapeutic effect.³ Therefore, new approaches are urgently needed, such as novel drugs that specifically target HCC.⁴

PP-26 is a monomer purified from *Paris polyphylla*, which has traditionally been used as an antimicrobial and hemostatic agent in China; it is used in treatment of abscesses, sore throat, snake bites, traumatic injuries, chronic bleeding, and tumors.⁵ Recent research has shown that some monomers purified from *P. polyphylla* exhibit anticancer activity towards multiple cancer cell lines. For example, we previously demonstrated that PP-10 induced apoptosis and autophagy in the BGC-823 human gastric cancer cell line through inhibition of PI3K/Akt signaling pathways by upregulation of p15.⁶ However, the anticancer activity and mechanism of PP-26, also

purified from *P. polyphylla*, have not been previously described.

In this study, we investigated the effects of PP-26 on the proliferation of various types of cancer cells and further explored the molecular mechanism by which PP-26 inhibits proliferation of HCC cells in vitro. Furthermore, because Akt-mediated GSK3 β and FoxO3a signaling is involved in apoptosis in cancer cells,^{7,8} we examined the effects of PP-26 on the AKT/FOXO3a/GSK-3 β signaling pathway.

Materials and methods

Chemicals and reagents

PP-26 was obtained from the Pharmacy College of Jinan University (Guangzhou, China) and dissolved in DMSO. 5-fluorouracil (5-FU) was purchased from MedChemExpress (Monmouth Junction, NJ, USA). Fetal bovine serum (FBS) and RPMI-1640 were purchased from Gibco (Grand Island, NY, USA). Trypsin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma (St. Louis, MO, USA). Antibodies against Akt (#9272), p-Akt (Ser473, #4060), p-Akt (Thr308, #13038), Bcl-2 (#4223), cyclin B1 (#12231), cyclin D1 (#2978), Bax (#14796), cyclin E2 (#4132), CDK4 (#12790), cdc2 (#28439), Bcl-xL (#2764), p-cdc2 (Tyr15, #4539), caspase-9

(#9502), cleaved caspase-9 (#52873), caspase-3 (#9665), cleaved caspase-3 (#9664), FOXO3a (#2497), p-FOXO3a (Ser318/321, #9465), GSK3 α/β , p-GSK3 β (Ser9, #5676), GAPDH (#2118), Mcl-1 (#39224), Myt-1 (#4282), PARP (#9532) and cleaved PARP (#5625) were purchased from Cell Signaling Technology Ltd. (Danvers, MA, USA).

Cell culture

One human hepatic cell line (LO2) and two human HCC cell lines (HepG2 and SMM-7721) were maintained in RPMI-1640 medium supplemented with 10% FBS in a 37°C incubator with 5% CO₂.

MTT assay

Cells were plated in 96-well plates at a density of 1.0×10^4 cells per well, then cultured for 24 hours. Cells were then treated with PP-26 at concentrations of 0.4, 0.8, 1.6, 3.2, 6.4, or 12.8 $\mu\text{mol/L}$ for 24 hours, 48 hours, or 72 hours. Subsequently, 20 μL of MTT (5 mg/mL) was added. After incubation for an additional 4 hours, supernatants were carefully discarded and 150 μL of DMSO was added. The absorbance of each well was measured at 490 nm. Cell viability was calculated as follows: cell viability rate (%) = $(\text{OD}_{\text{control}} - \text{OD}_{\text{test}}) / \text{OD}_{\text{control}} \times 100\%$.

For the drug sensitivity test, 1.0×10^4 HepG2 cells per well were plated in 96-well plates and cultured for 24 hours. Cells were then treated with 0.67 $\mu\text{mol/L}$ PP-26 and different concentrations (ranging from 0 to 800 $\mu\text{mol/L}$) of 5-FU for 48 hours. The coefficient of drug interaction (CDI) was used to test the following combination effect: $\text{CDI} = E_{(a+b)} / (E_a \times E_b)$. E_a and E_b represent the inhibition rates of PP-26 alone and 5-FU alone; E_{a+b} represents the inhibition rate of combination therapy. $\text{CDI} < 1$ indicates a synergistic effect, $\text{CDI} = 1$ indicates an additive effect,

and $\text{CDI} > 1$ indicates an antagonistic effect.

Nuclear staining with Hoechst 33258

HepG2 cells (3×10^5) were cultured in 10% FBS RPMI-1640 medium for 24 hours, and then treated with different concentrations of PP-26 for 24 hours. The cells were then collected and plated on slides. The slides were air-dried, fixed in methanol-acetone (3/1, v/v), and stained for 20 minutes with Hoechst 33258 for 37°C. The Hoechst 33258-stained cells were then air-dried, and nuclei of both live and apoptotic cells were examined by fluorescence microscopy.

