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Celastrol Enhances the Anti-Liver Cancer Activity of Sorafenib

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Background: Sorafenib, a multiple-target-point kinase inhibitor, has been used as a standard treatment for advanced liver cancer and has shown therapeutic benefits. However, resistance often occurs, prompting the need for identification of synergizing agents. Celastrol is a major active ingredient of *Tripterygium wilfordii,* which can increase the antitumor effect of traditional antitumor drugs. This work focused on the sensitization of liver cancers in use of celastrol combined with sorafenib.

Material/Methods: The IC50 values of sorafenib and celastrol on cancer cells were determined through MTT assays. The effects of sorafenib on AKT signaling and VEGF levels in sorafenib-treated cancer cells were analyzed by Western blotting and ELISA, respectively. After combined treatment with celastrol and sorafenib, the survival rate of tumor cells was determined by MTT and clonogenic assays, and the apoptosis rate was also determined by flow cytometry. In addition, the *in vivo* antitumor activity of celastrol combined with sorafenib was evaluated in Hepa1-6 tumor-bearing mice.

Results: Sorafenib treatment induced the compensatory activation of the AKT pathway and autocrine VEGF in hepatoma cells, which could be reversed by celastrol. Furthermore, celastrol enhanced the growth inhibition and apoptosis induction of cancer cells by sorafenib both *in vitro* and *in vivo* and reduced the dosage of sorafenib needed.

Conclusions: Celastrol enhances the antitumor activity of sorafenib in HCC tumor cells by suppressing the AKT pathway and VEGF autocrine system.

MeSH Keywords: Apoptosis • Carcinoma, Hepatocellular • Signal Transduction

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Background

Despite the development of various therapeutic strategies, hepatocellular carcinoma (HCC) is still one of the most common and fatal malignancies worldwide [1]. HCC patients are usually diagnosed at a late stage, and the tumors exhibit intrahepatic or distant organ metastasis, which is unsuitable for surgical treatment [2]. Targeted drugs are important clinical treatments for patients with liver cancer [3]. Sorafenib is a multiple-target-point kinase inhibitor approved as a first-line HCC treatment [4]. Although several large randomized controlled clinical studies have shown that sorafenib can effectively prolong the survival period of patients with liver cancer [5], the secondary drug resistance produced by long-term sorafenib therapy has become a bottleneck restricting further improvement of the efficacy of sorafenib [6,7]. Therefore, it is important to further understand the mechanism of liver cancer resistance to sorafenib and carry out molecularly targeted interventions.

The mechanism of tumor cell resistance is rather complicated, in which the compensatory activation of the anticipation signaling pathway is one of the main mechanisms. As a classic signal transduction pathway, PI3K/AKT signaling participates in the regulation of multiple cell activities, such as cell proliferation [8], survival [9], and apoptosis [10]. Chen et al. found that the long-term use of sorafenib activates the PDK/AKT signaling pathway and induces sorafenib resistance in hepatoma cells [11]. Some researchers indicated that activated AKT pathway mediated cellular resistance to sorafenib, and AKT inhibitor could enhance the growth inhibition of sorafenib in hepatoma cells [12,13]. Although it seems that inhibition of the AKT pathway can significantly enhance the efficacy of sorafenib, a problem that cannot be ignored is that the PI3K/AKT pathway also plays an essential role in the physiological process [14]. Therefore, the potentially toxic adverse effects of the combination therapy may limit its clinical applications.

Some natural medicinal plant components can be used as auxiliary or even alternative means for traditional or targeted therapy because of their minimal toxic adverse effects. Celastrol is a major active ingredient of *Tripterygium wilfordii*, and its anticancer effect has been confirmed in many types of tumors, such as breast cancer, melanoma, prostate cancer, and glioma [15,16]. However, whether celastrol can enhance the antitumor effect of sorafenib in hepatocellular carcinoma remains unknown. In this study, we focused on the inhibitory effects of sorafenib alone or in combination with celastrol on hepatoma cells. Our results showed that celastrol significantly enhanced the antitumor activity and reduce the dosage of sorafenib.

Material and Methods

Cell culture

The HCC cell lines HepG2 and Hepa1-6 were was obtained from ATCC and maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and conventional concentration antibiotics. All cells were cultured in a humidified 5% CO_2 incubator at 37°C.

Cell cytotoxicity assay

In vitro cytotoxicities of sorafenib and celastrol, alone or in combination, in the HCC cell lines were measured by MTT assay kit (Engene, Nanjing, China) as previously described [17]. In brief, HCC cells were plated in 96-well culture plates at a concentration of 5000 cells/well and treated with sorafenib and/or celastrol. At the indicated time points, 10 µL MTT solutions (5 mg/ml) were added and cells were then incubated for 2 h. After removing the medium, 500 µL DMSO was added to dissolve formazan crystals, and the absorbance was read at 570 nm on a Multiwell plate reader (Biotech, USA).

