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Review article

Angelica sinensis: A Chinese herb for brain cancer therapyPo-Cheng Lin^{a,b,1}, Po-Yen Liu^{c,1}, Shinn-Zong Lin^{b,d,**}, Horng-Jyh Harn^{e,f,*}^a Department of Stem Cell Applied Technology, Gwo Xi Stem Cell Applied Technology, Hsinchu, Taiwan^b Center for Neuropsychiatry, China Medical University and Hospital, Taichung, Taiwan^c Graduate Institute of Chinese Medical Science, China Medical University, Taichung, Taiwan^d Department of Neurosurgery, China Medical University Beigan Hospital, Yunlin, Taiwan^e Department of Medicine, China Medical University, Taichung, Taiwan^f Department of Pathology, China Medical University and Hospital, Taichung, Taiwan

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ABSTRACT

Herbs are an important aspect of traditional Chinese medicine, as well as a rich source of unique chemicals. Among the medicinal herbs, *Angelica sinensis* is the most popular used in Chinese medicine. The main compounds found in the acetone extract of *Angelica sinensis* (AS-AC) are ferulic acid, ligustilide, brefeldin A, butylidenephthalide, and polysaccharides, the latter with potential therapeutic effect on various human cancers. Based on molecular evidence from *in vitro* and *in vivo* studies, we discuss here how butylidenephthalide suppresses tumor cell proliferation and promotes tumor cell apoptosis. The molecular mechanisms involved include butylidenephthalide-stimulated translocation of Nur77 from the nucleus to the cytoplasm, leading to tumor apoptosis. Butylidenephthalide likewise suppresses telomerase activity, resulting in tumor senescence. Finally, a controlled release system can increase localized butylidenephthalide concentration. Importantly, butylidenephthalide can cross the blood–brain barrier. Current evidence suggests its efficacy against brain tumors and therefore potential clinical applications.

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1. Introduction

For centuries, natural plant remedies (many of them obtained from herbs) have been used to treat disease and maintain health, especially in China. More than 3200 herbs and 300 minerals (and even animal extracts) are routinely applied to patients as a mixture or formula. Practitioners of traditional Chinese medicine believe that disease primarily arises from imbalances in the body. The therapeutic purpose of a Chinese

doctor is therefore to bring the human body back into equilibrium.

Herbal drugs are used with the goal of restoring this balance by nourishing the body, including the energy, *qi* (breath circulation), and spirit, to maintain health rather than to treat a particular disease or medical condition. This mindset reflects an emphasis on preventive medicine. Treatments undertaken with this goal are called *Fu Zheng* and are given as complementary therapy intended to reduce the side effects of

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conventional Western medical treatment. Chinese herbal medicine is independent of conventional Western concepts of medical diagnosis and treatment.

One aspect of Chinese medicine is to bolster resistance to disease by strengthening a person's immunity. Chinese herbs attempt to prevent and treat physiological imbalances, such as those caused by cancer and other diseases, with combinations of herbs, minerals, and plant extracts. Diseases that cannot be cured by modern medical treatments, such as diabetes and cancer, may be alleviated by traditional Chinese medicine.

2. *Angelica sinensis* (dong quai) as a traditional Chinese medicine

Dong quai, the root of *Angelica sinensis*, is the most popular herbal medicine in China, present in many formulas that have been used over thousands of years. It mainly treats gynecological conditions, menstrual disorders, anemia, coronary heart disease, carbuncles, and sores. Several extracts or single compounds from *Angelica sinensis* have been investigated for their potential to increase myocardial blood flow, reduce radiation damage, and improve kidney function [1–4]. Polysaccharides, its main components, demonstrably protect against gastrointestinal damage and hepatic injury [5–8]. Diverse components from *Angelica sinensis* may thus have myriad pharmacological activities.

Natural products, such as plants, microorganisms, and marine life, are abundant sources of anticancer drugs [9]. In *Angelica sinensis*, the major compounds are ferulic acid, ligustilide, brefeldin A, butylidenephthalide, and polysaccharides [10]. Ferulic acid and ligustilide serve as chemical markers to assess the quality of *Angelica sinensis* plants [10]. In another report, Z-ligustilide was studied for its potential cytotoxicity against two tumor types, L1210 and K562 [11]. Butylidenephthalide is used as a standard profile for identifying *Ligusticum chuanxiong*. Ferulic acid can inhibit platelet aggregation, whereas ligustilide has antiasthmatic activity [12,13].

