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Dihydrotanshinone Enhances The Anticancer Effects of Sorafenib on Hepatocellular Carcinoma by Inhibiting Akt Signaling Pathway Activation*

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Abstract **Objective** Sorafenib is a first-line only drug approved for the treatment of advanced hepatocellular carcinoma (HCC). Resistance to sorafenib means that treatment outcomes are often unsatisfactory. Although the mechanism underlying sorafenib resistance remains unclear, resistance may occur through Akt signaling pathway activation in HCC. Dihydrotanshinone (DHT), a lipophilic component of traditional Chinese medicine *Salvia miltiorrhiza* Bunge, has multiple anti-tumor activities and inhibits Akt activation. The effect and mechanism of DHT combined with sorafenib on HCC have not been investigated. In this study, we investigate whether DHT potentiates the anti-cancer activities of sorafenib against HCC. **Methods** In this study, the effects of sorafenib and DHT on the viability, apoptosis and drug sensitivity of Huh7 and HepG2 cells were verified by Cell Counting Kit-8 (CCK-8) and flow cytometry. Akt, P-Akt, Caspase3, GSK-3β, P-GSK-3β, ribosomal protein S6 kinase (S6K), P-S6K, Cyclin D1, Bcl-xl, Bcl-2, and Bax expression levels were analyzed *via* Western blot. All data were statistically compared using one-way analysis of variance (ANOVA) and Dunnett test. Statistical analysis using SPSS 20.0 statistical software. **Results** DHT inhibit proliferation and promote apoptosis in HCC cells by reducing Akt activation. DHT inhibits the expression and activation of Akt downstream factors, including GSK-3β and S6K, which regulate the apoptotic response and are activated and upregulated by sorafenib treatment. Both sorafenib and DHT downregulate cyclin D1 expression and DHT upregulates Bax expression and downregulates Bcl-2 and Bcl-xl expression. However, sorafenib had little influence on Bcl-2 family protein expression. **Conclusion** DHT may enhance the proliferation inhibition and apoptosis induction of sorafenib in HCC cells by inhibiting the activation of Akt signaling pathway, thus enhancing the anticancer effect of sorafenib.

Key words dihydrotanshinone, sorafenib, Akt, hepatocellular carcinoma, apoptosis
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Hepatocellular carcinoma (HCC) is the most common primary malignant liver tumor, the sixth most common cancer, and the third leading cause of cancer-related death world-wide, and the second highest cancer related mortality rate globally[1-2]. The incidence rate of HCC is increasing, and is correlated with increasing age, reaching a peak at an average age of 70[3]. More than 700 000 people die of HCC each year[4]. Although surgery is the only way to cure HCC, most patients diagnosed with HCC are in an advanced stage, and the prognosis is not optimistic. There remains a need for the development of effective HCC treatments[4].

Sorafenib, an oral multi-kinase inhibitor, is the only drug approved for first-line systemic treatment of

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advanced HCC. However, increasing drug resistance has led to limited clinical response and limits the therapeutic effect of sorafenib[5]. Sorafenib suppresses tumor cell proliferation mostly through interruption of the Ras/Raf/MEK/ERK signaling cascade[6]. Acquired sorafenib resistance has been attributed to activation of the PI3K/Akt pathway[7]. The interaction between ERK and PI3K/Akt indicates that there is a relationship between the two in cancer progression[8]. Some research suggests that there are high levels of PI3K/Akt pathway activation in most sorafenib-resistant HCCs, and that inhibiting Akt activity could reverse sorafenib resistance[9]. In HCC cells, sorafenib can activate Akt and its downstream factors, including ribosomal protein S6 kinase (S6K) [10]. PI3K/Akt pathway blockade enhances sorafenib efficacy in the treatment of HCC[10-11]. These results suggest that Akt inhibition may enhance the anticancer activity of sorafenib against HCC.

Dihydrotanshinone (DHT) is one of the main components of *Salvia miltiorrhiza* Bunge. A large number of diterpenoid tanshinones have been extracted from *Salvia miltiorrhiza*, including dihydrotanshinone I (DHT), cryptotanshinone I, and tanshinone I, IIA. Compared with other *Salvia miltiorrhiza* components, DHT has higher cytotoxicity[12-13]. DHT can play an anti-tumor role by inhibiting proliferation, inducing differentiation, and promoting apoptosis in a variety of tumor cell lines including gastric cancer, colorectal cancer, and central nervous system tumors[12, 14-17]. DHT can also regulate Akt expression to influence cell proliferation and apoptosis[18-20]. Therefore, we studied the mechanism of DHT combined with sorafenib in HCC treatment.

There are several mechanisms of sorafenib resistance in hepatocellular carcinoma, including up-regulation of epidermal growth factor receptor (EGFR) expression of hepatocellular carcinoma itself and abnormal changes of its downstream signaling pathway, overexpression of silent message regulatory factor 1, enhancement of autophagy capacity of hepatocellular carcinoma cells and mesenchymal transformation. At present, a number of experiments have combined the targeted therapeutic drugs that inhibit the sorafenib resistance pathway with sorafenib in the treatment of HCC, and the efficacy is better than that of sorafenib alone. Combination therapy is a strategy to improve adverse reactions, overcome drug resistance, and improve the condition efficacy. Given that the current efficacy of sorafenib monotherapy in the treatment of hepatocellular carcinoma is relatively limited, and the therapeutic effect on advanced HCC patients is not even satisfactory, we urgently need to explore effective drugs that can be combined with sorafenib for hepatocellular carcinoma.