Flow cytometry analysis

HepG2 cells were plated in 6-well plates at a density of 1×10^6 cells per well. After attachment, cells were treated with PP-26 for 24 hours. After treatment with PP-26, the HepG2 cells were centrifuged at 650 g for 10 minutes and resuspended in PBS, and then stained by PI/RNase staining kits (Multisciences, Hangzhou, China) or annexin V fluorescein solution (Beyotime, Shanghai, China) at room temperature for 10 minutes. To assess onset of apoptosis, propidium iodide in HEPES buffer was added. Cell cycle stage and onset of apoptosis was analyzed by flow cytometry (FCM, Becton Dickinson, Franklin Lakes, NJ, USA), in accordance with the manufacturer's protocol. The percentages of cells in G0/G1, S, and G2/M phases, or the ratios of apoptotic cells, were calculated.

Western blot analysis

Cells were plated in 6-well plates at a density of 1.0×10^6 cells per well. After attachment, the cells were treated with PP-26 for 24 hours. Proteins were then extracted from the treated cells, and concentrations were determined by using Pierce™ BCA Protein Assay Kit (Thermo Fisher

Scientific, Waltham, MA, USA). Protein samples (50 μg) were separated by using 8%–15% sodium dodecyl sulfate-polyacrylamide electrophoresis, then transferred to 0.22- μm polyvinylidene fluoride membranes. The membranes were blocked with 5% evaporated skim milk for 1 hour at room temperature. Primary antibodies were diluted at 1:500–1:1000 in Tris-buffered saline + Tween (TBST). Membranes were incubated with diluted primary antibodies overnight at 4°C, followed by incubation with secondary antibody (BM2020, Boster, Wuhan, China) for 1 hour at 37°C. After each membrane was washed three times with 1X TBST, it was covered with Immobilon ECL luminescence liquid (Millipore, Burlington, MA, USA) and visualized using the Gel Image System (UVItec Ltd., Cambridge, UK). The intensity of each band was analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

Data were analyzed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Differences were compared by ANOVA and Student's *t*-test. All results are expressed as the mean \pm standard error.

All experiments were performed in triplicate. Only differences with $P < 0.05$ were considered statistically significant.

Results

PP-26 inhibited cell growth in HCC cells

PP-26 is a steroidal saponin compound extracted from *P. polyphylla* (Figure 1). To reveal the effects of PP-26 on cell proliferation, the MTT assay was performed to analyze the proliferation of HepG2, SMMC-7721, and LO2 cells. The results showed that the proliferation of HepG2 cells (Figure 2a) and SMMC-7721 cells (Figure 2b) was significantly inhibited upon treatment with different concentrations of PP-26 for 24, 48, or 72 hours. However, PP-26 treatment showed a poor growth-inhibitory effect on LO2 cells (Figure 2c). These results indicated that the viability of HCC cells was significantly reduced by PP-26 treatment in a dose- and time-dependent manner. When cells were treated for 48 h, the respective IC_{50} values for LO2 cells, HepG2 cells, and SMMC-7721 cells were $6.98 \pm 0.99 \mu\text{mol/L}$, $1.91 \pm 0.45 \mu\text{mol/L}$, and $1.85 \pm 0.25 \mu\text{mol/L}$. Thus, PP-26 treatment resulted in less