Angelica sinensis has been used to relieve the side effects of radiation treatment on lung tissue. Other studies highlight the potential clinical application of *Angelica sinensis* extracts, e.g., to inhibit the progress of radiation-induced pulmonary fibrosis by downregulating tumor necrosis factor alpha (TNF- α) and transforming growth factor beta-1 [14–16].

3. *Angelica sinensis* in anticancer studies

To test the antitumor effects of *Angelica sinensis* extracts, Cheng et al used bio-based assays with different human cancer cell lines [17]. Their study showed that AS-AC had a dose-dependent antiproliferative effect on human cancer cell lines derived from the lung (A549), brain (DBTRG-05MG), liver (J5), and colorectal tissue (HT29). Their results also demonstrated that the AS-C induced the arrest of human cancer cells and activated the mechanism of apoptosis. In human brain tumors, both AS-AC and AS-C significantly inhibited proliferation by 30–50%; both treatments also suppressed cathepsin B and vascular endothelial growth factor

expression. In an animal study, AS-AC or AS-C alone suppressed tumor growth by 30% and 60%, respectively [18]. Further, AS-AC and AS-C were able to inhibit microvessel formation in tumors in nude mice [18].

In 1994, Choy and colleagues identified a low-molecular-weight polysaccharide from the *Angelica sinensis* rhizome and proved its strong antitumor and immunostimulatory activities [19]. Additional polysaccharides have since been identified from *Angelica sinensis*: APS-1, APS-3a, APS-3b, and APS-3c [20,21]. These display diverse structural features and antitumor activities: e.g., the backbone of APS-1 consists of (1,4)- α -D-glucopyranosyl residues, its branches being (1,6)- α -D-glucopyranosyl residues with a terminal β -L-arabinofuranose residue. Notably, APS-1 had antitumor effects *in vitro*, especially against human cervical cancer HeLa cells [20]. This antitumor activity coincided with a greater expression of mRNAs encoding interferon-gamma, interleukin-2, and interleukin-6 in splenocytes, as well as greater nitric oxide and TNF-gamma production in macrophages [21].

Butylidenephthalide was recently identified as the active component of AS-AC [22]. Natural phthalide compounds such as butylidenephthalide are considered to be candidate antitumor agents [23,24]. Indeed, butylidenephthalide has proven anticancer potential against colon cancer. As well as its antitumor effect, butylidenephthalide has demonstrated an ability to prevent benzo[a]pyrene-induced forestomach cancer in mice [25].

Butylidenephthalide and other agents (senkyunolide A and Z-ligustilide) act synergistically to reduce tumor cell proliferation [26]. A synergistic antiproliferative effect is noted when butylidenephthalide is combined with the chemotherapeutic drug 1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine or BCNU) [27]. This synergism is mediated by downregulation of the gene MGMT, which encodes the DNA repair enzyme O-6-methylguanine-DNA methyltransferase [27].

4. Antitumor effects of AS-C *in vitro* and *in vivo*

Throughout its long record of use in traditional Chinese medicine, *Angelica sinensis* has exhibited many biological activities, e.g., immune system regulation, relief from menopausal symptoms, and improvement in myocardial blood flow [28–33]. Currently, few drugs treat malignant brain tumors, partly owing to the difficulty of producing drugs that cross the blood–brain barrier.

Tsai et al [34] were the first to confirm that AS-C displayed strong activity against glioblastoma (GBM) *in vitro* and *in vivo*. In *in vitro* cytotoxic assays, AS-C exhibited efficacy against not only brain tumors, but also other tumor cells; normal fibroblast cells were resistant to AS-C in the same experiment. Importantly, there was no evidence of AS-C-induced cytotoxicity in either the liver or kidney after injection of 500 mg/kg AS-C (either intraperitoneally or subcutaneously). In that study, AS-C had better anticancer capacity than carmustine and proved less cytotoxic to normal cells than Taxol [34].

In clinical studies, various antitumor drugs, such as temozolomide, only slightly extended survival time for patients with GBM [29,35–42]. In line with the *in vitro* cytotoxic

results, AS-C had a correspondingly greater cytotoxic effect on GBM cells than on other tumor cells. In a subcutaneous tumor model, AS-C inhibited rat GBM tumor growth and extended survival, especially reducing the tumor volume of rat GBM *in situ* [34], suggesting that AS-C crosses the blood–brain barrier and inhibits GBM cell growth. As such, it inhibits GBM tumor growth and induces GBM tumor cell apoptosis, as demonstrated both *in vitro* and *in vivo*.