ELISA assay

VEGF levels in the cell culture medium supernatants were determined using human and mouse ELISA kits (NeoBioscience, Shenzhen, China) following the manufacturer's protocol. Briefly, samples were added to plates (100 µL/well) supplied with the kit, and incubated at 37°C for 90 min. After washing 5 times, biotinylated antibodies were added (100 µL/well), and incubated at 37 °C for 60 min. After another round of washing, avidin-peroxidase was added (100µL/well) and incubated at 37°C for 30 min. Plates were then washed 5 times and reacted with 100 µL/well TMB for 15 min at room temperature. Finally, termination reagent was added, and absorbance was measured at 450 nm on a microplate reader (Biotek Instruments, USA).

Western blot

Proteins from tumor cells were extracted by RIPA lysis buffer (Keygen, Nanjing, China), separated by 12% SDS-PAGE, and subjected transferred to PVDF membranes. The membranes were incubated with P-AKT (Ser473) or total AKT antibodies, followed by hybridization with the secondary HRP-conjugated antibody. Detection was performed by an enhanced chemiluminescence assay (Wanleibio, Shenyang, China).

Colony formation assay

Cancer cells were seeded in 12-well culture plates at a concentration of 1000 cells/well and then incubated in 5% CO_2 at 37°C. After treatment with indicated agents for 16 days, cells

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were stained with 0.5% crystal violet for 20 min. Colony numbers in each plate were counted using an inverted microscope.

Apoptosis assay

The apoptotic effects of sorafenib and celastrol, alone or in combination, on the HCC cell lines were measured by staining with FITC – Annexin V and propidium iodide (PI) kit (4ABio, Beijing, China) in accordance with the provided instructions, and the data were analyzed with CellQuest software (BD Biosciences, San Jose, CA, USA).

In vivo experiment

The animal experiment was approved by the Ethics Committee of the Experimental Animal Center of Shanxi Medical University, and all the C57bl/6 mice (4–6 weeks old) used for the experiment were well fed before inoculation. Hepa1-6 single-cell suspension cells (2×10^7/mL) were injected subcutaneously at a volume of 0.1 mL in the right flank of each mouse. After 7 days, the skins of mice were palpable, showed bumps, and were irregularly shaped. After providing medicine randomly, the size of the subcutaneous tumor was observed regularly every 7 days. After 21 days of administration, the mice were anesthetized and killed, completely removing the transplanted tumor. The inhibition rate of tumor volume was then calculated.

Immunohistochemistry

The stripped mouse tumor blocks were fixed in 10% neutral formalin and embedded in paraffin. After slicing (with a thickness of approximately 5 μm), dewaxing, antigen retrieval, and sealing, VEGF, p-AKT, and cleaved-caspase 3 were detected using the corresponding primary antibodies. After washing, they were incubated with secondary antibodies marked with HRP or Alexa Fluor 488 (Keygene, Nanjing, China) and finally were made chromogenic *in situ* through DAB or observed under a fluorescence microscope.

Statistical analysis

All data were the results of 3 independent experiments, expressed as means ± s.d. The t test and single-factor ANOVA were performed using SPSS13.0 software, and $P<0.05$ indicated statistical significance.

Results

The effects of sorafenib and celastrol on HCC cell growth

To investigate the effects of sorafenib and celastrol on the hepatoma cell lines Hep G2 and Hepa1-6 growth, we performed MTT assay to detect the proliferation inhibition of different concentrations of sorafenib (0, 1, 2, 5, and 10 μmol/L) or celastrol (0, 1, 2, 4, and 8 μmol/L) at different time points (24 and 48 h). As shown in Figure 1, with the increase in concentration and the prolongation of action time, the inhibitory effect of sorafenib on the proliferation of tumor cells gradually increased, in which the IC_{50} values of sorafenib at 48 h for Hep G2 and Hepa1-6 cells were 7.35 and 9.46 μm, respectively (Figure 1A). In the celastrol-treated group, as shown in Figure 1B, HCC cell proliferation was also inhibited in a concentration- and time-dependent manner. After treating for 48 h, the IC50 values of celastrol for HepG2 and Hepa1-6 cells were 3.37 and 2.55 μm, respectively.

Sorafenib enhanced VEGF autocrine and activated the AKT pathway

Activation of the PI3K/AKT signaling pathway and autocrine VEGF mediate acquired resistance to sorafenib in hepatocellular carcinoma cells [10,11]. Thus, we checked the P-AKT and VEGF level in tumor cells after sorafenib treatment. The results of our study showed that sorafenib single-drug therapy significantly increased the expression levels of P-AKT (Figure 2A, 2B) and VEGF (Figure 2C, 2D) compared with the control group, which indicated the activation of P-AKT and enhanced VEGF autocrine induced by sorafenib.

Celastrol enhanced sorafenib-induced HCC cell growth inhibition and apoptosis

According to the above results, hepatoma cells were treated with 1 mol/L celastrol combined with 1 mol/L sorafenib. As shown in Figure 3, celastrol significantly enhanced the growth inhibition of sorafenib on the hepatoma cells. Under the combined action of celastrol, the inhibitory rate of sorafenib on the hepatoma cells at 48 h was increased by approximately 5 and 3 times. The effect of celastrol on the apoptosis of hepatoma cells induced by sorafenib through ANNEXIN V/PI staining was further examined. Results showed that the combined treatment significantly increased the apoptotic-staining-positive cells of the cancer cells after 48 h. The apoptosis rate of cancer cells induced by sorafenib was 2.3 and 4 times enhanced by celastrol.