1 Materials and methods

1.1 Cell culture， antibodies， and reagents

The human Huh7 HCC cell line was obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), and the HepG2 cell line was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco BRL; Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) (ExCellBio, Shanghai, China). Cells were incubated in a standard cell culture incubator in 5% CO_2 and humidified air at 37℃ . Sorafenib was purchased from Jinan Trio Pharmatech Co., Ltd. (Jinan, China), dissolved in dimethyl sulfoxide, and stored at −20℃ . Perifosine (Akt inhibitor) were purchased from Jinan Trio Pharmatech Co., Ltd. (Jinan, China), and it was prepared in dimethyl sulfoxide as a 10 mmol/L stock solution. After cell attachment, cells were treated with perifosine (at a final concentration of 10 μmol/L) in complete medium for 48 h and the treated cells were harvested. DHT was purchased from Yida Pharmaceutical, Harbin Medical University, China. The Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories (Mashikimachi, Japan). The Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit was obtained from BD Biosciences. β-Actin, P-Akt (Ser473), Akt, Caspase3, Pro-Caspase3, GSK-3β, P-GSK-3β, S6K, P-S6K, CyclinD1, Bcl-2, Bcl-xl, and Bax antibodies were purchased from Cell Signaling Technology (Danvers, USA).

1.2 Methods

1.2.1 Cell proliferation assay

Cell proliferation was measured using the CCK-8 assay. HepG2 and Huh7 cells at logarithmic growth phase were seeded into 96-well plates at

concentrations of 3 000 cells/well and treated with sorafenib (0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, and 20 μmol/L) and DHT (0, 0.125, 0.25, 0.5, 1, 2, 4, and 8 μmol/L). Huh7 cells were incubated with sorafenib (10 μmol/L) and DHT (5 μmol/L) or combination with two drugs. After 48 h, 10 μl CCK-8 solution was added to each well and the mixture was incubated for 4 h at 37°C. Cell viability was measured every hour. The optical density was measured at 450 nm with a reference of 600 nm. The 50% inhibitory concentrations (IC_{50}) values were calculated from the concentration-response curves. The effects of sorafenib, DHT, perifosine, sorafenib combined with DHT, and sorafenib combined with perifosine on cell viability were determined based on IC_{50} value. All experiments above were repeated at least three times independently.

1.2.2 Apoptosis assay

Apoptosis was examined using the Annexin V-FITC Apoptosis Detection Kit (BD Biosciences). Cells were seeded (5×10^4 cells per well) in a 6-well culture plate and pretreated with the indicated drugs. HepG2 and Huh7 cells were treated with a concentration gradient of sorafenib (0, 2.5, 5, and 10 μmol/L), DHT (0, 1, 2, and 4 μmol/L) and Huh7 cells were treated with sorafenib (5 μmol/L), DHT (2.5 μmol/L), and sorafenib (5 μmol/L) combined with DHT (2.5 μmol/L) for 48 h. After 48 h of culture, cells were washed twice with phosphate-buffered saline PBS, treated with trypsin, and collected by centrifugation at 1 000 r/min for 5 min and resuspended in Annexin V-FITC binding buffer, followed by incubation with 5 μl Annexin V-FITC and 5 μl PI for 15 min in the dark. The apoptosis rate (%) in each group was detected using a Beckman Coulter Epics Altra II cytometer (Beckman Coulter; California, USA). Experiments were performed in triplicate, and the average results were calculated.

1.2.3 Western blot

Cells were washed with PBS and proteins extracted by lysing on ice with RIPA buffer (Beyotime Biotechnology; Beijing, China) containing proteinase inhibitors and phosphatase inhibitors. Supernatants were collected and the protein assay kit (Bio-Rad; Richmond, CA, USA) was used to determine protein concentration. Samples were resolved on a 10% sodium dodecyl sulfate (SDS)-acrylamide gradient gel and transferred onto polyvinylidene difluoride (PVDF) membranes (US Millipore). After transfer, the PVDF membranes were washed three times with Tris-buffered saline with 0.1% Tween 20 (TBST) and blocked in 5% skim milk in TBST for 2 h at room temperature. Membranes were washed in TBST and incubated with primary antibodies (Akt, P-Akt, Caspase3, GSK-3β, P-GSK-3β, S6K, P-S6K, Cyclin D1, Bcl-xl, Bcl-2, and Bax) overnight at 4°C. After washing four times (5 min each) with TBST, membranes were incubated with secondary antibodies for 2 h at room temperature and washed four times (5 min each) with TBST. Protein bands were visualized using an enhanced chemiluminescence plus detection reagent (Pierce Chemical; Rockford, IL, USA) and recorded using a Molecular Imager ChemiDoc™ XRS+Imaging System (Bio-Rad, USA). Images were quantified by Image Lab™ software. β-Actin was used as the loading control. All experiments were repeated three times.

1.3 Statistical analysis

All data are presented as mean values±standard deviations and each assay was performed at least three independent times. Statistical comparisons were made by one-way analysis of variance (ANOVA) followed by Dunnett test. Statistical analyses were performed using the SPSS 20.0 statistical software (SPSS 224 Inc., IL, USA). $P<0.05$ was considered statistically significant.

2 Results

2.1 Sorafenib increases Akt activation， reduces viability， and induces apoptosis in HCC cells

Huh7 and HepG2 cells were incubated with a sorafenib concentration gradient for 48 h and cell viability was determined using the CCK-8 assay. The IC_{50} were 12.8 μmol/L and 13.2 μmol/L respectively. Consistent with results previously reported[21-23], we observed a dose-dependent inhibition of cell viability and increased apoptosis (Figure 1a, b). Western blot revealed that sorafenib treatment up-regulated P-Akt expression and downregulated Pro-caspase3 expression in a dose-dependent manner, but Akt expression was not affected (Figure 1c).