Further, human GBM cells may have a different metabolism than rat GBM cells, as a result possessing a distinct susceptibility to AS-C treatment. AS-C can also inhibit human GBM tumor growth in a subcutaneous tumor model [34]. These *in vivo* cytotoxicity studies suggest that AS-C should be further developed to yield effective and safe anti-GBM drugs.

5. Butylidenephthalide: *in vitro* and *in vivo* antitumor effect of *Angelica sinensis*

The antitumor activity of AS-C against human GBM [34] made it critical to isolate the antitumor components from AS-C. The major ones were found to be BP (molecular weight 188.22 Dalton) and K2 (molecular weight 190.23 Dalton). Butylidenephthalide emerged as key anticancer component (>30% of crude AS-C) [22]. Our study shows that BP induces cell cycle arrest (at G0/G1) and apoptosis in GBM and hepatocellular carcinoma [22,43,44]. BP promotes apoptosis through both the p53-dependent and p53-independent pathways *in vitro*. In an *in situ* rat model of GBM, BP inhibited tumor growth while decreasing GBM volume, suggesting an anticancer potential [22,43,44].

Studies from the Harn group indicate that *Angelica sinensis* suppresses telomerase activity [22,43,44]. BP may target telomerase to effect its dramatic antitumor activity both *in vitro* and *in vivo*. To test this hypothesis, we monitored its inhibition of telomerase activity in brain cancer cell lines DBTRG-05MG and GBM 8401. BP did indeed inhibit telomerase mRNA transcription and reduced telomerase protein expression in these cells, whereas the expression of the telomerase RNA component was not affected by BP treatment. Reduced telomerase expression emanated from transcriptional inhibition of human telomerase reverse transcriptase, with a consequent reduction in telomerase activity. Results suggest that BP inhibits GBM by downregulating both telomerase expression and activity [45].

6. Antitumor effects of BP in animal models of autologous GBM

This activity has been probed *in vivo* in animal experiments. BP inhibited the growth of RG2, a spontaneous GBM tumor type in rat, whereas tumors continued to grow in the control group treated with the vehicle [22]. There was no significant intergroup variance in body weight [22]. The expression of Ki-67 apparently declined, indicating BP's antiproliferation activity *in vivo*. On the other hand, the level of the apoptotic marker cleaved caspase 3 increased after BP treatment, with a consequent apoptosis of tumor cells *in vivo* [22].

Tsai and co-workers implanted RG2 tumors into rat brain and subsequently treated the animals with BP. Magnetic resonance imaging revealed a significant reduction in tumor size upon subcutaneous administration of BP (300 mg/kg/d) for 5 days [22]. This result is similar to those obtained with a subcutaneous RG2 tumor model, suggesting that BP exhibited equivalent potency across the blood–brain barrier. Finally, on Day 19, the BP-treated group had a significantly higher survival rate (50%; 3/6) than the control group (16.7%; 1/6).

7. Antitumor effect of BP in a nude mouse model of heterologous human GBM

The antitumor effect of BP has been tested in a human GBM xenograft nude mouse model. After mice were grafted with human GBM, BP was administered subcutaneously in larger doses (70, 150, 300, 500, and 800 mg/kg) from Days 1 to 5. As noted in the RG2 experiments, tumor growth was significantly suppressed in all five dosage groups compared with the control group.

At 200 days post-treatment, survival rates were 16.7% (1/6), 33.3% (2/6), 50% (3/6), 80% (4/5) and 100% (6/6) for the respective BP dosage groups [22]. In the control group (vehicle only), the average tumor size was over 1000 mm³ compared with 171.7 mm³ in the 800 mg/kg BP treatment group. In this nude mouse model, survival at Day 200 was significantly prolonged by BP treatment. The survival rate in the various groups was as follows: untreated, 0% (0/6); 70 mg BP, 20% (1/5); 300 mg BP, 50% (3/6); 800 mg BP, 83.3% (5/6). Immunohistochemical staining for cleaved caspase 3 indicated an increased expression of this protein, while that of Ki-67 decreased, hinting that BP can inhibit tumor proliferation and induce tumor cell apoptosis. Collectively, this evidence indicates that BP can decrease tumor size and increase survival rate in mice.

