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Honokiol, a natural therapeutic candidate, induces apoptosis and inhibits angiogenesis of ovarian tumor cells

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Abstract

Objectives: To observe the anti-tumor activities of honokiol on human ovarian tumor *in vitro* and *in vivo*.
Study design: Cells were treated with honokiol, and the effects on proliferation and apoptosis were examined by MTT, DNA ladder, Hoechst staining, and flow cytometry assays. Expression of Bcl-2 members and caspase-3 were assessed. Measurements of tumor volume and microvessel densities (MVDs) were performed.
Results: Honokiol significantly inhibited proliferation and induced apoptosis, with alteration of Bcl-2 members and caspase-3. Administration of honokiol to tumor-bearing animals decreased MVD and resulted in inhibition of tumor growth.
Conclusions: Honokiol could induce apoptosis and inhibit angiogenesis *in vitro* and *in vivo*, suggesting a novel and attractive therapeutic candidate for ovarian tumor treatment.

Keywords: Honokiol; Ovarian tumor; Apoptosis; Angiogenesis

1. Introduction

As an oriental medicine, the root and stem bark of *Magnolia officinalis* has been widely used in thrombotic stroke, gastrointestinal complaints, anxiety and nervous stroke, etc. Honokiol, one of the major phenolic constituents of magnolia bark, has several pharmacological effects such as anti-oxidant, anti-thrombosis, anti-inflammatory, xanthine oxidase inhibition, and anxiolytic effect [1,2]. Recently, some studies have revealed the remarkable anti-tumor effects of honokiol on human colon cancer RKO cells [3], lung cancer CH27 cells [4], leukemia HL-60 cells [5] and Molt 4B cells [6], etc., in which apoptosis induction might be involved. Up to date, however, little is known about its activities on ovarian tumor cells.

Ovarian tumor is one of the most common tumors in female genital organs, and the 5-year survival rate of advanced ovarian cancer remains only 20–30% [7]. The growth, persistence and metastasis of ovarian tumors are angiogenesis dependent and, as a result, targeting angiogenesis represents a novel therapeutic strategy, in which anti-angiogenic agents attempted to prevent new vessels from growing around tumor mass and destroy the existing abnormal vascular net and, as a result, blocked the feed resource of solid tumor. In the present study, we aimed to characterize the events in apoptosis and angiogenesis triggered by honokiol.

2. Study design

2.1. Cell culture and cell proliferation assay

The human ovarian tumor SKOV3 and COC1 cells were maintained in RPMI-1640 and Angelen and A2780

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maintained in DMEM medium, supplemented with 10% fetal bovine serum, 2 mM glutamine and antibiotics, at 37 °C in humidified atmosphere of 5% CO_2.

Cells were seeded on 96-well plates and cultured with honokiol (Sikehua Biotechnology, Chengdu, China) at various doses for indicated durations, and MTT was then added for 4-h incubation. The MTT formazan precipitate was then dissolved in DMSO, and the absorbance was measured at a 595 nm wavelength.

2.2. Cell apoptosis assays

Cells were collected and then resuspended in lysis buffer (50 mM Tris–HCl, pH 8.0, 20 mM EDTA and 5% Triton X-100). Fragmentation DNA was extracted routinely by phenol/chloroform protocol; electrophoresed on 2% agarose gel and then visualized by transillumination with UV light.

To further confirm the nuclear morphology of apoptotic cells, adherent cells were stained with Hoechst 33258, and examined immediately with a fluorescence microscope. The nuclei were visualized with blue staining under fluorescence and apoptotic cells were defined on the basis of nuclear morphology changes including nuclear shrinkage and chromatin condensation, etc.

2.3. Flow cytometry

The PI-stained cells were analyzed on a EPICS ELITE ESP flow cytometer (Beckman Coulter, USA). DNA-bound PI fluorescence was measured with 15 mW air-cooled argon ion laser at 488 nm as excitation sources. Analysis of cell cycle was performed with Coulter Elite 4.5 Multicycle software, 10,000 events for each sample.

2.4. RT-PCR analysis

Total RNA was extracted using Trizol (Invitrogen, USA) and RT-PCR was performed as follows: reverse transcription at 50 °C 30 min and boiling at 94 °C 2 min; then amplification for 30 cycles at 94 °C 0.5 min, annealing at 47–55 °C 0.5 min, and extension at 72 °C 0.5 min; then cycled at 72 °C 10 min to complete the elongation. Samples were electrophoresed on 1.5% agarose gel and visualized by transillumination with UV light.

2.5. Western blotting analysis

Cells or dissected fresh tissues were lysed in RIPA lysis buffer, and lysates were subjected to 12% SDS-PAGE, transferred to PVDF membrane, and immunoblotted with primary antibodies according to the manufacturer's instruction. The blots were labeled with HRP-conjugated secondary antibodies and visualized with DAB staining. The specific antibodies were mouse anti-human caspase-3 (Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-human vascular endothelial growth factor (VEGF) (Abcam, Cambridge, UK) antibodies. The intensity of each band was measured using Quantity One 4.6 software, and expressed as optical density (OD).