Celastrol enhanced the antitumor effect of sorafenib *in vivo*

To further evaluate the inhibitory activity of celastrol combined with sorafenib on hepatoma cells, Hepa1-6-cell-bearing mice were established and treated with drugs. After sorafenib or celastrol single-drug treatment, the size of the transplanted tumor was significantly reduced. This reduction was the most significant in the celastrol+sorafenib group, which reduced the volume of the transplanted tumor at a maximum value (Figure 4A, 4B).

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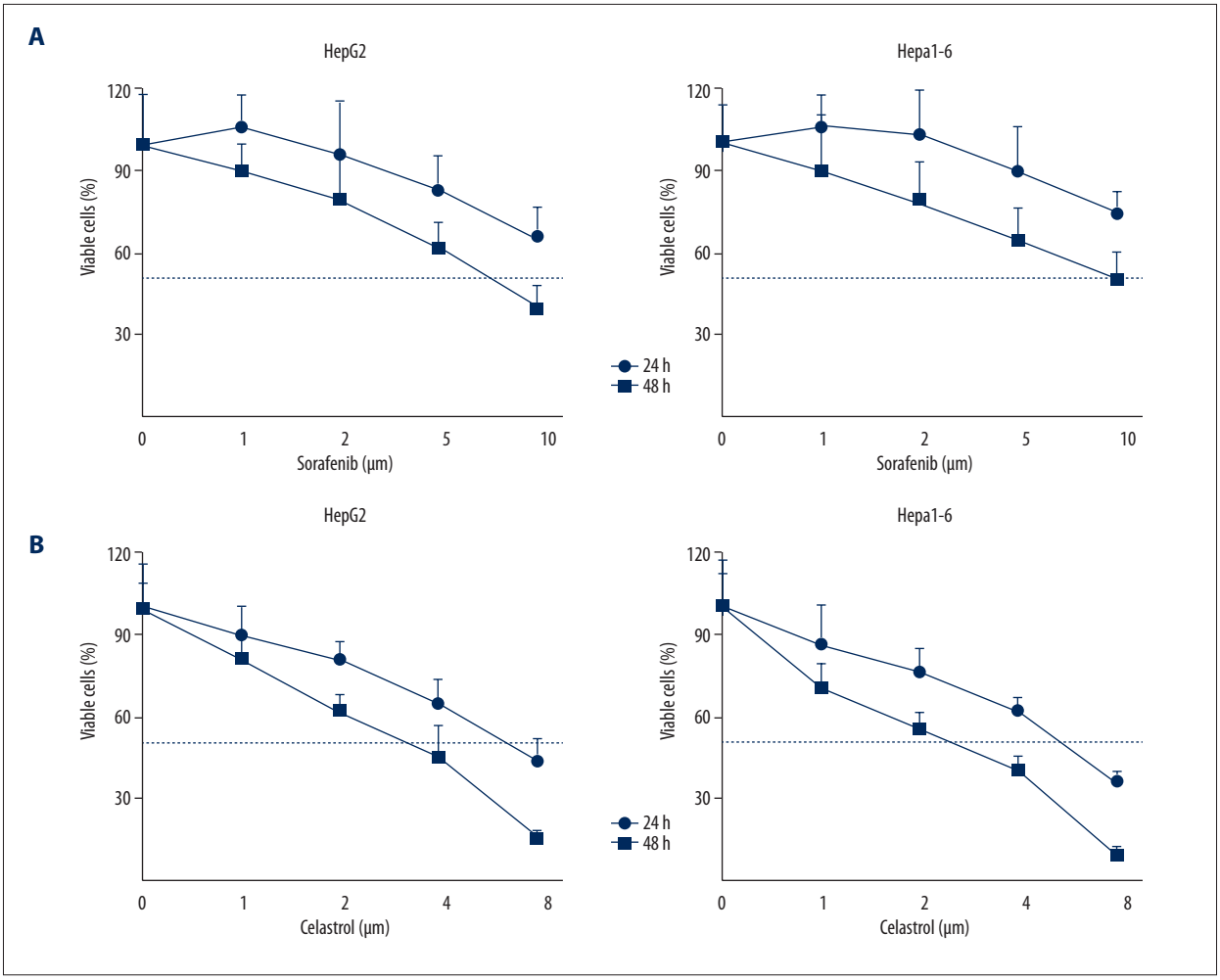

Figure 1. Effects of sorafenib and celastrol on the growth of hepatoma cells. Cell viability percentages in hepatoma cells treated with various concentrations of sorafenib (**A**) or celastrol (**B**) *in vitro* for 24 h and 48h. The dotted lines represent the corresponding concentration of IC50.