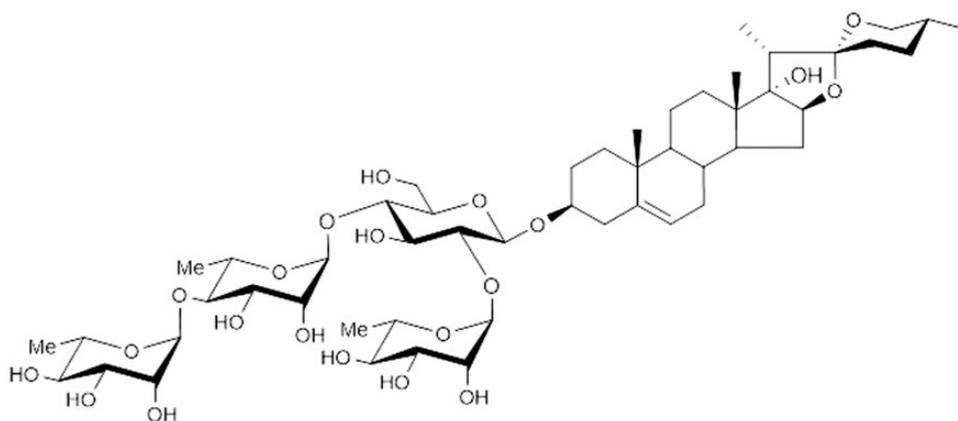


Figure 1. Chemical structure of PP-26

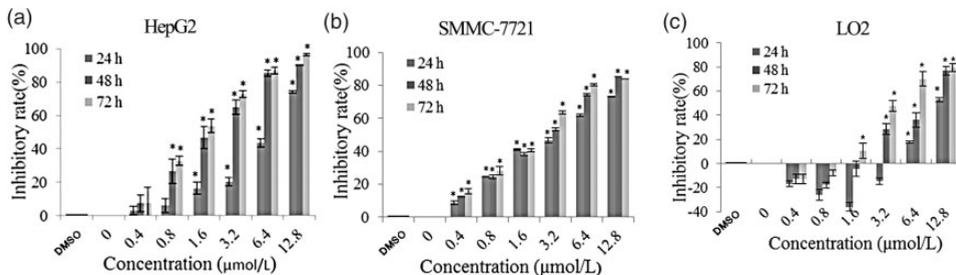


Figure 2. PP-26 inhibited the growth of HepG2, SMMC-7721, and LO2 cells. (a) Growth-inhibition effects of PP-26 on HepG2 cells. (b) Growth-inhibition effects of PP-26 on SMMC-7721 cells. (c) Growth-inhibition effects of PP-26 on LO2 cells. The cells were incubated with different concentrations (0.4, 0.8, 1.6, 3.2, 6.4, or 12.8 μmol/L) of PP-26 for 24 h, 48 h, and 72 h, then subjected to MTT assays. Results represent three independent experiments (* $P < 0.05$, ANOVA)

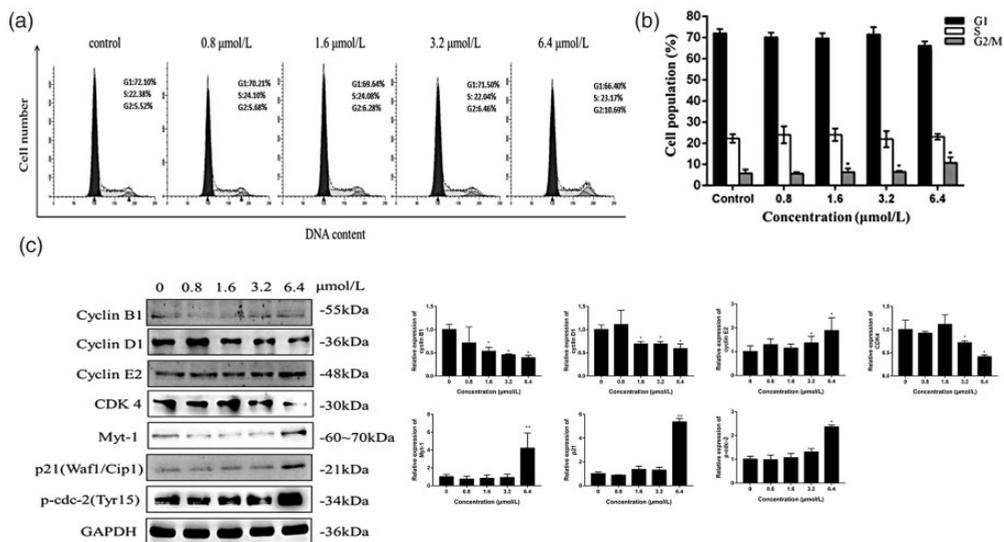


Figure 3. PP-26 induced G2/M arrest in HepG2 cells. (a) Flow cytometry and (b) cell cycle distribution of HepG2 at 24 hours (* $P < 0.05$, ** $P < 0.01$, ANOVA). (c) Cell cycle-related proteins were analyzed by western blot

cytotoxicity in normal liver cells than in HCC cells.

PP-26 triggered G2/M-phase arrest in HepG2 cells

To investigate whether PP-26 could affect the cell cycle, cells were treated with PP-26 at different concentrations for 24 hours and then assessed by using flow cytometry with

propidium iodide staining. Upon exposure to different concentrations of PP-26 for 24 hours, the proportion of HepG2 cells in G1 phase gradually decreased, while the proportion of HepG2 cells in S phase remained unchanged (Figures 3a and b). These results suggested that PP-26 caused arrest of cell cycle distribution in G2/M phases.

We evaluated the effect of PP-26 on the expression of cell cycle-related proteins by

using western blot analysis (Figure 3c). The results showed that the expression levels of cyclin D1 and CDK4 were significantly suppressed in HepG2 cells by 24 hours of PP-26 treatment, in a dose-dependent manner. However, the expression levels of cyclin B1 and cyclin E2 remained unchanged. Myt1 protein kinase functions to negatively regulate Cdc2-cyclin B complexes by phosphorylating Cdc2 on tyrosine 15.⁹ The expression levels of Myt1, p21, and p-cdc2 (Tyr15) were increased, suggesting that the activity of the cyclin B/cdc2 complex was inhibited by treatment with PP-26 in HepG2 cells (Figure 3b).