2.6. Constructions of animal model and treatment

The following studies were approved and granted by the institutional animal care and treatment committee of Sichuan University. BALB/C healthy female nude mice were injected subcutaneously with SKOV3 cells (1×10^7) via right axilla and assigned into three groups at random, treated with 100 μl PBS, 0.6 mg liposome/100 μl PBS or 1 mg liposome-encapsuled honokiol (40%)/100 μl PBS by intraperitoneally injection every day, respectively. During the session, signs of possible toxicity were recorded.

2.7. Microvessel density (MVD) assay

Tumor volumes were measured every 8 days after inoculation, determined by the following formula: tumor volume (mm^3) = 0.52 × length × width × width. The dissected tumors were fixed in 10% neutral buffered formalin and embedded in paraffin. For MVD analysis, the sections were stained against rat anti-mouse CD31 monoclonal antibody (BD Biosciences Phamingen, San Diego, CA). MVD was determined by examining vascular hot spots as described previously [8], with some slight modifications. Five hot vascular areas without necrosis were selected and counted at high magnification (200×), and the five values were averaged for each sample.

2.8. Statistics

Values were given as mean ± S.D. Comparisons between two groups were performed by Student's *t*-test; comparisons among multiple groups were performed by analysis of variance (ANOVA), or Dunnet *t*-test, with SPSS11.0 software (SPSS, Chicago, IL). It was statistically significant when $p < 0.05$.

3. Results

3.1. Honokiol inhibits proliferation and induces apoptosis

To identify the therapeutic potential of honokiol, four ovarian tumor cell lines were cultured with honokiol for 72 h. MTT assays showed that honokiol treatment resulted in a dose- and time-dependent inhibition of cell proliferation (Fig. 1), which occurred at a dose over 5 μg/ml, and 50% inhibition (IC_{50}) at 24 h were 16.7, 19.6, 16.4, and 14.9 μg/ml for SKOV3, Coc1, Angelen and A2780 cells, respectively. Doses over 20 μg/ml did not significantly increase the inhibition effect.

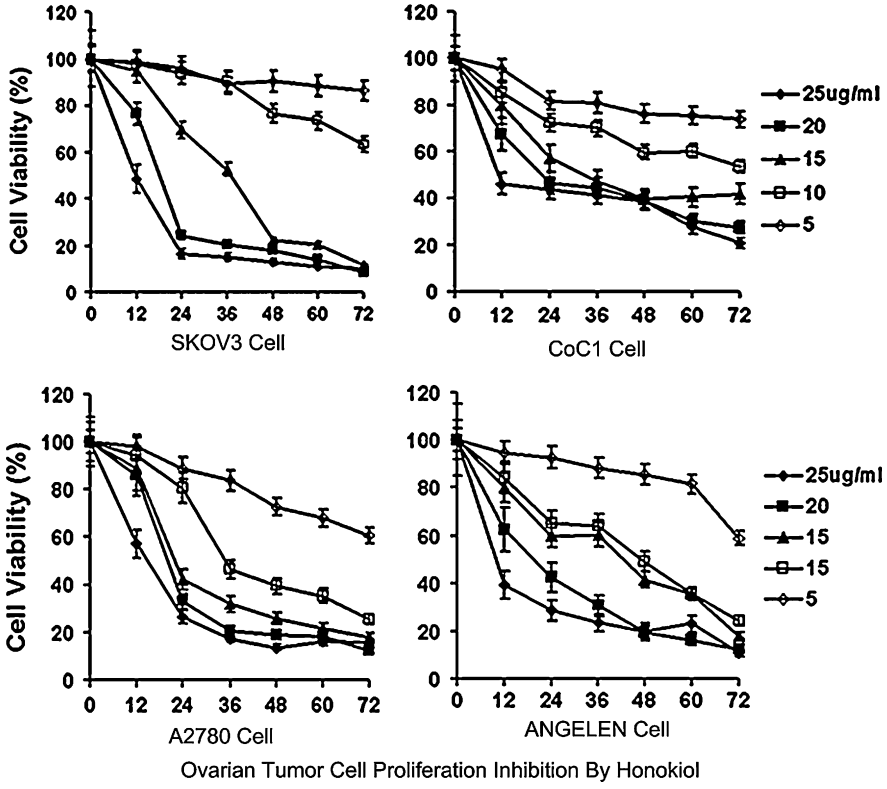

Fig. 1. Inhibition of cell proliferation by honokiol was assessed by MTT assay. SKOV3, Coc1, A2780, and Angelen cells were cultured with honokiol (5, 10, 15, 20, and 25 μg/ml) for 72 h. Cell proliferation was expressed as a percentage of viable cells cultured in the absence of honokiol, and shown as mean ± S.D. Results were representative of three separate experiments.