Interestingly, the results of our study showed that sorafenib single-drug therapy significantly increased the expression levels of VEGF and P-AKT compared with the control group, whereas celastrol decreased the levels of VEGF and P-AKT, suggesting that combined celastrol therapy can reverse the upregulation of VEGF and P-AKT induced by sorafenib (Figure 4C, 4D). Furthermore, the results of immunohistochemistry showed that, except for the control group, all groups displayed significant cell apoptosis after drug intervention. More cell apoptosis was observed using the combined intervention of sorafenib with celastrol (Figure 4E). These results indicate that celastrol and sorafenib can inhibit the growth of HCC tumors and increase the apoptosis of tumor cells.

Discussion

Sorafenib is a first-line treatment for advanced hepatocellular carcinoma that can improve the survival of patients. However, although the patient's initial clinical response to the treatment had a certain effect, the long-term use of sorafenib has obvious adverse effects and drug resistance [18–20]. Combined therapy, as an important clinical medication in recent years, has the advantages of enhancing the efficacy, reducing the dosage, and lowering the toxicity of a single drug, as well as effectively slowing drug resistance. Thus, researchers seek drugs that can effectively enhance the efficacy of sorafenib for combination therapy.

Celastrol is one of the main active components of *Tripterygium wilfordii* Hook F that has a wide range of biological functions, such as antibacterial, anti-inflammatory, cholesterol-reducing, antitumor, and other pharmacological effects [21]. Many studies have shown that celastrol can inhibit the growth of multiple tumors *in vivo* and *in vitro*. It has a wide range of targets that can regulate apoptosis-related signaling pathways [22], inhibit the growth-factor-mediated signaling pathway [23], and reduce the release of growth and angiogenic factors [24].

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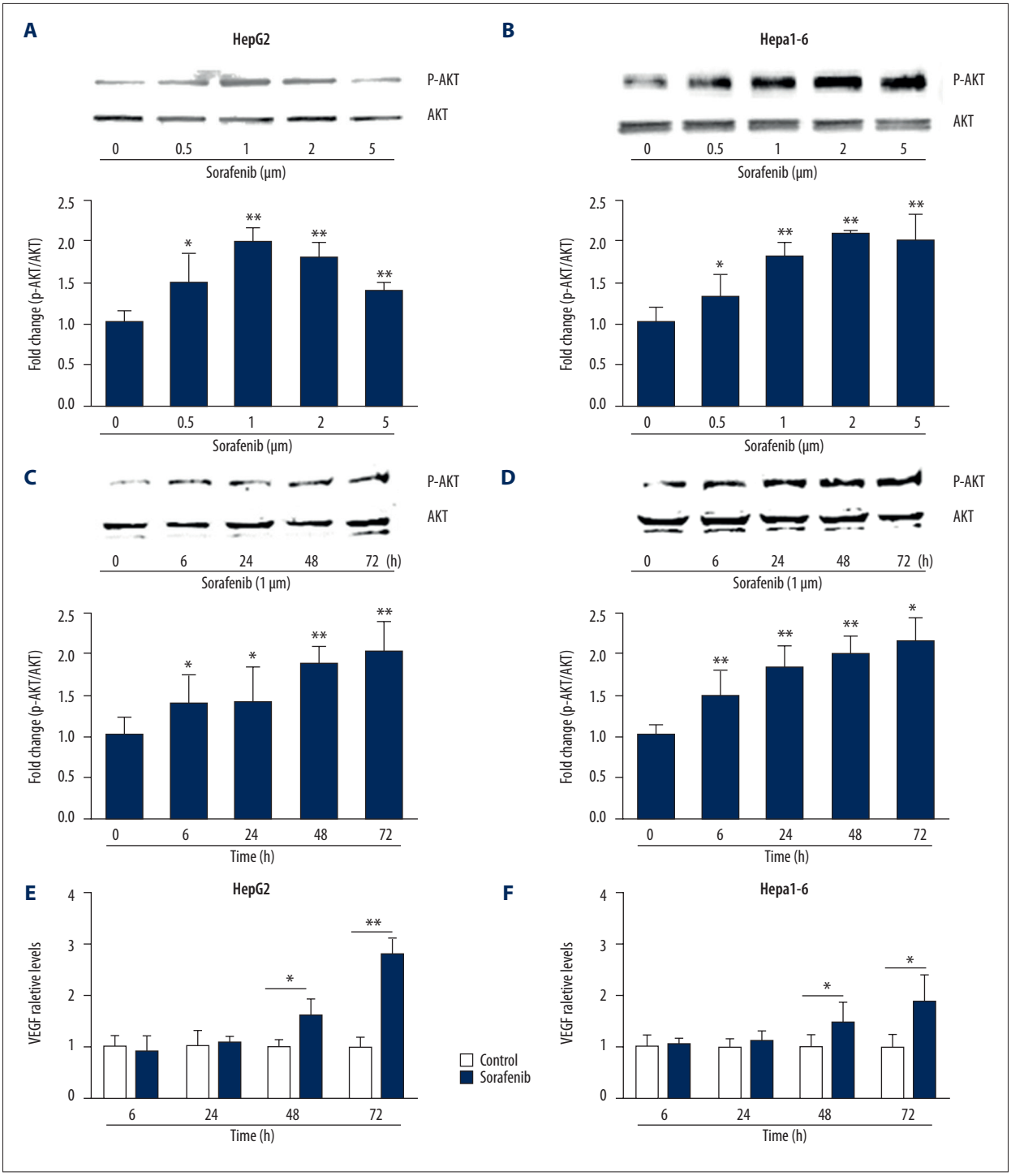