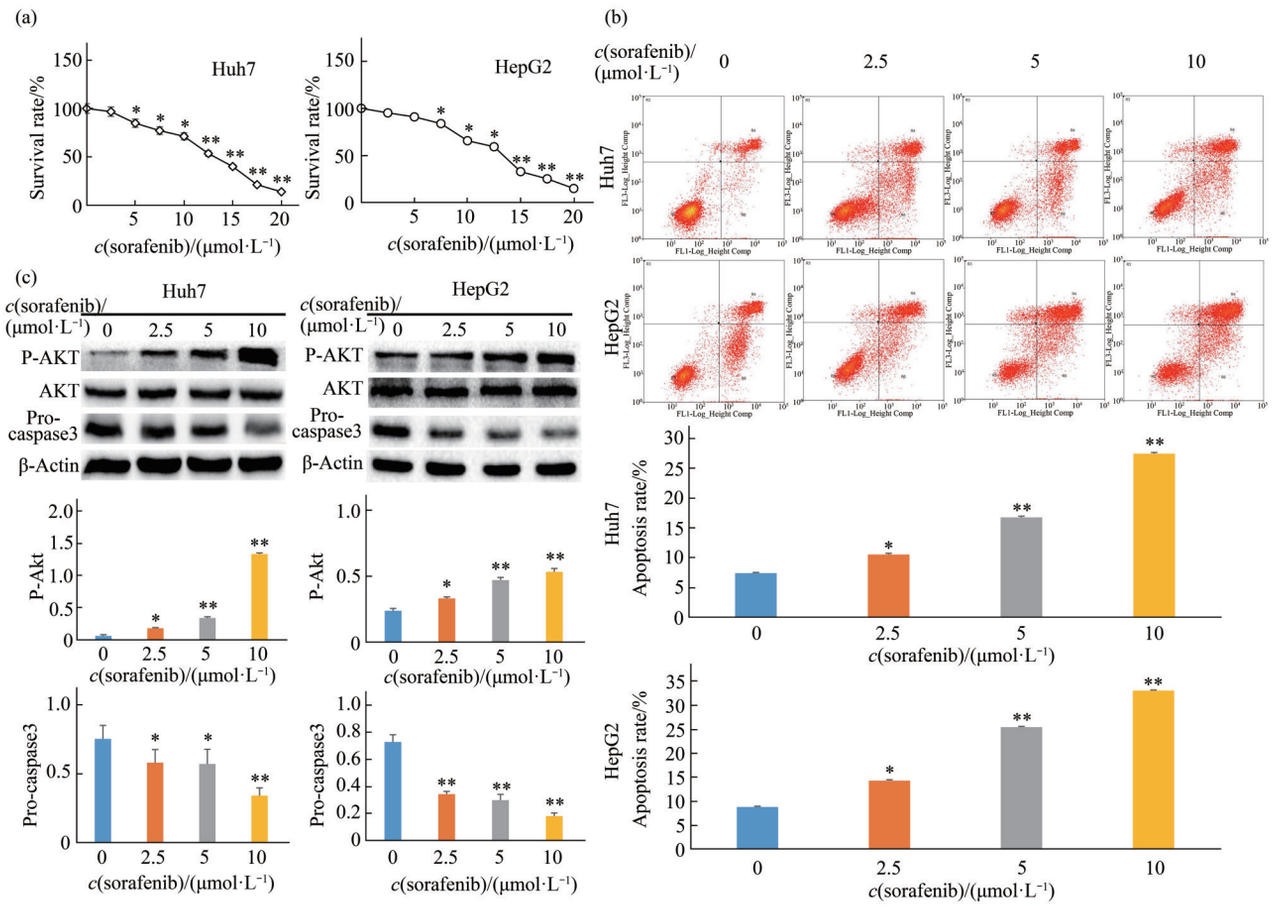

Fig. 1　Sorafenib inhibits the proliferation, induces the apoptosis, and increases Akt activation of HCC cells

(a) HepG2 and Huh7 cells were incubated with sorafenib at serial of concentrations (2.5, 5, 7.5, 10, 12.5, 15, 17.5, or 20 μmol/L) for 48 h. Cell viability was assessed and the survival rate (in percent) was calculated. (b) The above cells incubated with sorafenib at concentrations of 0, 2.5, 5 or 10 μmol/L were subjected to flow cytometry for analyzing apoptosis rates. (c) The lysates of cells were subjected to Western blot. **P*<0.05 and ***P*< 0.01 indicate a significant difference from vehicle-treated cells.

2.2　Inhibition of Akt enhances the effects of sorafenib against HCC cells

To explore whether inhibition of Akt expression strengthened the anticancer activity of sorafenib, we used perifosine, a specific Akt inhibitor. Cell viability was significantly reduced in Huh7 cells treated with perifosine and sorafenib for 48 h. Combination treatment with sorafenib and perifosine had a more significant effect on growth inhibition than did treatment with either drug alone (Figure 2a). The value for the coefficient of drug interaction (CDI) was calculated to be 0.53, indicating a synergistic effect of sorafenib and perifosine in reducing cell viability. Sorafenib treatment increased P-Akt expression and perifosine treatment decreased P-Akt expression. Treatment with both sorafenib and perifosine down-regulated the expression of Pro-caspase3 and had no effect on Akt expression. Furthermore, perifosine reduced the increase of Akt expression induced by sorafenib, and their combination further reduced the expression of Pro-caspase3 (Figure 2b).