To characterize the apoptosis in cells, DNA fragmentation assays were performed. As shown in Fig. 2A, 24 h treatment with 15 μg/ml honokiol resulted in degradation of chromosomal DNA into small internucleosomal fragments, a hallmark of cells undergoing apoptosis. In addition, the changes of nuclear morphology in SKOV3 cells were detected by Hoechst fluorescence staining, which revealed that the number of smaller nuclei and condensed chromatin increased in dose- and time-dependent manners, and most apoptotic nuclei containing condensed chromatin were observed in 15 μg/ml for 36 h (Fig. 2B; ANOVA, $p < 0.01$). These results showed that honokiol-induced apoptosis in SKOV3 and Coc1 cells. Cell cycle analysis by flow cytometry also revealed the significant augmentation of sub-G_0/G_1 cells accompanied with increase in dose, reaching the peak value in 15 μg/ml (Fig. 3; ANOVA, $p < 0.01$).

3.2. Involvement of Bcl-2 family and caspase-3

RT-PCR assay revealed that treatment with honokiol led to significant down-regulation of Bcl-X_L and up-regulation of Bad in a time-dependent manner. No apparent changes of other members were observed, including Bcl-2, Bak, Bax, and Bid (Fig. 4A).

Western blotting assay revealed a significant time-dependent decrease of 32 kDa caspase-3 in SKOV3 and Coc1 cells followed by honokiol treatment, suggesting that honokiol caused the proteolytic activities of caspase-3 precursor into active subunits. As shown in Fig. 4B, activation of caspase-3 was first observed at 24 h, reaching a maximum at 48 h. These results indicated the involvement of Bcl-2 members and caspase cascade in honokiol-induced apoptotic process.

3.3. Honokiol inhibits tumor growth and angiogenesis in vivo

Tumor volumes were measured every 8 days after SKOV3 cells inoculation and significant differences observed during the treatment session. As shown in Fig. 5A, all tumor volumes increased over the duration, but the group treated with liposome-encapsuled honokiol presented significantly lower growth rate in comparison with the control groups. Until 56 days after inoculation, tumor volumes in control groups treated with PBS or lipofectamine (LIPO) reached approximately 3653 ± 465 or 3297 ± 416 mm^3, respectively; whereas, in treatment group were 1060 ± 298 mm^3, only 30–33% of controls. These data indicated the significant inhibition of tumor growth by honokiol (Dunnet t-test, $p < 0.01$), and no significant difference between PBS and LIPO groups (Student's t-test, $p > 0.05$). During the therapeutic session, no evident changes in gross measures were observed, including weight loss, feeding, and behavior, etc.

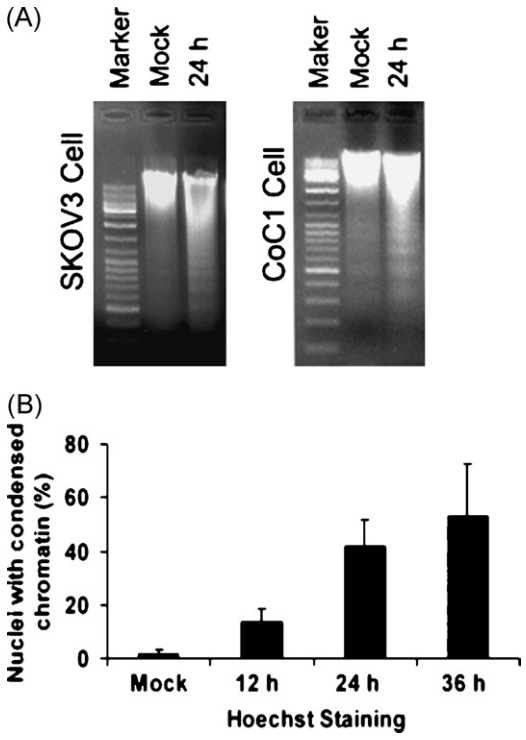

Fig. 2. (A) Induction of apoptosis by honokiol was assessed by DNA ladder. SKOV3 and Coc1 cells were cultured with 15 μg/ml honokiol for 24 h, and resulted in formation of DNA ladder on 2% agarose gel. Lane 1: DNA ladder marker; lane 2: control culture; lane 3: 15 μg/ml honokiol treatment for 24 h. (B) SKOV3 cells were cultured with 15 μg/ml honokiol for 12, 24, and 36 h, and the increase of smaller nuclei and condensed chromatin, in time-dependent manner, was visualized by Hoechst staining under a fluorescence microscope.

Angiogenesis was evaluated by microvessel density assay of frozen sections stained against an antibody reactive to CD31, with high specific affinity for vascular endothelial cells. A significant lower MVD was observed in treatment group, compared with control groups (Fig. 5B; Dunnet-*t* test, $p < 0.01$). The number of microvessels per vascular hot spot was 17.5 ± 2.5 in treatment group, 47.8 ± 6.7 and 43.7 ± 6.1 in PBS and LIPO groups, respectively, which showed that MVD in treatment group was at least two times lower than in control groups. In addition, VEGF expressions in the tumor tissues were also examined by immunoblotting against VEGF, and the results showed a remarkable decrease of VEGF expression in honokiol-treated group, in comparison with the PBS or LIPO group, as quantified by Quantity One 4.6 software (Fig. 5C; PBS group: 54.7; LIPO group: 51.8; treatment group: 17.4; Dunnet *t*-test, $p < 0.01$).