Finally, after 200 days, there were no changes in the specific histology of the hollow organs or in body weight, even in the highest dosage group (800 mg/kg BP for 5 consecutive days). This suggests that BP has no acute or subacute toxicity.

8. Assessing target genes for BP using oligodeoxynucleotide-based microarrays

To investigate the gene(s) targeted by BP in malignant brain tumors, a BP-treated GBM cell line was subjected to RNA microarray analysis after 3 hours or 24 hours of treatment. Subsequently, western blotting and real-time polymerase chain reaction confirmed the microarray results compared with the vehicle group (the tumor cell line treated with only dimethyl sulfoxide). Interesting, among the 30,000 genes assessed, 422 were upregulated in response to BP (49 in 3 hours, and 373 in 24 hours).

Subsequent clustering analysis highlighted a particular family of receptor-encoding genes that included NOR-1, Nurr-1, and Nur77. The proteins encoded by these genes have a similar structure; because they are orphan receptors, however, their ligand(s) are unknown [46]. NOR-1, Nurr-1, and Nur77 are early response genes, and their encoded receptors can be induced by apoptotic stimuli, serum, growth factors,

and ligand-binding [47–49]. Nur77, NOR-1, and Nurr-1 are reportedly related to tumor apoptosis and cell growth [50].

Moreover, the mechanism of Nur77-mediated apoptosis in T-cells and several cancers has been extensively studied [46,47,51–53]. There are two proposed mechanisms for Nur77-mediated tumor cell apoptosis. First, upon translocation of Nur77 from the nucleus to the cytoplasm and mitochondria, as well as the conversion of Bcl-2 into its phosphorylated form, cytochrome c is released into the cytoplasm, which leads to tumor cell apoptosis. This mechanism has been reported in prostate cancer and other cancers [46,51].

Second, Nur77 can act as a transcription factor that binds the promoters of the apoptosis genes, e.g., those encoding Fas-ligand, the Nur77 downstream gene 1, and the Nur77 downstream gene 2; Nur77 also participates in TNF-related apoptosis [54–56]. Many chemicals induce apoptosis mediated by Nur77 overexpression, e.g., retinoid-related 6-[3'-(1-adamantyl)-4'-hydroxyphenyl]-2-naphthalenecarboxylic acid (also known as CD437), tetradecanoylphorbol-1, 3-acetate, etoposide (VP-16), cisplatin, and insulin-like growth factor binding protein-3 [36,46,52,57].

9. BP-induced Nur77 nucleo-cytoplasmic translocation leads to tumor cell apoptosis

To determine the role of Nur77 in BP-induced cancer cell apoptosis, Lin and co-workers used a fluorescently labeled Nur77-specific antibody to trace the location of Nur77, reporting that Nur77 was translocated from nucleus to cytoplasm for 24 hours after BP treatment [44]. Nur77 translocation to cytoplasm was recently shown to be induced by activation of c-Jun N-terminal kinase or inhibition of AKT [16].

In addition, the role of T-cells in thymocyte apoptosis and the protein kinase C activation pathway that results in Nur77 expression have also been confirmed [58]. Lin et al separated cytosolic and nuclear proteins and confirmed Nur77 translocation by western blotting. In addition, Nur77 translocation was able to trigger cytochrome C release into the cytoplasm, accompanied by activation of caspase 9 and then caspase 3, resulting in tumor cell apoptosis [44]. Importantly, when small interfering RNA (siRNA) targeting Nur77 mRNA was used to downregulate the Nur77 level in the presence of BP, BP-induced tumor apoptosis was reversed. In sum, BP-induced GBM apoptosis is mediated by a targeted overexpression of Nur77 and its subsequent translocation from nucleus to cytoplasm.

10. BP suppresses telomerase activity, resulting in tumor senescence

The expression of the Nur77 siRNA in BP-treated cells restored their viability by only 40%, suggesting that BP has yet another target(s) besides Nur77 [44]. Because BP can suppress telomerase activity in a dose-dependent manner, it is reasonable to speculate that BP induces cell senescence by (in)directly inhibiting telomerase activity, resulting in tumor cell apoptosis. Since cell viability is a function of both proliferative capacity and apoptotic effects, BP-stimulated suppression of

telomerase activity can induce cytostatic effects and thereby decrease cell viability. In our studies, transfection of a telomerase-encoding transgene and the subsequent restoration of telomerase activity increased the viability of BP-treated GBM cells by 90% when combined with Nur77 siRNA [44]. At present there exist two potential targets: Nur77, which mediates cytotoxicity, and telomerase, which promotes cytostatic state. These dual effects contribute almost equally to reducing brain tumor cell viability in the presence of BP.