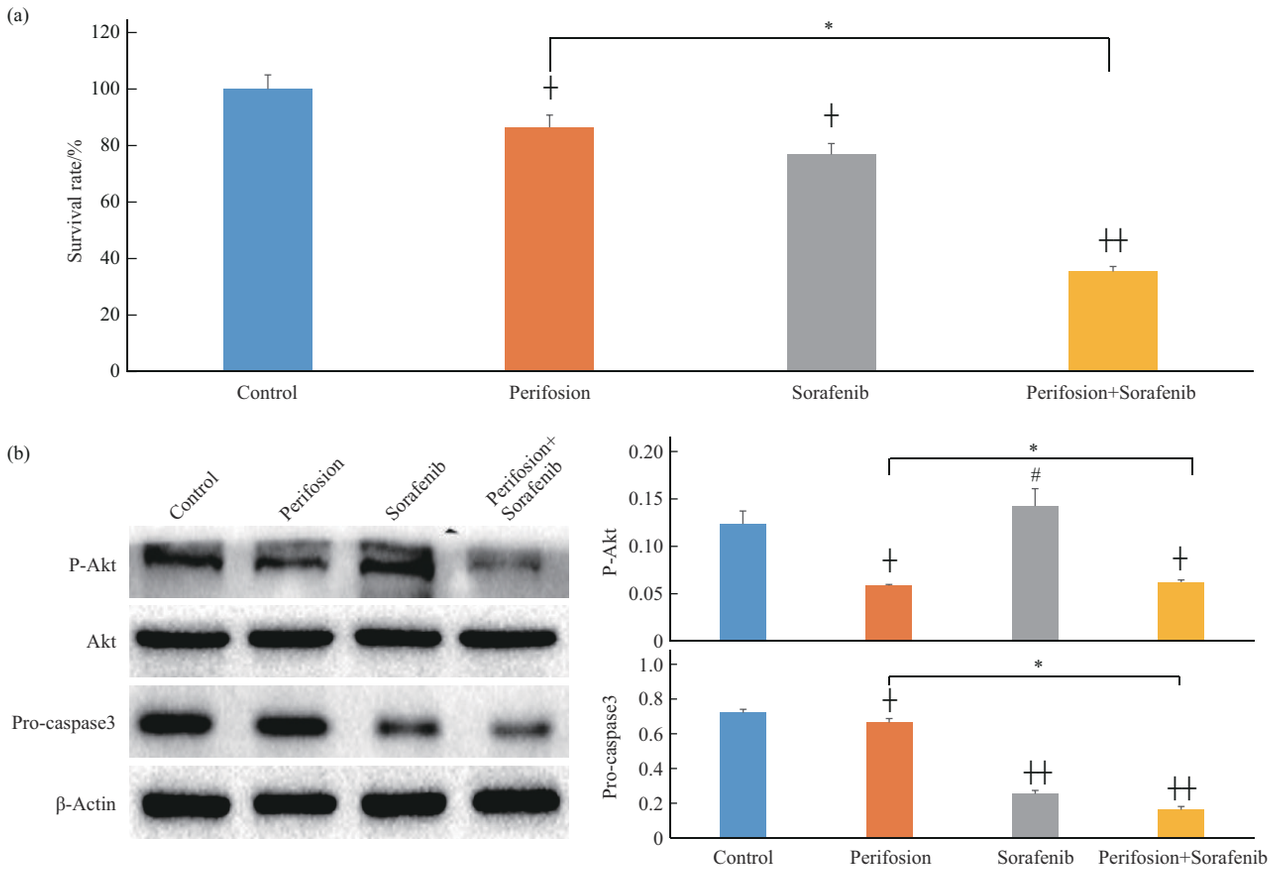

Fig. 2　Inhibition of Akt enhances the sensitivity of HCC cells to sorafenib

(a) Huh7 cells were incubated for 48 h with sorafenib (5 μmol/L), perifosine (10 μmol/L), or the combination the two drugs. Cell viability (in percent) was assessed and normalized to control group. (b) The cells were subjected to Western blot. **P*<0.05 indicate a significant difference. ┼*P*<0.05 and ┼┼*P*<0.01 indicate a significant reduction and #*P*<0.05 a significant increase from controls.

2.3　DHT reduces HCC cell viability， induces apoptosis， and inhibits Akt activation

Cell viability and apoptosis were detected in Huh7 and HepG2 cells after treatment with a DHT concentration gradient for 48 h. The results show that DHT reduced cell viability (Figure 3a). The 50% inhibitory concentrations (IC_{50}) were 2.8 μmol/L and 1.9 μmol/L respectively. DHT promote apoptosis of Huh7 and HepG2 cells in a concentration-dependent manner (Figure 3b). Western blot analyses show DHT significantly inhibits P-Akt and Pro-caspase3 expression in a concentration-dependent manner, but has little effect on Akt expression (Figure 3c).