4. Discussion

A number of chemotherapeutic agents, with properties including apoptosis induction and anti-angiogenesis, have been isolated from natural products and characterized to inhibit the development of malignancies, such as curcumin from Curcuma longa [9,10], epicatechin gallate from tea [11] and paclitaxel from Pacific yew [12,13]. Honokiol, a biphenyl extract from *Magnolia obovata* bark, has been reported to induce apoptosis in a number of tumor cell lines [3–6]. Furthermore, inhibition of transformed endothelial SVR cells growth [14] and tube formation by human umbilical vein endothelial cells might indicate its anti-angiogenic effect [15].

In the present study, we investigated the effects of honokiol on ovarian tumor cells. Our results showed significant proliferation inhibitions with 50% inhibition (IC_{50}) at 24 h of 14–20 μg/ml, and apoptosis induction confirmed by DNA ladder and Hoechst staining assays. Furthermore, flow cytometry assay revealed that these effects were accompanied with G_1 arrest in the cell cycle. Consistently, a recent study about breast cancer also suggested that honokiol treatment resulted in cell cycle

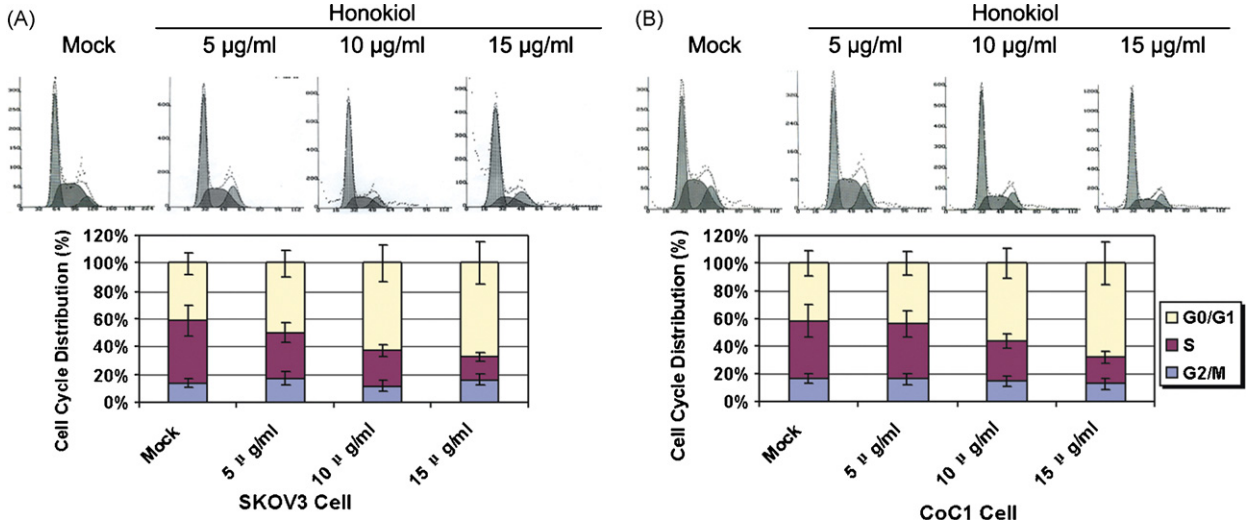

Fig. 3. Effect of honokiol on cell cycle distribution was analyzed by flow cytometry. Treatment with 5, 10, and 15 μg/ml honokiol for 24 h led to significant G_1 arrest in SKOV3 (A) and Coc1 (B) cells. Results were representative of three separate experiments.