PP-26 induced apoptosis in HepG2 cells

To assess whether PP-26 causes apoptosis in HepG2 cells, different concentrations (0, 0.8, 1.6, 3.2, 6.4 μmol/L) of PP-26 were used to treat HepG2 cells for 24 h; the results were analyzed by using flow cytometry. Compared with the control (untreated) cells, the ratios of apoptotic cells in each group were 1.48%, 2.08%, 7.50%, and 23.58%, respectively

(Figures 4a and b). The data showed that PP-26 could induce apoptosis in HepG2 cells in a dose-dependent manner. Hoechst staining was also performed. The ratio of apoptotic cells in PP-26-treated groups was higher than in the control group (Figure 4c). Moreover, we performed western blotting to study the mechanisms by which PP-26 induces apoptosis in HepG2 cells. The data showed that the expression levels of PARP, caspase-3, and caspase-9 were downregulated in a dose-dependent manner (Figure 4d). Additionally, a subset of apoptosis-related upstream proteins (Bcl-xL, Bcl-2, and Mcl-1) were downregulated, while Bax was upregulated upon treatment of HepG2 cells with PP-26 (Figure 4e). These results indicated that PP-26 induced apoptosis in HepG2 cells through a mitochondrial signaling pathway.

PP-26 inhibited Akt kinase and its downstream signaling pathway

To investigate whether PP-26 induced apoptosis in HepG2 cells through the Akt

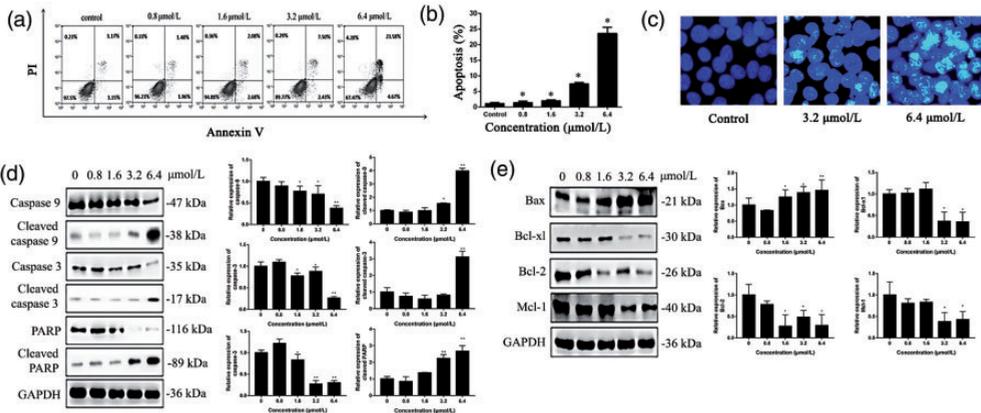


Figure 4. PP-26 induced HepG2 cell apoptosis. (a) Flow cytometry and (b) annexin V-FITC and PI staining showed that apoptosis of HepG2 cells increased at 24 hours (*P < 0.05, **P < 0.01, ANOVA). (c) Hoechst 33258 staining showed typical apoptotic morphological changes of HepG2 cells, such as nuclear condensation, fragmentation, and disappearance of nucleoli. (d) Western blot analysis showed that caspase 9, caspase 3, and PARP protein expression were downregulated. (e) Western blot analysis revealed that the pro-apoptotic protein, Bax, was upregulated and the anti-apoptotic proteins, Bcl-2, Bcl-xL, and Mcl-1, were down-regulated (*P < 0.05, **P < 0.01, ANOVA)

signaling pathway, we measured the effects of PP-26 on phosphorylation of Ser473-Akt and Thr308-Akt protein levels. The results showed that the expression levels of p-Akt (Thr308), p-Akt (Ser473), p-GSK3β (ser9), and p-FOXO3a (Ser318/321) were reduced, while those of AKT, GSK3α/β, and FOXO3a showed no significant changes (Figures 5a and b). Therefore, the phosphorylation of Akt was inhibited by PP-26. As expected, the data suggested that treatment with PP-26 also inhibited phosphorylation of FOXO3a and GSK-3β.