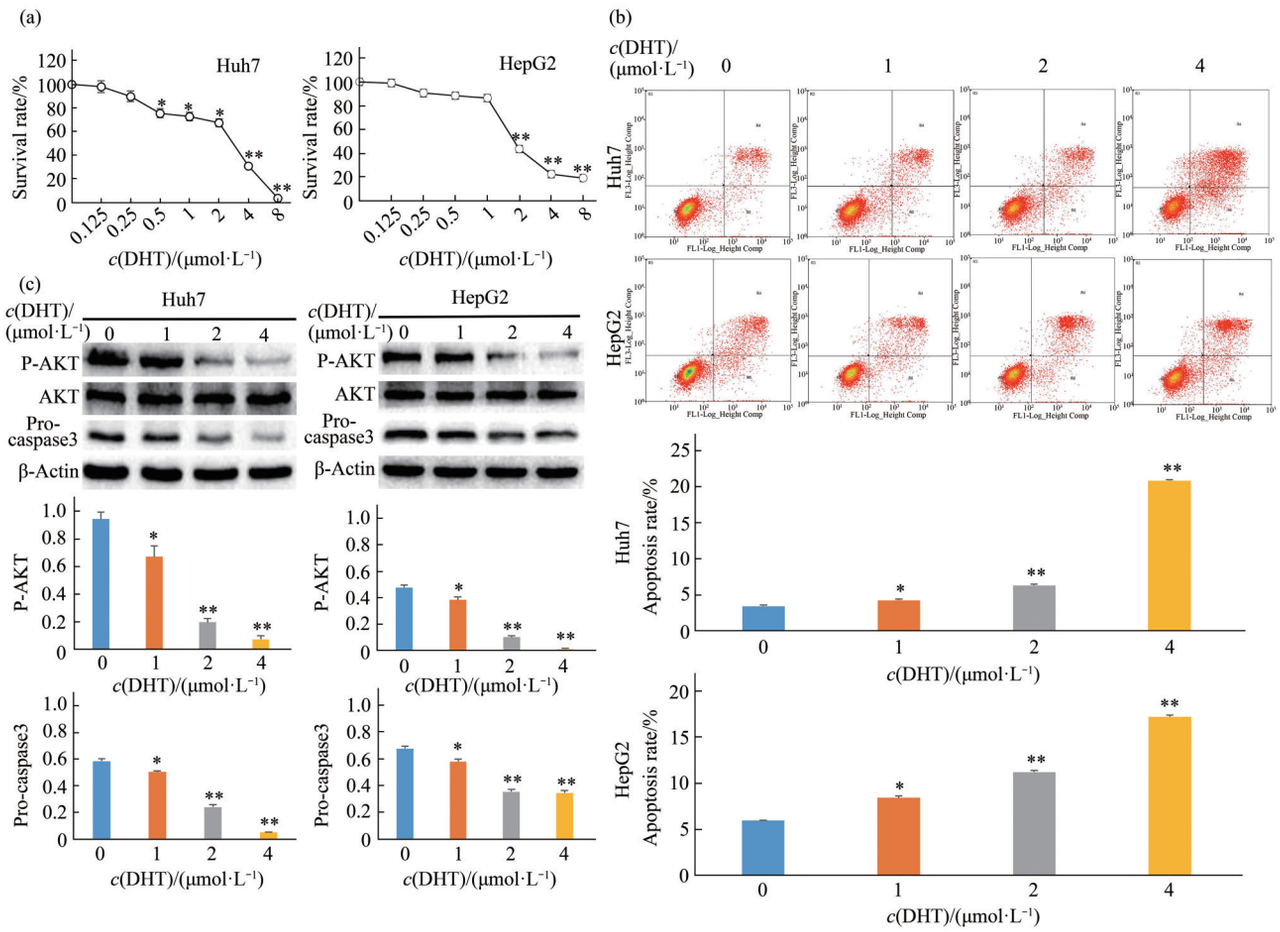

Fig. 3　DHT reduces the viability, induces the apoptosis of HCC cells and inhibits Akt activation

(a) HepG2 and Huh7 cells were incubated with DHT at serial of concentrations (0.125, 0.25, 0.5, 1, 2, 4, or 8 μmol/L) for 48 h. Cell viability was assessed and the inhibitory rate was calculated. (b) The above cells incubated with vehicle or DHT at concentrations of 1, 2, or 4 μmol/L were subjected to flow cytometry for analyzing apoptosis rates. (c) The lysates of cells were subjected to Western blot. **P*<0.05 and ***P*<0.01 indicate a significant difference from vehicle-treated cells.

2.4　DHT synergizes with sorafenib to inhibit proliferation and induce apoptosis in HCC cells

Our results showed that sorafenib and DHT treatment alone significantly inhibited Huh7 and HepG2 cell proliferation and promoted apoptosis. Next, we explored the effects of sorafenib and DHT in combination. Huh7 cells were treated with sorafenib (5 μmol/L), DHT (2 μmol/L), and sorafenib combined with DHT for 24, 48, or 72 h. The results showed that cell viability gradually decreased in a time-dependent manner and that combined sorafenib and DHT treatment had a greater effect on cell viability and apoptosis than did either treatment alone (Figure 4a, b). The value of CDI was calculated to be 0.65, 0.61, and 0.79, when cells were incubated for 24, 48, or 72 h, respectively, indicating their inhibitory effects were synergistic. The value of CDI was 0.16, indicating that DHT synergized with sorafenib to induce the apoptosis of HCC cells. Western blot analysis revealed that combined sorafenib and DHT treatment resulted in down-regulation of Pro-caspase3 expression. Examination of Bcl-2 family protein expression, including anti-apoptosis proteins Bcl-2 and Bcl-xl, and the Bax apoptosis protein, revealed that treatment with DHT alone or in combination with sorafenib downregulated Bcl-2 and Bcl-xl expression and upregulated Bax expression. However, treatment with sorafenib alone had little effect on Bcl-2, Bcl-xl, and Bax expression (Figure 4c). These results indicate that a synergistic interaction between sorafenib and DHT has a significant effect on proliferation and apoptosis in Huh7 cells.