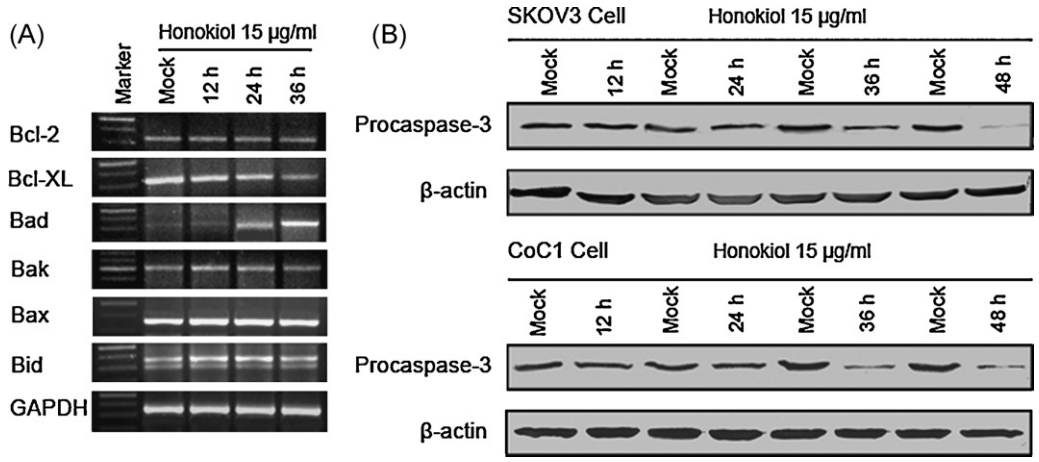

Fig. 4. (A) Expression of Bcl-2 members was assessed by semi-quantitative RT-PCR. SKOV3 cells were cultured with 15 μg/ml honokiol for 12, 24, and 36 h. Results showed the significant up-regulation of Bad and down-regulation of Bcl-X_L, and no apparent changes of other members were observed, normalized by G3PDH gene. (B) Caspase-3 activation was assessed by Western blot assay. SKOV3 (a) and Coc1 (b) cells were cultured with 15 μg/ml honokiol for 12, 24, 36, and 48 h. Results showed the decrease of 32KD caspase-3 precursor in time-dependent manner, indicating its activation via the proteolytic activities into active subunits.

arrest at lower dose and resulted in induction of apoptosis at higher dose [16]. Furthermore, honokiol also could activate the p38 MAP kinase pathway and led to G_1 arrest and growth inhibition in vascular smooth muscle cells [17], as well as in cancer cells.

In our results, the honokiol-dependent apoptosis was accompanied with significant increase of Bad and decrease of Bcl-X_L. Bad promoted apoptosis by hetero-dimerization with Bcl-2 or Bcl-X_L, and sequential release of free Bax [18]. Bcl-X_L, a mitochondrial membrane protein [19], prevented apoptosis by blocking the release of mitochondrial cytochrome C and association with caspase-1, 8, 9 [20,21]. Similar outcomes were obtained in lung cancer CH27 cells [4]; whereas, no similar results were obtained in colorectal cancer RKO cells [3] and multiple myeloma cells [22]. Therefore, whether regulations of Bcl-2 members' expressions by honokiol are dependent on cell types and, if so, the exact roles of these proteins in honokiol-induced apoptosis remain to be elucidated. In addition, no apparent alterations of Bcl-2 or Bax expression were shown from our data. Since pro-apoptotic Bax was a P53 downstream target, the lack of change of its expression suggested that honokiol probably should not trigger the P53-mediated apoptosis pathway, which had also been indicated by previous study on colorectal cancer RKO cell [3].

Increase in the ratio of Bad/Bcl-X_L could lead to the release of cytochrome C from mitochondria into cytoplasm, and sequentially, the formation of apoptosome and the activation of caspase cascade. In the present study, consistent with the observation in prostate cancer cells [23], it was observed that the caspase-3 precursor expression decreased significantly followed by honokiol treatment, suggesting the proteolytic activities of caspase-3 precursor into active subunits caused by honokiol. Moreover, it was notable that the Bcl-2 members' changes and caspase-3 activation started to occur 12 and 24 h after honokiol treatment, respectively, suggesting the alterations of Bcl-2 members should participate in the caspases activation, and the honokiol-induced apoptosis was caspase-dependent. In other studies, with complete inhibition of caspase-3 activation by pretreatment with z-VAD-fmk, inhibition of honokiol-induced cytotoxicity and apoptosis was only partial, and honokiol also induced apoptosis in caspase-3-deficient cells [22]. These results indicated that both caspase-dependent and -independent pathways would be involved in honokiol-induced apoptosis.

Besides its potent activities to induce apoptosis, honokiol could also be demonstrated to play anti-tumor roles from some other mechanisms, such as enhancement of retinoid-induced differentiation of leukemia cells [5], induction of mitochondrial permeability transition pore [24], activation of poly-adenosine diphosphate ribose polymerase [23], etc. Furthermore, synergistic effects of honokiol with commonly used chemotherapeutics, and sequentially, even overcoming chemotherapy resistance to MDR breast cancer MCF-7/ADR cells, was also discovered, probably due to down-regulation of P-glycoprotein [25]. The activities of honokiol against multiple anti-tumor targets should be studied intensively for further clinical application either as a natural agent alone, or in combination with other commonly used chemotherapeutics.