Figure 2. (**A–F**) Effects of sorafenib on the phosphorylated AKT levels in hepatoma cells. (**A**) After treating with indicated concentrations of sorafenib for 6 h, the HepG2 and Hepa1-6 cellular proteins were extracted and the phosphorylated and total AKT levels were detected by Western blot analysis. * $P<0.05$, ** $P<0.01$. (**B**) The levels of phosphorylated and total AKT were detected through Western blot analysis at different time points after treatment with 1 μm sorafenib. * $P<0.05$, ** $P<0.01$. (**C**) After treatment with sorafenib (1 μm) for different times, the VEGF levels in culture supernatant of hepatoma cells were detected by ELISA. * $P<0.05$, ** $P<0.01$.

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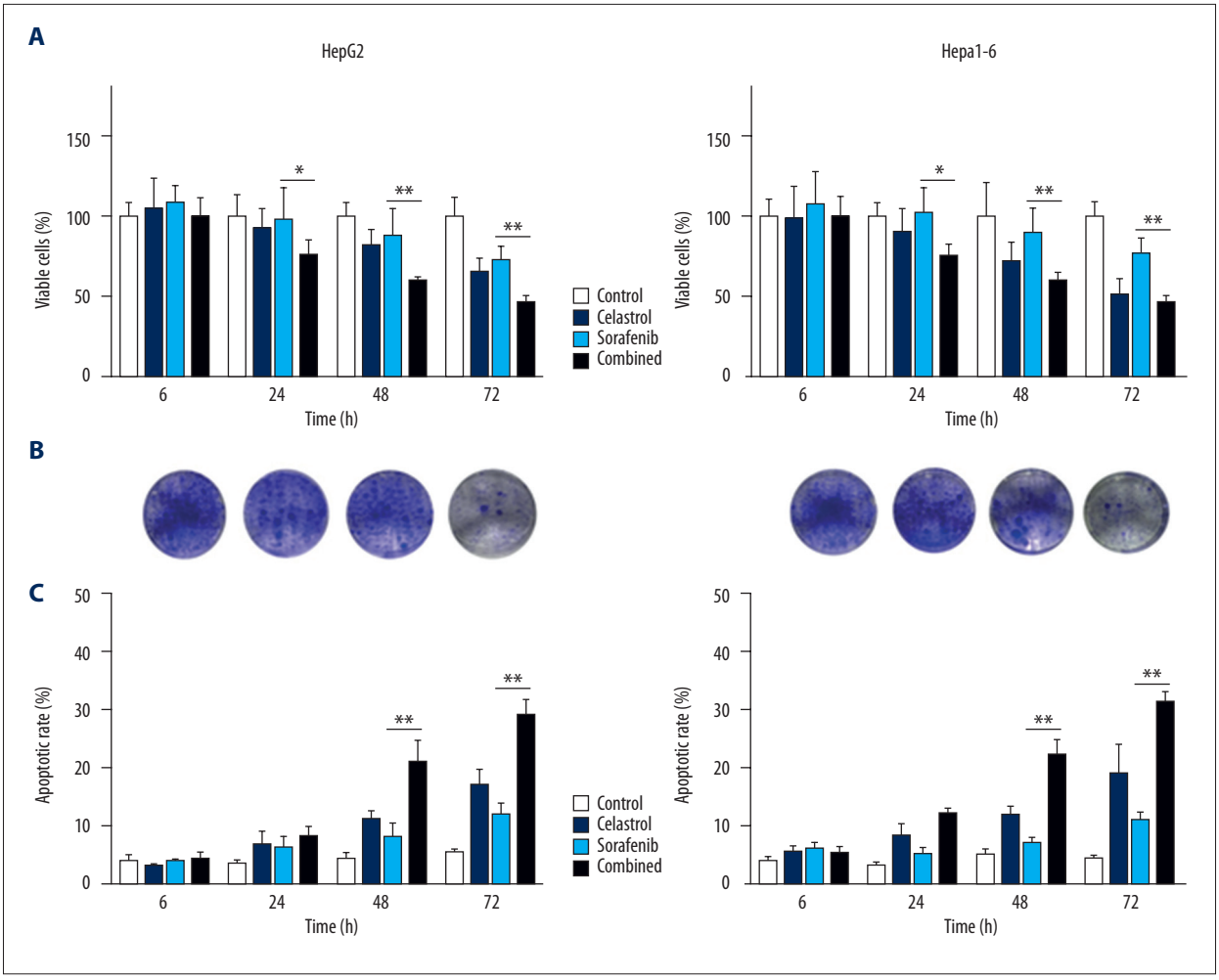

Figure 3. Effects of sorafenib combined with celastrol on the growth and apoptosis of hepatoma cells. (**A**) After treating with control agents (equal volume of PBS), sorafenib (1 μm), celastrol (1 μm), and the same amount of sorafenib and celastrol for different time, the cell viability percentages were detected by MTT assays, ** $P<0.01$. (**B**) After treating with the above agents for 16 days, crystal violet staining to detect the number of clones formed in hepatoma cells. (**C**) After treating with the above agents for different time, cell apoptotic rates were analyzed by Annexin V-FITC and PI staining, ** $P<0.01$.