PP-26 sensitized HepG2 cells to 5-FU in vitro

The effect of PP-26 on chemotherapy sensitivity was further examined in HepG2 cells. The MTT assay was performed to assess the growth inhibition effect of PP-26 on HepG2 cells, in combination with different concentrations of 5-FU. The results showed that

the cytotoxicity of 5-FU in HepG2 cells was enhanced upon combination with PP-26 (Figure 6). The combination of PP-26 and 5-FU produced a significant synergistic effect, as indicated by $CDI < 1$. Furthermore, when HepG2 cells were treated with 5-FU combined with PP-26, the IC_{50} of 5-FU was 43.81 μmol/L. In contrast, the IC_{50} of 5-FU was 386.5 μmol/L when HepG2 cells were treated with 5-FU alone.

Discussion

Some studies regarding the mechanism of progression of HCC^{10,11} have shown that *P. polyphylla* could inhibit proliferation of various tumor cell lines.¹² For instance, Qin et al.¹³ demonstrated that pp-7 has an inhibitory effect on HepG2 and HEK293 cells, with respective IC_{50} values of $2.9 \pm 0.5 \mu M$ and $5.0 \pm 0.6 \mu M$. Ke et al.⁶

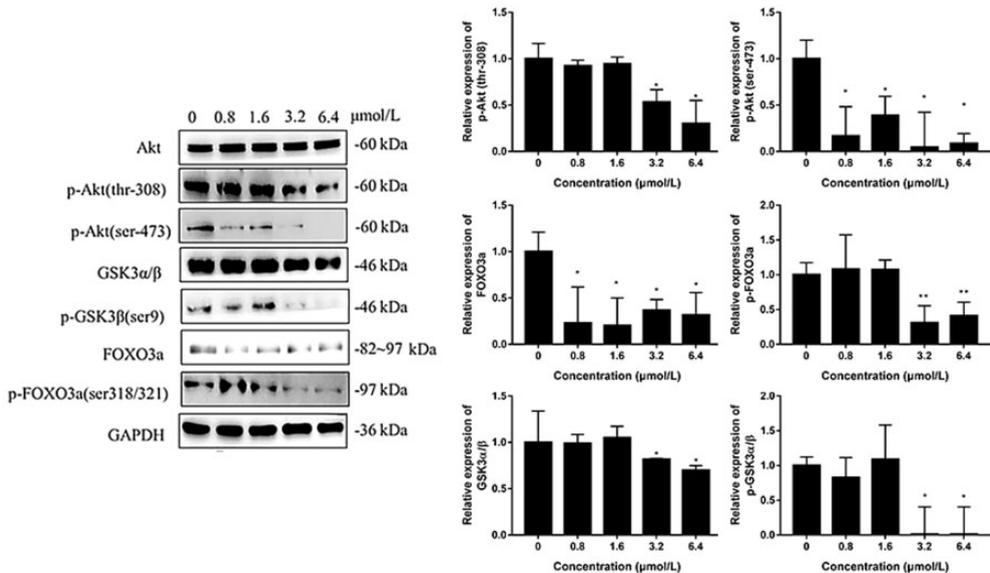


Figure 5. PP-26 inhibited the expression of PI3K/Akt signaling components. The expression levels of p-Akt (thr308), p-Akt (ser473), p-GSK3β (ser9), and p-FoxO3a (Ser318/321) were reduced by treatment with PP-26 for 24 h, while the expression levels of Akt, GSK3α/β, and FOXO3a did not change significantly. *P < 0.05, **P < 0.01, ANOVA

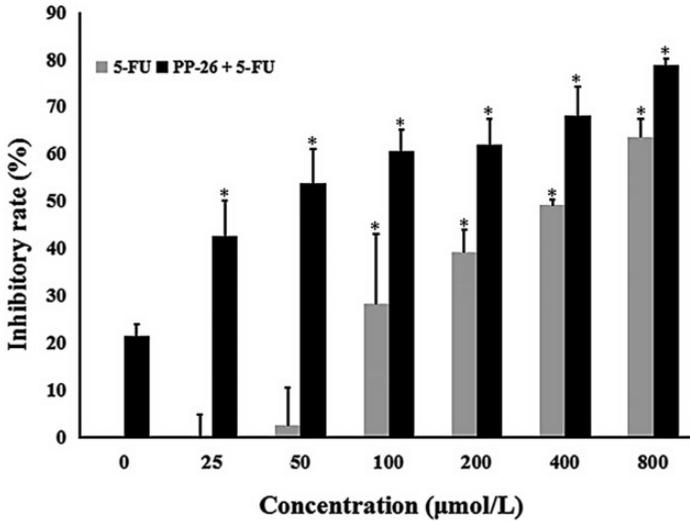


Figure 6. Effects of 5-FU alone or combined with PP-26 on cell viability of HepG2 cells. The PP-26 concentration was 0.67 μmol/L and treatment time was 48 h (* $P < 0.05$, Student's t-test)