A variety of natural products have been reported previously to have potent anti-tumor activities *in vitro*, but the effects *in vivo* remain poor. From our data, it was well established that honokiol could inhibit ovarian tumor cell proliferation and induce apoptosis *in vitro*, but its activities remained unknown *in vivo*. In order to obtain insight into the activity of honokiol *in vivo*, a SKOV3 tumor xenograft nude mice model was constructed. The dose and schedule of honokiol administration were well tolerated by all mice. It was noteworthy that, consistent with the outcomes obtained *in vitro* in cultured ovarian tumor cells, administration of

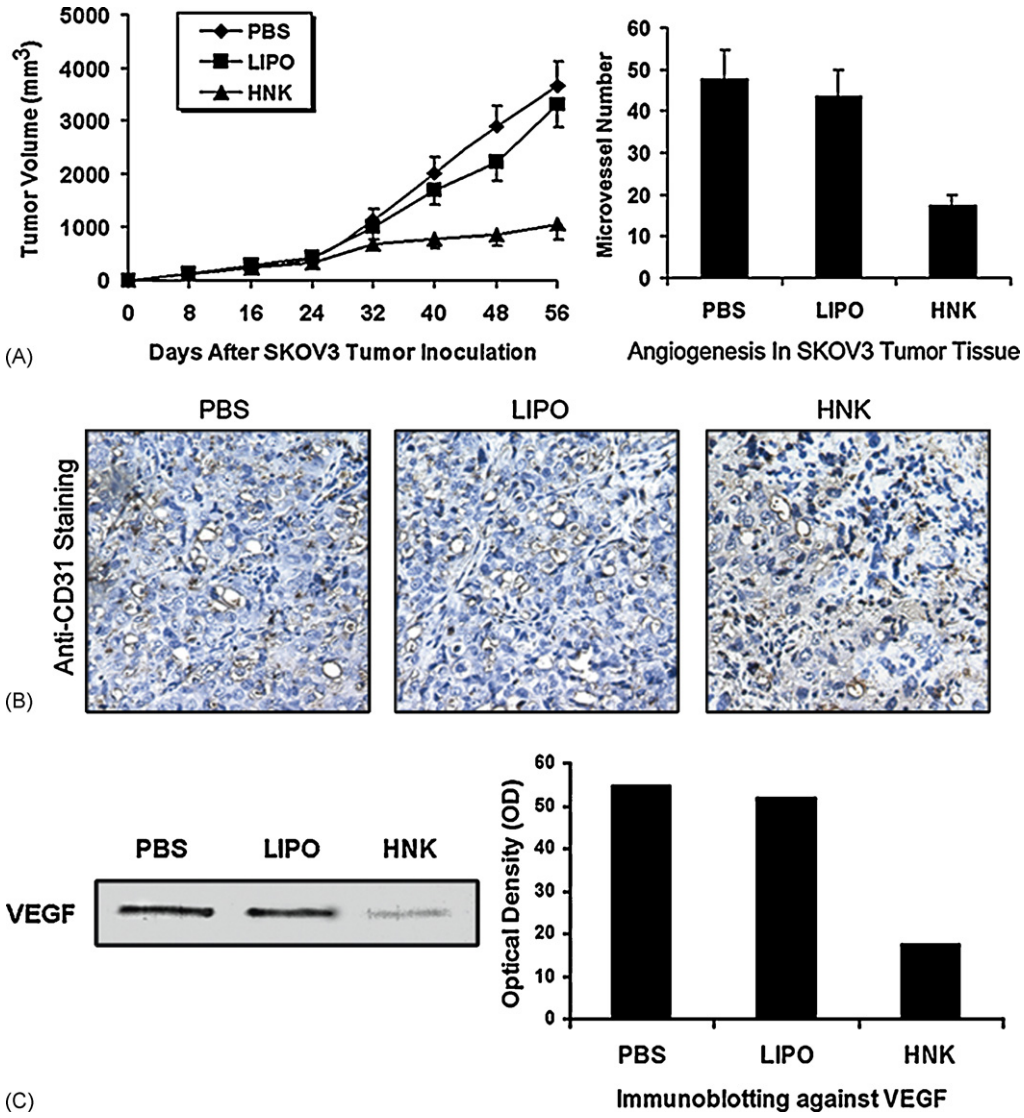

Fig. 5. (A) Tumor growth inhibition by honokiol. Eight days after SKOV3 cells inoculation, mice were assigned randomly and treated as follows: (1) PBS, 100 μl; (2) 0.6 mg liposome/100 μl PBS; (3) 1 mg liposome-encapsuled honokiol (40%)/100 μl PBS for each mouse by intraperitoneally injection every day, as described in text. Tumor volumes were measured every 8 days. Over the therapeutic session, treatment resulted in tumor volume inhibition (Dunnet *t*-test, $p < 0.01$), compared with the two controls. Data were shown as mean ± S.D. (B) Inhibition of angiogenesis by honokiol was assessed by microvessel density (MVD) in tumor tissues. The microvessel endothelial cells, with specific reaction to CD31 antibody, were brown-stained and visualized in microscopy. MVD was determined by counting the number of microvessels, as described in text. The number of microvessels was significantly lower in the honokiol-treated group compared with the control groups (Dunnet *t*-test, $p < 0.01$), with no difference observed between two control groups (Student's *t*-test, $p > 0.05$). Data were shown as mean ± S.D. (C) Expression level of VEGF in tumor tissues was examined using immunoblotting analysis, quantified as optical density (OD). Expression of VEGF significantly decreased in treatment group compared with the control groups (Dunnet *t*-test, $p < 0.01$).

honokiol was shown to result in significant suppression of tumor growth. Over the course of treatment session all tumor volumes increased, but the tumors in mice treated with honokiol presented growth rate remarkably smaller, only 30–33% of those with PBS or LIPO ($p < 0.05$). During treatment session, in addition, no evident changes in gross measures, such as weight loss, feeding, or other signs of possible side effects, were observed, indicating that honokiol treatment seems to be lack of toxicity and well-tolerated *in vivo*, at least at the present dose we used.