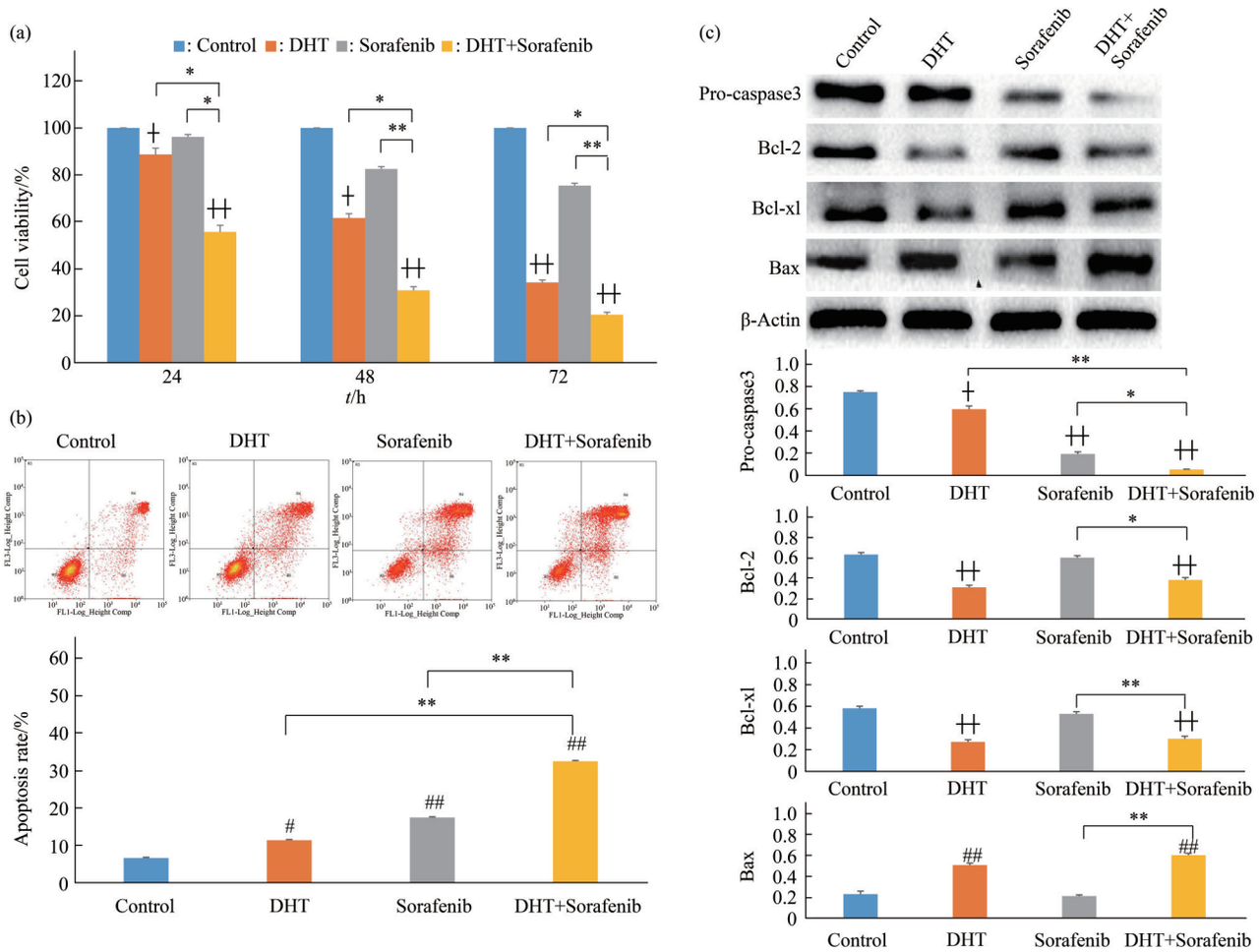

Fig. 4　DHT synergizes with sorafenib to inhibit the proliferation of HCC cells and induces the apoptosis of HCC cells

(a) Cell viability (in percent) was assessed and normalized to control cells. (b) Apoptosis rates (in percent) were plotted. (c) Cell lysates were immunoblotted to detect the expression of Pro-caspase-3, Bcl2, Bcl-xl, and Bax. **P*<0.05 and ***P*<0.01 indicate a significant difference. ┼*P*<0.05 and ┼┼*P*<0.01 indicate a significant reduction and #*P*<0.05 and ##*P*<0.01 a significant increase from controls.

2.5　DHT synergizes with sorafenib to inhibit HCC cell proliferation by regulating the Akt pathway

To investigate the mechanisms involved in the suppression of cell growth observed following combination DHT and sorafenib treatment, we examined the expression of key proteins that function downstream of the PI3K/Akt signaling pathway. Huh7 cells were treated with the indicated concentrations of sorafenib (5 μmol/L) and DHT (2 μmol/L) alone and in combination for an additional 48 h. Western blot analysis show that DHT dramatically decreased the expression of Akt, GSK-3β, and S6K phosphorylation, increased Akt phosphorylation, and decreased the expression of GSK-3β and S6K phosphorylation induced by sorafenib (Figure 5). Cyclin D1 levels decreased after DHT or sorafenib treatment, and this decrease was more evident following combination treatment (Figure 5). Together, these data indicate that sorafenib combined with DHT may induce HCC cell proliferation through the PI3K/Akt signaling pathway.