found that pp-22 inhibited the growth of SCC-15 human tongue squamous cells in a dose- and time-dependent manner. We isolated 51 active monomers (PP-01-PP-51) from *P. polyphylla*. Among these monomers, 16 had significant inhibitory effects on the proliferation of CNE1 cells.^{12,14} We selected PP-26 for further investigation of its inhibitory effect on HepG2 cell proliferation in vitro. PP-26 is also known as (3β, 17α,25R)-spiros-5-ene-3, 17-diol-3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside; its chemical formula is C₅₁H₈₂O₂₁. The present study investigated the inhibitory effect of PP-26 on various cells and provided an experimental basis for its use in cancer treatment. Here, we found that PP-26 inhibited the proliferation of HepG2 cells in a dose- and time-dependent manner, but exhibited reduced cytotoxicity in LO2 cells, a normal liver cell line. However, an extremely low concentration (< 3.2 μM) of PP-26 induced proliferation of LO2, suggesting that

concentrations of PP-26 should be carefully monitored during cancer treatment.

The cell cycle is an important aspect of eukaryotic cell division, with four key checkpoints in its progression. At the G2/M phase checkpoint, Myt1 causes cell cycle arrest by phosphorylating Tyr14 and Thr15 of cdc2.¹⁵ The CDK and cyclin complexes are important in the regulation of cell cycle progression; cyclin B and cdc2 complexes can guide G2/M transition.¹⁶ In the present study, we found that the proportion of cells in the G2/M phase increased in a time- and dose-dependent manner, upon treatment with PP-26. In addition, western blotting analysis of cell cycle-related proteins showed that PP-26 treatment led to down-regulation of the expression levels of cyclin D1, cyclin B1, and CDK4; however, such treatment did not affect expression levels of cyclin E2 and cyclin B1. Moreover, the expression levels of Myt-1, p21, and p-cdc2 (Tyr15) were upregulated. It has been shown that the expression of p21 inhibits the activity of cyclin B/cdc2 complexes.¹⁶

The expression of Myt1 led to phosphorylation of Tyr15, which inhibited cdc2 activity and reduced the binding of the cyclin B-cdc2 complex. Thus, HepG2 cell cycle was arrested in the G2 phase.

Apoptosis is a process of cell death under pathological or normal physiological conditions, which occurs via extrinsic and intrinsic signaling pathways.^{17,18} In the present study, using annexin V-FITC/PI double staining, we found that the rate of apoptosis in HepG2 cells was positively correlated with PP-26 concentration, and that there was a typical apoptotic change in morphology in HepG2 cells. The mitochondrial apoptotic pathway is controlled by members of the Bcl-2 family and plays an important role in pro-apoptotic and anti-apoptotic

processes.^{19,20} We found that PARP, caspase-9, caspase-3, Mcl-1, Bcl-2, and Bcl-xL proteins were downregulated, while the pro-apoptotic protein, Bax, was upregulated in HepG2 cells. As a specific substrate of caspase-3, PARP is regarded as an indicator of caspase-3 activation and an important indicator of apoptosis.²¹⁻²⁴ The results of this study showed that PP-26 induced HepG2 cell apoptosis through the mitochondrial pathway.

The PI3K-Akt signaling pathway plays an important role in cell proliferation, cell cycle regulation, cell growth, and metabolism; moreover, it is closely related to the development of human tumors.²⁵ Yu et al.²⁶ found that curcumin induced apoptosis in SKOV3 cells via modulation of the