Angiogenesis was also known to be essential for solid tumor growth and metastasis, and consequently became an attractive target against anti-tumor treatment. To obtain the potential role of honokiol in angiogenesis, we observed the microvessel density in dissected tumor tissues by anti-CD31 immunostaining. Our results showed that the MVD were significantly decreased when treated with honokiol ($p < 0.05$), in comparison with those controls with PBS or LIPO, suggesting that the potent anti-tumor efficiency of honokiol *in vivo* should be partially due to the anti-angiogenic activities. Among the molecules with specific effects on vascular endothelial cells, VEGF is considered to be the most powerful and specific growth factor responsible for tumor angiogenesis [26]. Thus, we examined the

expression of VEGF in tumor tissues, with the aim to evaluate the potential role of VEGF in the honokiol-induced anti-angiogenic effects. Our data showed a significant decrease of VEGF expression in honokiol-treated group, accompanied with decreased MVD counting. Another similar study also indicated that this decrease was involved in the modulation of nuclear factor-kappaB activation pathway [27]. Additionally, other studies showed that honokiol inhibited angiogenesis by interfering with VEGFR2 autophosphorylation [14] and subsequently blocking the VEGF-induced Rac activation [28], which is required for endothelial cell migration and proliferation [29]. In addition, regulation of platelet-derived endothelial cell growth factor and TGF-β expression [30], inhibition of nitric oxide synthesis and TNF-α [31] might be involved in this event. Further studies will be conducted to elucidate the intensive mechanism of honokiol in angiogenesis.

Taken together, honokiol, a natural compound, has remarkable activities both *in vivo* and *in vitro* on ovarian tumors. Honokiol could significantly suppress cell proliferation and induce apoptosis in ovarian tumor cells, and restrained tumor growth and inhibited angiogenesis *in vivo*, with no indication of evident cytotoxicity. These results demonstrated honokiol, with both anti-tumor and anti-angiogenesis properties, might be a novel and attractive therapeutic candidate for ovarian tumor treatment in clinical practice, with potential effectiveness by various activities.