Importantly, celastrol increases the antitumor effect of traditional antitumor drugs or targeted antitumor drugs, reversing the resistance of tumor cells to multiple drugs [25,26]. In the present work, we assessed the feasibility of combining celastrol to enhance sorafenib and reduce its dosage.

Consistent with our expectations, celastrol significantly enhanced the growth inhibition of sorafenib on a variety of hepatoma cells, both *in vivo* and *in vitro*; suggesting that combined therapy can be widely used for liver cancer. Under the action of sorafenib with the same concentration, celastrol can increase its antitumor activity by more than 5 times. The significant inhibitory effect was manifested in a significant decline in the tumor proliferation index, indicating one of the mechanisms by which combined therapy achieved strong suppression of the proliferation of hepatoma cells. Anti-cancer drugs are prone to exhibit drug resistance after long-term administration. However, the inhibitory effect of liver cancer in this study, especially *in vivo*, did not decrease with time, indicating the stability of combined therapy to a certain extent.

In particular, we observed that, compared with sorafenib monotherapy, combined celastrol can cause more intense apoptotic death in hepatocellular carcinoma cells. Notably, sorafenib can activate the PI3K/AKT signaling pathway in hepatoma cells while achieving certain therapeutic effects. As a classic signal transduction pathway against apoptosis and promoting survival, the PI3K/AKT pathway participates in the regulation of multiple cell activities and plays an unusual role in tumor-targeted drug resistance [27]. Other studies have also confirmed that AKT is involved in the drug resistance of hepatocellular carcinoma cells to sorafenib. Therefore, the strong suppression of

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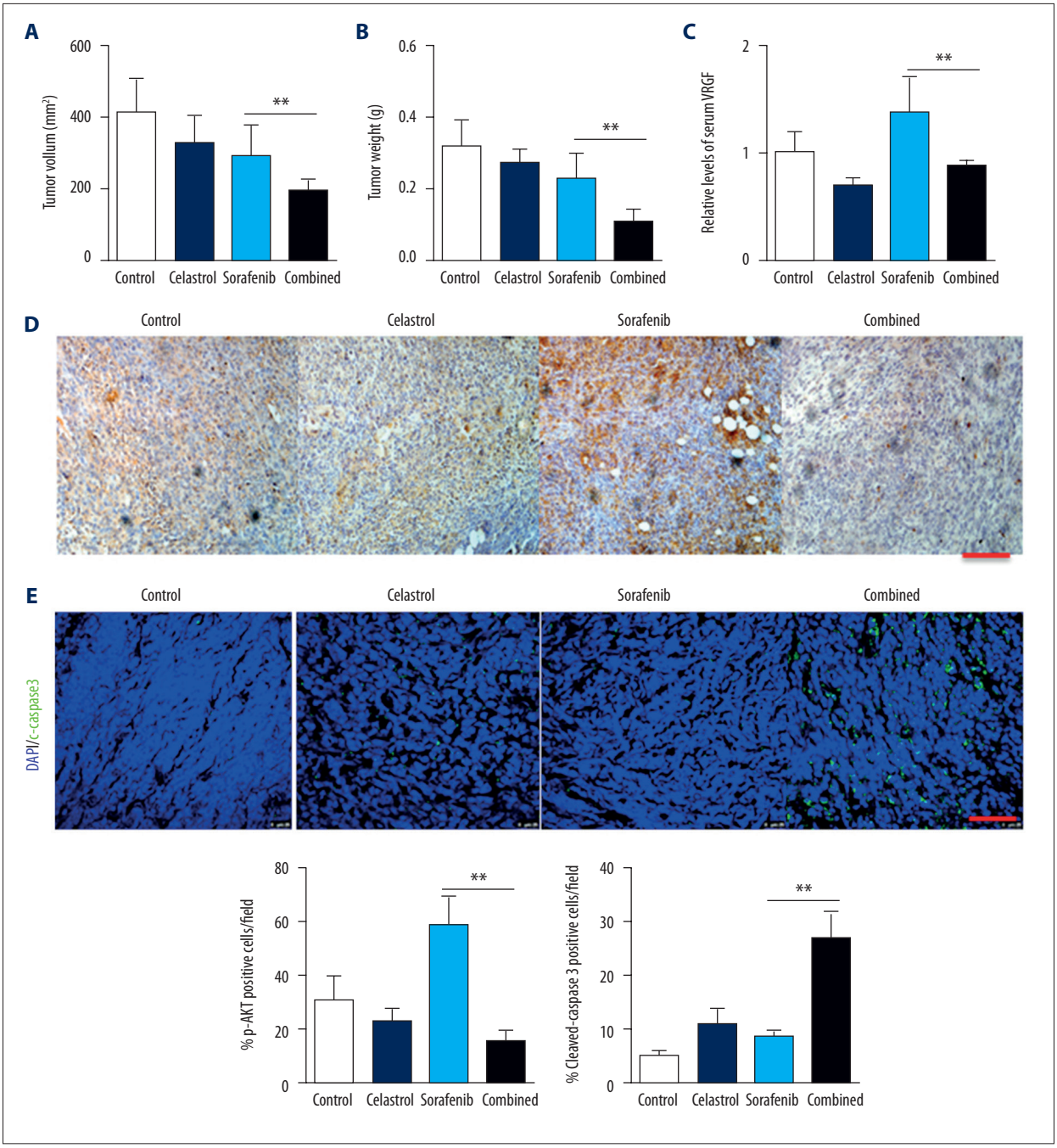