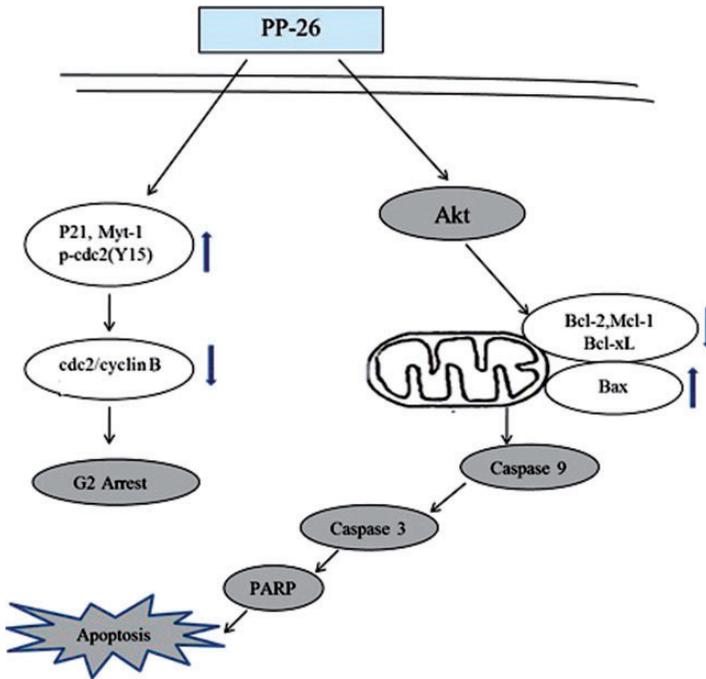


Figure 7. Schematic representation of the mechanism of PP26 induction of G2/M-phase arrest in HepG2 cells. The Myt1 protein kinase functions to negatively regulate Cdc2-cyclin B complexes by phosphorylating Cdc2. Akt-mediated GSK3β and FoxO3a signaling is involved in the apoptosis of cancer cells. Our study showed that PP-26 caused upregulation of p21 and Myt-1 expression, phosphorylation of cdc2 at Tyr15, and inhibition of the production of the cdc2/cyclin B complex. Inhibition of the Akt pathway is important in PP-26 mitochondrial-associated apoptosis in HepG2 cells

PI3K/Akt-signaling pathway. Additionally, Kawiak et al.²⁷ found that ramentaceone induced apoptosis in breast cancer cells through inhibition of PIK/Akt signaling. In the present study, western blotting analysis showed that PP-26 inhibited Akt kinase activity and blocked initiation of the downstream signaling pathway. The level of total Akt kinase remained unchanged, while the levels of p-Akt (Ser473) and p-Akt (Thr308) were reduced. Akt exerts its cellular functions through phosphorylation of multiple proteins. Because GSK-3 β and FOXO3a are downstream of Akt, decreased phosphorylation of these proteins implies suppression of Akt activity. In the present study, p-GSK-3 β and p-FOXO3a were downregulated when cells were treated with different concentrations of PP-26. Taken together, these results indicated that PP-26 could inhibit Akt kinase activity, block initiation of its downstream signaling pathway, and ultimately induce apoptosis. However, the role of Akt should be validated by overexpression of Akt in future studies.

Additionally, 5-FU is widely used for the treatment of hepatoma in the clinic.²⁸ However, 5-FU has limited efficacy, due to its inherent non-specificity, drug resistance, and toxicity. We hypothesized that PP-26 would enhance the sensitivity of HepG2 cells to 5-FU. Use of the MTT assay showed that the growth-inhibition rate of combined 5-FU plus PP-26 was increased, relative to that of 5-FU alone; furthermore, synergistic analysis revealed that the combination of PP-26 and 5-FU produced significant synergistic cytotoxicity when used for treatment of HepG2 cells (CDI < 1).

Conclusion

PP-26 inhibited proliferation and caused apoptosis in HepG2 cells; moreover, it exhibited weaker cytotoxicity in LO2 cells.

PP-26 treatment led to upregulation of p21 and Myt-1 expression, phosphorylation of cdc2 at Tyr15, and inhibition of the production of the cdc2/cyclin B complex. Notably, the Akt/FOXO3a/GSK-3 β signaling pathway was involved in this process. A schematic diagram summarizing our findings is shown in Figure 7.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This work was supported by grants from the Cultivation Fund of the First Affiliated Hospital of Jinan University (2014203).

ORCID iD

Zexuan Su  <http://orcid.org/0000-0002-0063-7680>

References

1. Siegel RL, Miller KD and Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* 2017; 67: 7–30.
2. Zhong JH, Wu FX and Li H. Hepatic resection associated with good survival for selected patients with multinodular hepatocellular carcinoma. *Tumour Biol* 2014; 35: 8355–8358.
3. Cao MR, Li Q, Liu ZL, et al. Harmine induces apoptosis in HepG2 cells via mitochondrial signaling pathway. *Hepatobiliary Pancreat Dis Int* 2011; 10: 599–604.
4. Zeng Y. Advances in mechanism and treatment strategy of cancer. *Cell Mol Biol (Noisy-le-grand)* 2018; 64: 1–3.
5. Long FY, Chen YS, Liang Z, et al. Pennogenyl saponins induce cell cycle arrest and apoptosis in human hepatocellular carcinoma HepG2 cells. *J Ethnopharmacol* 2015; 162: 112–120.
6. Ke JY, Zhang W, Gong RS, et al. A monomer purified from Paris polyphylla (PP-22) triggers S and G2/M phase arrest and apoptosis in human tongue squamous cell carcinoma SCC-15 by activating the p38/cdc25/