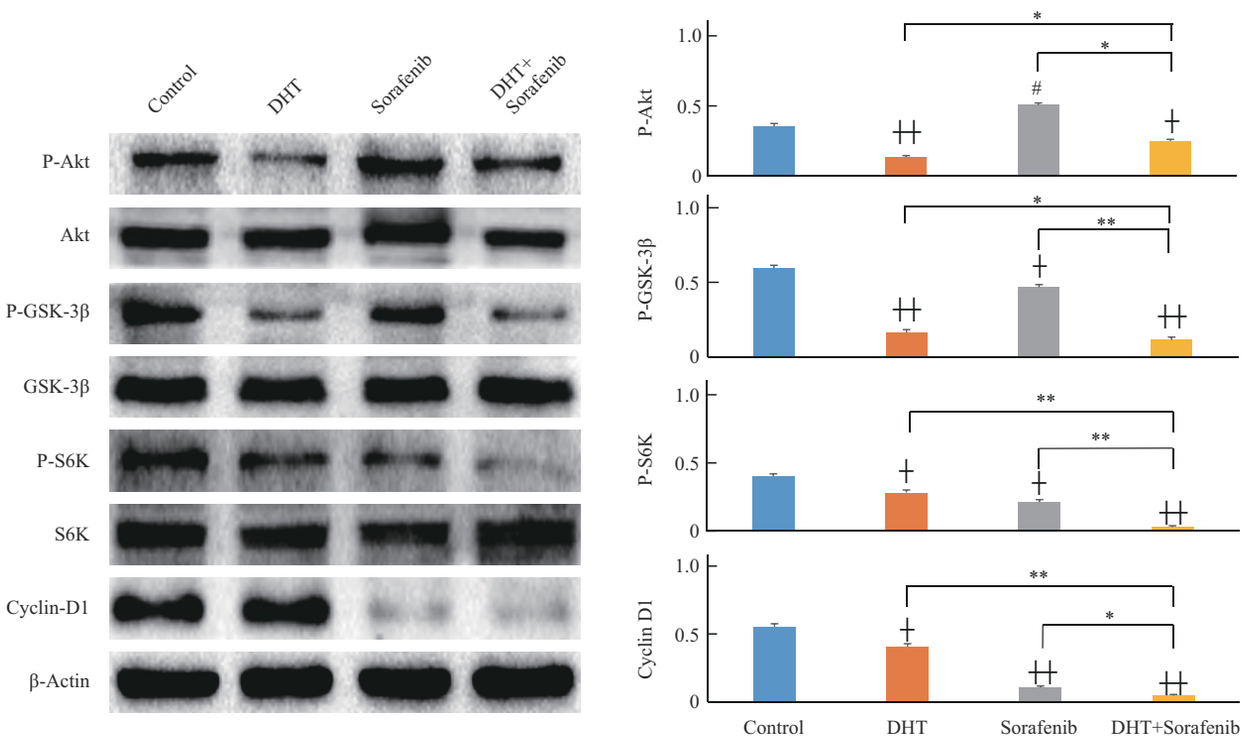

Fig. 5　DHT synergizes with sorafenib to inhibit the proliferation of HCC cells by regulating the Akt pathway

Huh7 cells were incubated with vehicle (control), sorafenib (5 μmol/L), DHT (3 μmol/L) or the combination of the two drugs for 48 h, and the cells were subjected to Western blot. **P*<0.05 and ***P*<0.01 indicate a significant difference. ┼*P*<0.05 and ┼┼*P*<0.01 indicate a significant reduction and #*P*<0.05 a significant increase from controls.

3　Discussion

Previous studies have shown that sorafenib, a multi-RTK inhibitor used as first-line treatment in patients with advanced HCC, represses HCC progression with clear survival benefits[24-26]. However, in patients with advanced HCC, sorafenib treatment only extends survival by 3 months[27-28]. Promisingly, combination therapies have shown increased efficacy[29-32]. Our results show that sorafenib inhibits proliferation and promotes apoptosis in Huh7 and HepG2 cells in a dose-dependent manner, which is consistent with previous findings[21, 33]. Our results show that co-treatment with sorafenib and DHT enhances sorafenib-induced suppression of cell viability and apoptosis induction in HCC cells. The PI3K/Akt signaling pathway is highly complex[34] and is considered an important regulator in HCC[35]. PI3K/Akt signaling plays a key role in hepatocarcinogenesis and affects various signaling and biological processes[36-39]. Activation of Akt signaling induces acquired resistance to sorafenib in HCC cells[40]. Multiple studies have established that PI3K/Akt signaling pathway inhibition has strong anti-cancer effects against HCC[41-43]. However, our results suggest that DHT can strengthen sorafenib anti-hepatocarcinoma activity by suppressing Akt activation, and are consistent with those of previous studies. Currently, the mechanism of DHT-mediated Akt inhibition is not clear. Akt is a proto-oncogene that mediates cell survival and growth signals by phosphorylating GSK-3β and regulating expression of mTOR, which inactivates pro-apoptotic proteins[44-45]. Therefore, we used perifosine, a specific Akt inhibitor, to inhibit Akt expression and downstream factors to drive HCC cells towards apoptosis. Perifosine treatment inhibited Pro-caspase3 expression and combined perifosine and sorafenib treatment had a significantly greater inhibitory effect[8]. Bcl-2 family proteins are critical regulators of apoptosis and can affect tumor metastasis through regulation of the mitochondrial apoptotic pathway[46-47]. Bcl-2 family members include the pro-apoptosis protein Bax, a

central mediator of apoptosis, and anti-apoptotic proteins Bcl-xl and Bcl-2, crucial controllers of the mitochondrial pathway of pancreatic β-cell apoptosis induced by lipotoxicity[48-51]. At present, how sorafenib functions to influence the Bcl-2 family is not clear. Our results show that sorafenib treatment has little effect on Bcl-2, Bcl-xl, and Bax protein expression. These results are supported by those previously reported[52-55]. Our results show that DHT treatment decreases Bcl-2 and Bcl-xL expression and increased Bax expression. However, combination treatment with DHT and sorafenib inhibits Bcl-2 and Bcl-xl expression and increases Bax expression. Caspase3 is a downstream effector of Caspase9 and a molecule in apoptosis. Caspase3 cleaves key structural proteins leading to DNA fragmentation and membrane blebbing, and the interaction between antiapoptotic Bcl-2 and pro-apoptotic Bax proteins plays a key role in Caspase3 activation[56-58]. During apoptotic cell death, Pro-caspase3, the Caspase3 precursor, is activated by exogenous (death ligand cascade involving Caspase8) and endogenous (involving a mitochondrial cascade of Caspase9) factors [59]. Our results show that treatment with both sorafenib and DHT reduced Pro-caspase3 expression and that combination treatment with sorafenib and DHT had a more significant effect than did either drug alone. The downstream factors GSK-3β and S6K of the Akt signaling pathway can regulate cell apoptosis response, and our research results indicate that DHT can inhibit GSK-3β And S6K expression, but activated and upregulated by sorafenib treatment. The cyclin D1 proto-oncogene plays a key role in regulating the mitotic cell cycle, and cyclin D1 overexpression is frequently observed in carcinogenesis[60-62]. Our results show that treatment with sorafenib and DHT alone downregulate Cyclin D1 expression, and that that combination treatment with sorafenib and DHT of sorafenib and DHT had a more significant effect than did either drug alone. The proposed mechanism by which DHT synergizes with sorafenib to suppress the growth and induce the apoptosis of HCC cells is summarized (Figure 6). Sorafenib induces the activation of Akt, which contributes to the resistance of HCC to sorafenib[10- 11]. DHT blocks Akt activation, there by inhibiting the expression or phosphorylation of Akt downstream factors, such as GSK-3β And S6K[18-20]. DHT down-regulates the expression of Bcl-2 and Bcl-xL, and up-regulates the expression of Bax, which leads to the activation of Caspase-3 and the process of sequential apoptosis. Both DHT and sorafenib downregulated cyclin D1 expression, resulting in cell cycle arrest and inhibition of proliferation. The field of HCC therapy is long awaited. Combining certain drugs with sorafenib, and making an informed choice among them, will be the next challenge. Related studies are needed to better elucidate resistance pathways and inform future treatment strategies. As a traditional Chinese medicine with anti-tumor effects, DHT can enhance the therapeutic effect of sorafenib on hepatocellular carcinoma by regulating the Akt signaling pathway, which may provide us with a revelation or evidence to prove that combined