Figure 4. After the administration of control agents (equal volume of PBS), celastrol (5 mg/kg), sorafenib (6 mg/kg), or a combination of the same amount of the celastrol and sorafenib, the effects of combined therapy on the growth of Hepa1-6 transplanted tumor in C57bl/6 mice. (**A**) The tumor sizes in all groups were measured at the end of drug administration, ** $P<0.01$. (**B**) The tumors of mice in all groups were weighed at the end of drug administration, ** $P<0.01$. (**C**) The VEGF levels in mice serum were measured by ELISA at the end of drug administration, ** $P<0.01$. IHC was used to detect the expressions of p-AKT (**D**) and cleaved-caspase 3 (**E**) in tumor tissue sections, bar=100 μm, ** $P<0.01$.

sorafenib-induced AKT activation by celastrol is probably one of the major causes for the higher apoptosis rate induced by combination therapy. The complexity of the mechanism of tumor resistance is reflected in the release of multiple growth factors in addition to compensatory activation in the regulation of anti-apoptosis and survival-related signaling pathways [28,29]. We also found that sorafenib increased the secretion of VEGF in tumor cells, and the increased VEGF undoubtedly helped cancer

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cells to resist sorafenib therapy. However, celastrol can inhibit the secretion of VEGF, which further enhances the efficacy of sorafenib. Whether increased VEGF is regulated by activated AKT and whether celastrol can inhibit VEGF secretion by inhibiting the AKT pathway requires further confirmation.

Surprisingly, celastrol can enhance the efficacy of sorafenib while reducing its dosage. The limited clinical efficacy of sorafenib is also due to the adverse effect of long-term medication, including circulatory system, skin reaction, and gastrointestinal reaction. Although some targeted drugs, such as AKT pathway inhibitors, can enhance the antitumor effect of sorafenib, the targets of these drugs also play important physiological functions in the body, and the potential hazards caused by their oversuppression need further research. In view of the widespread targets and low toxicity of celastrol, the combined strategy with sorafenib is an effective and safe treatment.

Conclusions

Overall, our study shows that sorafenib monotherapy can trigger the compensatory activation of the AKT pathway and oversecrete VEGF, which can be reversed by celastrol, leading to better treatment results. Low-dose sorafenib combined with celastrol exhibited a strong therapeutic potential in the cell model of hepatocellular carcinoma, accompanied by a significant pro-apoptotic effect. However, further research is needed to better understand the antitumor mechanism and safety of this combination therapy.

References:

1. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Akinyemiju TF, Al Lami FH et al: Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2016: A systematic analysis for the global burden of disease study. JAMA Oncol, 2018; 4(11): 1553–68
2. Ang C, Shields A, Xiu J et al: Molecular characteristics of hepatocellular carcinomas from different age groups. Oncotarget, 2017; 8(60): 101591–98
3. Marijon H, Faivre S, Raymond E: Targeted therapies in hepatocellular carcinomas: Recent results and future development. Bull Cancer, 2009; 96(5): 553–61
4. S Leathers J, Balderramo D, Prieto J et al: Sorafenib for treatment of hepatocellular carcinoma: A survival analysis from the South American liver research network. J Clin Gastroenterol, 2018 [Epub ahead of print]
5. Aktas G, Kus T, Emin Kalender M et al: Sorafenib with TACE improves the survival of hepatocellular carcinoma patients with more than 10 cm tumor: A single-center retrospective study. J BUON, 2017; 22(1): 150–56
6. Zhang H, Wang Q, Liu J, Cao H: Inhibition of the PI3K/AKT signaling pathway reverses sorafenib-derived chemo-resistance in hepatocellular carcinoma. Oncol Lett, 2018: 15(6): 9377–84
7. Wu CH, Wu X, Zhang HW: Inhibition of acquired-resistance hepatocellular carcinoma cell growth by combining sorafenib with phosphoinositide 3-kinase and rat sarcoma inhibitor. J Surg Res, 2016; 206(2): 371–79
8. Zhang MH, Niu H, Li Z et al: Activation of PI3K/AKT is involved in TINAG-mediated promotion of proliferation, invasion and migration of hepatocellular carcinoma. Cancer Biomark, 2018; 23(1): 33–43
9. Wang X, Zeng J, Wang L et al: Overexpression of microRNA-133b is associated with the increased survival of patients with hepatocellular carcinoma after curative hepatectomy: Involvement of the EGFR/PI3K/AKT/mTOR signaling pathway. Oncol Rep, 2017; 38(1): 141–50
10. Zhang H, Cao Y, Chen Y et al: Apatinib promotes apoptosis of the SMMC-7721 hepatocellular carcinoma cell line via the PI3K/AKT pathway. Oncol Lett, 2018; 15(4): 5739–43
11. Chen KF, Chen HL, Tai WT et al: Activation of phosphatidylinositol 3-kinase/AKT signaling pathway mediates acquired resistance to sorafenib in hepatocellular carcinoma cells. J Pharmacol Exp Ther, 2011; 337(1): 155–61
12. Zhai B, Hu F, Jiang X et al: Inhibition of AKT reverses the acquired resistance to sorafenib by switching protective autophagy to autophagic cell death in hepatocellular carcinoma. Mol Cancer Ther, 2014; 13(6): 1589–98
13. Jilkova ZM, Kuyucu AZ, Kurma K et al: Combination of AKT inhibitor ARQ 092 and sorafenib potentiates inhibition of tumor progression in cirrhotic rat model of hepatocellular carcinoma. Oncotarget, 2018; 9(13): 11145–58
14. Franke TF: PI3K/AKT: Getting it right matters. Oncogene, 2008; 27(50): 6473–88
15. Kashyap D, Sharma A, Tuli HS et al: Molecular targets of celastrol in cancer: Recent trends and advancements. Crit Rev Oncol Hemat, 2018; 128: 70–81
16. Yang Y, Cheng S, Liang G et al: Celastrol inhibits cancer metastasis by suppressing M2-like polarization of macrophages. Biochem Biophys Res Commun, 2018; 503(2): 414–19
17. Shrivastava S, Jeengar MK, Reddy VS et al: Anticancer effect of celastrol on human triple negative breast cancer: possible involvement of oxidative stress, mitochondrial dysfunction, apoptosis and PI3K/AKT pathways. Exp Mol Pathol, 2015; 98(3): 313–27
18. Berk V, Kaplan MA, Tonyali O et al: Efficiency and side effects of sorafenib therapy for advanced hepatocellular carcinoma: A retrospective study by the Anatolian society of medical oncology. Asian Pac J Cancer Prev, 2013; 14(12): 7367–69
19. Schmidt TM, Liu LI, Abraham IE et al: Efficacy and safety of sorafenib in a racially diverse patient population with advanced hepatocellular carcinoma. Anticancer Res, 2018; 38(7): 4027–34
20. Abdel-Rahman O, Lamarca A: Development of sorafenib-related side effects in patients diagnosed with advanced hepatocellular carcinoma treated with sorafenib: A systematic-review and meta-analysis of the impact on survival. Expert Rev Gastroent Hepatol, 2017; 11(1): 75–83
21. Kannaiyan R, Shanmugam MK, Sethi G: Molecular targets of celastrol derived from Thunder of God Vine: Potential role in the treatment of inflammatory disorders and cancer. Cancer Lett, 2011; 303(1): 9–20
22. Ren B, Liu H, Gao H et al: Celastrol induces apoptosis in hepatocellular carcinoma cells via targeting ER-stress/UPR. Oncotarget, 2017; 8(54): 93039–50
23. Zhang R, Zhu Y, Dong X et al: Celastrol attenuates cadmium-induced neuronal apoptosis via inhibiting Ca (2+) -CaMKII-dependent Akt/mTOR pathway. J Cell Physiol, 2017; 232(8): 2145–57
24. Huang S, Tang Y, Cai X et al: Celastrol inhibits vasculogenesis by suppressing the VEGF-induced functional activity of bone marrow-derived endothelial progenitor cells. Biochem Bioph Res Commun, 2012; 423(3): 467–72
25. He D, Xu Q, Yan M et al: The NF-kappa B inhibitor, celastrol, could enhance the anti-cancer effect of gambogic acid on oral squamous cell carcinoma. BMC Cancer, 2009; 9: 343
26. Yan YY, Guo Y, Zhang W et al: Celastrol enhanced the anticancer effect of lapatinib in human hepatocellular carcinoma cells *in vitro*. J BUON, 2014; 19(2): 412–18
27. Brotelle T, Bay JO: PI3K-AKT-mTOR pathway: Description, therapeutic development, resistance, predictive/prognostic biomarkers and therapeutic applications for cancer. Bull Cancer, 2016; 103(1): 18–29
28. Kieran MW, Kalluri R, Cho YJ: The VEGF pathway in cancer and disease: Responses, resistance, and the path forward. CSH Perspect Med, 2012; 2(12): a006593
29. Stanton MJ, Dutta S, Zhang H et al: Autophagy control by the VEGF-C/NRP-2 axis in cancer and its implication for treatment resistance. Cancer Res, 2013; 739(1): 160–71

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