Acknowledgments

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References

[1] Teng CM, Chen CC, Ko FN, et al. Two antiplatelet agents from *Magnolia officinalis*. Thromb Res 1988;50(6):757–65.
[2] Kuribara H, Stavinoha WB, Maruyama Y. Behavioural pharmacological characteristics of honokiol, an anxiolytic agent present in extracts of Magnolia bark, evaluated by an elevated plus-maze test in mice. J Pharm Pharmacol 1998;50(7):819–26.
[3] Wang T, Chen F, Chen Z, et al. Honokiol induces apoptosis through p53-independent pathway in human colorectal cell line RKO. World J Gastroenterol 2004;10(15):2205–8.
[4] Yang SE, Hsieh MT, Tsai TH, Hsu SL. Down-modulation of Bcl-X_L, release of cytochrome c and sequential activation of caspases during honokiol-induced apoptosis in human squamous lung cancer CH27 cells. Biochem Pharmacol 2002;63(9):1641–51.
[5] Fong WF, Tse Anfernee KW, Poon KH, Wang C. Magnolol and honokiol enhance HL-60 human leukemia cell differentiation induced by 1,25-dihydroxyvitamin D_3 and retinoic acid. Int J Biochem Cell Biol 2005;37(2):427–41.
[6] Hibasami H, Achiwa Y, Katsuzaki H, et al. Honokiol induces apoptosis in human lymphoid leukemia Molt 4B cells. Int J Mol Med 1998;2(6):671–3.
[7] Hoskins WJ, Mcguire WP, Brady MF, et al. The effect of diameter of largest residual disease on survival after primary cytoreductive surgery in patients with suboptimal residual epithelial ovarian carcinoma. Am J Obstet Gynecol 1994;174(4):974–9.
[8] Wu Y, Li ZY, Zhao X, Kan B, Wei YQ. Inhibition of ovarian tumor growth by gene therapy with recombinant soluble vascular endothelial growth factor receptor 2. Hum Gene Ther 2006;17(9):941–8.
[9] Chakraborty S, Ghosh U, Bhattacharyya NP, Bhattacharya RK, Roy M. Inhibition of telomerase activity and induction of apoptosis by curcumin in K-562 cells. Mutat Res 2006;596(1/2):81–90.
[10] Singh S, Khar A. Biological effects of curcumin and its role in cancer chemoprevention and therapy. Anticancer Agents Med Chem 2006;6(3):259–70.
[11] Baek SJ, Kim JS, Jackson ER, Eling TE, McEntee MF, Lee SH. Epicatechin gallate-induced expression of NAG-1 is associated with growth inhibition and apoptosis in colon cancer cells. Carcinogenesis 2004;25(12):24425–32.
[12] Young M, Plosker GL. Paclitaxel: a pharmacoeconomic review of its use in the treatment of ovarian caner. Pharmacoeconomics 2001;19(12):1227–59.
[13] Symmans FW. Breast cancer response to paclitaxel in vivo. Drug Resist Update 2001;4(5):297–302.
[14] Bai X, Cerimele F, Ushio-Fukai M, et al. Honokiol, a small molecular weight natural product, inhibits angiogenesis in vitro and tumor growth in vivo. J Biol Chem 2003;278(37):35501–7.
[15] Chen F, Wang T, Wu YF, et al. Honokiol: a potent chemotherapy candidate for human colorectal carcinoma. World J Gastroenterol 2004;10(23):3459–63.
[16] Wolf I, O'Kelly J, Wakimoto N, et al. Honokiol, a natural biphenyl, inhibits in vitro and in vivo growth of breast cancer through induction of apoptosis and cell cycle arrest. Int J Oncol 2007;30(6):1529–37.
[17] Beobyi L, Cheorl HK, Sung KM. Honokiol causes the p21WAF1-mediated G(1)-phase arrest of cell cycle through inducing p38 mitogen activated protein kinase in vascular smooth muscle cells. FEBS Lett 2006;580(22):5177–84.
[18] Yang E, Zha J, Jockel J, Boise LH, Thompson CB, Korsmeyer SJ. Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. Cell 1995;80(2):285–91.
[19] Gonzalez GM, Perez BR, Ding L, et al. Bcl-XL is the major bcl-x mRNA form expressed during murine development and its product localizes to mitochondria. Development 1994;120(10):3033–42.
[20] Hu Y, Benedict MA, Wu D, Inohara N, Nunez C. Bcl-XL interacts with Apaf-1 and inhibits Apaf-1-dependent caspase-9 activation. Proc Natl Acad Sci USA 1998;95(8):4386–91.
[21] Chinnaiyan AM, O'Rourke K, Lane BR, Dixit VM. Interaction of CED-4 with CED-3 and CED-9: a molecular framework for cell death. Science 1997;275(5303):1122–6.
[22] Ishitsuka K, Hideshima T, Hamasaki M, et al. Honokiol overcomes conventional drug resistance in human multiple myeloma by induction of caspase-dependent and independent apoptosis. Blood 2005;106(5):1794–800.
[23] Shigemura K, Arbiser JL, Sun SY, et al. Honokiol, a natural plant product, inhibits the bone metastatic growth of human prostate cancer cells. Cancer 2007;109(7):1279–89.
[24] Li L, Han WD, Gu Y, et al. Honokiol induces a necrotic cell death through the mitochondrial permeability transition pore. Cancer Res 2007;67(10):4894–903.
[25] Xu D, Lu QH, Hu X. Down-regulation of P-glycoprotein expression in MDR breast cancer cell MCF-7/ADR by honokiol. Cancer Lett 2006;243(2):274–80.
[26] Chodorowska G, Chodorowski J, Wysokinski A. Vascular endothelial growth factor (VEGF) in physiological and pathological conditions. Ann Univ Mariae Curie Sklodowska 2004;59(3):8–14.
[27] Ahn KS, Setti G, Shishodia S, Sung B, Arbiser JL, Aggarwal BB. Honokiol potentiates apoptosis, suppresses obsteoclastogenesis, and inhibits invasion through modulation of nuclear factor-kappaB activation pathway. Mol Cancer Res 2006;4(9):621–33.

[28] Zeng H, Dvorak HF, Mukhopadhyay D. Vascular permeability factor (VPF)/vascular endothelial growth factor (VEGF) receptor-1 down-modulates VPF/VEGF receptor-2-mediated endothelial cell proliferation, but not migration, through phosphatidylinositol 3-kinase-dependent pathways. J Biol Chem 2001;276(29):26969–7.

[29] Soga N, Connolly JO, Chellaiah M, Kawamura J, Hruska KA. Rac regulates vascular endothelial growth factor stimulated motility. Cell Commun Adhes 2001;8(1):1–13.

[30] Lee BC, Doo HK, Lee HJ, et al. The inhibitory effects of aqueous extract of *Magnolia officinalis* on human mesangial cell proliferation by regulation of platelet-derived growth factor-BB and transforming growth factor-beta 1 expression. J Pharmacol Sci 2004;94(1): 81–5.

[31] Son HJ, Lee HJ, Yun Choi HS, Ryu JH. Inhibitors of nitric oxide synthesis and TNF-alpha expression from *Magnolia obovata* in activated macrophages. Planta Med 2000;66(5):469–71.