- cdc2 and caspase 8/caspase 3 pathways. *Tumour Biol* 2016; 37: 14863–14872.
7. Li Y, Wang Z, Kong D, et al. Regulation of Akt/FOXO3a/GSK-3beta/AR signaling network by isoflavone in prostate cancer cells. *J Biol Chem* 2008; 283: 27707–27716.
 8. Dey G, Bharti R, Dhanarajan G, et al. Marine lipopeptide Iturin A inhibits Akt mediated GSK3beta and FoxO3a signaling and triggers apoptosis in breast cancer. *Sci Rep* 2015; 5: 10316.
 9. Liu F, Rothblum-Oviatt C, Ryan CE, et al. Overproduction of human Myt1 kinase induces a G2 cell cycle delay by interfering with the intracellular trafficking of Cdc2-cyclin B1 complexes. *Mol Cell Biol* 1999; 19: 5113–5123.
 10. Zeng Y, Liu X, Yan Z, et al. Sphingosine 1-phosphate regulates proliferation, cell cycle and apoptosis of hepatocellular carcinoma cells via syndecan-1. *Prog Biophys Mol Biol* 2017. <https://doi.org/10.1016/j.pbiomolbio.2017.11.006>
 11. Zeng Y, Yao X, Chen L, et al. Sphingosine-1-phosphate induced epithelial-mesenchymal transition of hepatocellular carcinoma via an MMP-7/syndecan-1/TGF-beta autocrine loop. *Oncotarget* 2016; 7: 63324–63337.
 12. Chen YS, He Y, Chen C, et al. Growth inhibition by pennogenyl saponins from *Rhizoma paridis* on hepatoma xenografts in nude mice. *Steroids* 2014; 83: 39–44.
 13. Qin XJ, Yu MY, Ni W, et al. Steroidal saponins from stems and leaves of *Paris polyphylla* var. *yunnanensis*. *Phytochemistry* 2016; 121: 20–29.
 14. Wu X, Wang L, Wang GC, et al. Triterpenoid saponins from rhizomes of *Paris polyphylla* var. *yunnanensis*. *Carbohydr Res* 2013; 368: 1–7.
 15. Mueller PR, Coleman TR, Kumagai A, et al. Myt1: a membrane-associated inhibitory kinase that phosphorylates Cdc2 on both threonine-14 and tyrosine-15. *Science* 1995; 270: 86–90.
 16. Sanchez I and Dynlacht BD. New insights into cyclins, CDKs, and cell cycle control. *Semin Cell Dev Biol* 2005; 16: 311–321.
 17. Portt L, Norman G, Clapp C, et al. Anti-apoptosis and cell survival: a review. *Biochim Biophys Acta* 2011; 1813: 238–259.
 18. Mohammad RM, Muqbil I, Lowe L, et al. Broad targeting of resistance to apoptosis in cancer. *Semin Cancer Biol* 2015; 35: S78–S103.
 19. Czabotar PE, Lessene G, Strasser A, et al. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nat Rev Mol Cell Biol* 2014; 15: 49–63.
 20. Nikolettou V, Markaki M, Palikaras K, et al. Crosstalk between apoptosis, necrosis and autophagy. *Biochim Biophys Acta* 2013; 1833: 3448–3459.
 21. Benafif S and Hall M. An update on PARP inhibitors for the treatment of cancer. *Onco Targets Ther* 2015; 8: 519–528.
 22. Hou ZY, Tong XP, Peng YB, et al. Broad targeting of triptolide to resistance and sensitization for cancer therapy. *Biomed Pharmacother* 2018; 104: 771–780.
 23. Timucin AC, Basaga H and Kutuk O. Selective targeting of antiapoptotic BCL-2 proteins in cancer. *Med Res Rev* 2018; 39: 146–175.
 24. Wang M, Law ME, Castellano RK, et al. The unfolded protein response as a target for anticancer therapeutics. *Crit Rev Oncol Hematol* 2018; 127: 66–79.
 25. Butt G, Attar R, Tabassum S, et al. Regulation of signal transduction cascades by Pterostilbenes in different cancers: is it a death knell for oncogenic pathways? *Cell Mol Biol (Noisy-le-grand)* 2017; 63: 5–10.
 26. Yu Z, Wan Y, Liu Y, et al. Curcumin induced apoptosis via PI3K/Akt-signalling pathways in SKOV3 cells. *Pharm Biol* 2016; 54: 2026–2032.
 27. Kawiak A and Lojkowska E. Ramentaceone, a naphthoquinone derived from *Drosera* sp., induces apoptosis by suppressing PI3K/Akt signaling in breast cancer cells. *PLoS One* 2016; 11: e0147718.
 28. Tournoux C, Paoletti X and Barbare JC. Treatment outcomes for hepatocellular carcinoma using chemoembolization in combination with other therapies. *Cancer Treat Rev* 2007; 33: 762–763.