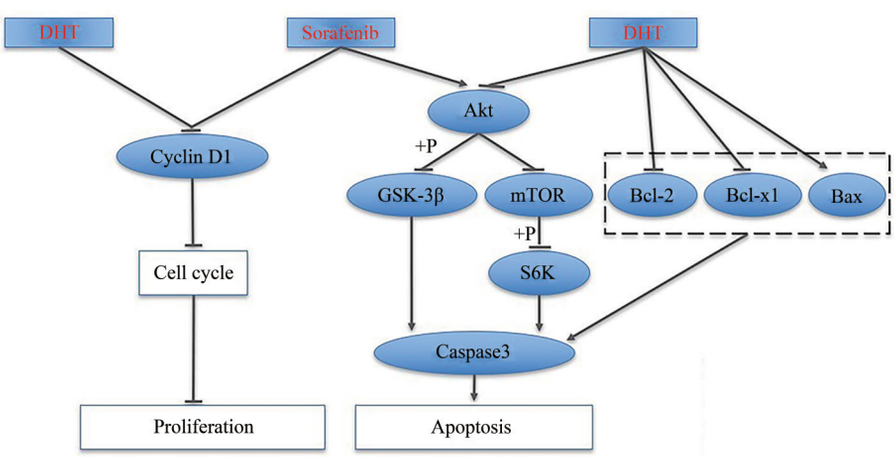

Fig. 6 Proposed mechanisms by which DHT synergizes with sorafenib to execute antitumor activity

"→", positive regulation or activation; "⊥", negative regulation or blockade; +P, regulation by phosphorylation. Abbreviations: Akt/PKB, protein kinase B; DHT, dihydrotanshinone; GSK-3β, glycogen synthase kinase 3β; mTOR, mammalian target of rapamycin; S6K, ribosomal protein S6 kinase.

administration is a therapeutic strategy for hepatocellular carcinoma at this stage.

4 Conclusion

In conclusion, DHT enhances sorafenib-induced proliferation suppression and apoptosis induction in HCC cells. This may occur through DHT-mediated inhibition of the Akt signaling pathway activation enhancing the anti-cancer effect of sorafenib. The combination of sorafenib and DHT may be an effective strategy for HCC treatment. However, further *in vivo* experiments and clinical studies are needed to confirm the findings presented here.

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二氢丹参酮通过抑制Akt信号通路激活来增强索拉非尼对肝细胞癌的抗癌作用*

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摘要　目的　索拉非尼是唯一被批准用于治疗晚期肝细胞癌（hepatocellular carcinoma，HCC）的一线药物。然而索拉非尼的耐药性使得治疗效果并不理想。尽管索拉非尼耐药性的机制尚不清楚，但在HCC中的耐药性可能通过Akt信号通路的激活而发生。二氢丹参酮（dihydrotanshinone，DHT）是中药丹参的亲脂性成分，具有多种抗肿瘤活性并可抑制Akt活化。DHT联合索拉非尼治疗HCC的作用机制尚未明确。本文旨在研究DHT是否可增强索拉非尼对HCC的抗癌活性。**方法**　采用细胞计数试剂盒8（cell counting kit-8，CCK-8）和流式细胞仪检测索拉非尼和DHT对HCC细胞Huh7和HepG2细胞活力、细胞凋亡和药物敏感性的影响。通过蛋白质印迹分析Akt、P-Akt、Caspase3、GSK-3β、P-GSK3-β、核糖体蛋白S6激酶（S6K）、P-S6K、细胞周期蛋白D1、Bcl-xl、Bcl-2和Bax的表达水平。使用单因素方差分析（analysis of variance，ANOVA）和Dunnett检验对所有数据进行统计学比较。采用SPSS 20.0统计软件进行统计分析。**结果**　DHT通过减少Akt的激活来抑制HCC细胞的增殖和促进细胞凋亡。DHT抑制Akt下游因子的表达和激活，包括GSK-3β和S6K，这些因子调节细胞凋亡反应，并被索拉非尼激活和上调。索拉非尼和DHT均下调细胞周期蛋白D1的表达，DHT上调Bax的表达并下调Bcl-2和Bcl-xl的表达。索拉非尼对Bcl-2家族蛋白质表达的影响不大。**结论**　DHT可能通过抑制Akt信号通路的激活来增强索拉非尼的HCC细胞增殖抑制作用和凋亡诱导作用。

关键词　二氢丹参酮，索拉非尼，Akt，肝细胞癌，细胞凋亡

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