In terms of the mechanism by which a telomerase gene is regulated, several transcription binding sites have been reported, including the Myc/Mad sites (E-boxes) [59]. Wu et al reported c-Myc binding to E-boxes and activating telomerase mRNA transcription in tumor cells [60].

A binding site for transcription factor Sp1 is another vital motif present in the telomerase promoter. Kyo et al reported five binding sites for the transcription factor Sp1 in the telomerase core promoter region, indicating Sp1 as vital regulator of the telomerase gene [61]. Most tumor cells have high telomerase activity due to upregulation of Sp1 level. Kyo et al also report a marked decrease in telomerase activity after Sp1 mutation [61].

Our western blot analysis showed no significant decrease in c-Myc level after BP treatment in GBM cells, whereas telomerase activity markedly decreased [44]. The electrophoretic mobility shift assay indicated, however, that Sp1 protein expression declined after BP treatment, with a concomitant drop in Sp1 binding activity at the telomerase promoter. The Sp1 regulatory element in the telomerase promoter may prove to be critical for telomerase transcription in BP-treated GBM cells [44].

The absence of telomerase activity is associated with cell senescence and cell cycle proliferation. Our study showed BP has an antiproliferative effect and induces tumor cell senescence [45]. Cellular proliferation capacity can be restored, with concomitant resistance to senescence, after transfection and overexpression of telomerase – even in the presence of BP. This implicates BP-mediated inhibition of cell proliferation as a central factor in promoting cell senescence and subsequent apoptosis.

Immunohistochemical staining of X-gal, a senescence marker, indicated that BP not only increases cell age by decreasing telomerase activity, but also affects the expression of three major proteins involved in cell cycle regulation and senescence: p53, p21, and p16 [62]. On the other hand, senescence-associated markers p21 and p16 increased after BP treatment [45]. Future experiments must confirm the mechanisms underlying BP-induced tumor cell senescence.

11. Conclusion and future perspectives

Current information suggests BP as potential treatment against brain tumors, with minimal toxicity. However, brain tumors are hard to treat, since any efficacious drug must cross the brain–blood barrier. As such, the means by which this prospective drug could be delivered (including the possibility of local interstitial delivery) poses a formidable task. BP is oil-like and thus less water soluble owing to its hydrophobic character, another issue warranting resolution. Delivering it via

nanoparticles or as prodrug may constitute a viable drug formulation. Because BP targets genes encoding Nur77 and human telomerase reverse transcriptase, we view it as a potential target drug for use against brain tumors, and therefore worth developing for clinical use.

REFERENCES

- [1] Kim SH, Lee SE, Oh H, Kim SR, Yee ST, Yu YB, et al. The radioprotective effects of bu-zhong-yi-qi-tang: a prescription of traditional Chinese medicine. *Am J Chin Med* 2002;30:127–37.
- [2] Wang X, Wei L, Ouyang JP, Muller S, Gentils M, Cauchois G, et al. Effects of an angelica extract on human erythrocyte aggregation, deformation and osmotic fragility. *Clin Hemorheol Microcirc* 2001;24:201–5.
- [3] Xie F, Li X, Sun K, Chu Y, Cao H, Chen N, et al. An experimental study on drugs for improving blood circulation and removing blood stasis in treating mild chronic hepatic damage. *J Tradit Chin Med* 2001;21:225–31.
- [4] Yim TK, Wu WK, Pak WF, Mak DH, Liang SM, Ko KM. Myocardial protection against ischaemia-reperfusion injury by a Polygonum multiflorum extract supplemented 'Dang-Gui decoction for enriching blood', a compound formulation, ex vivo. *Phytother Res* 2000;14:195–9.
- [5] Ye YN, Koo MW, Li Y, Matsui H, Cho CH. Angelica sinensis modulates migration and proliferation of gastric epithelial cells. *Life Sci* 2001;68:961–8.
- [6] Ye YN, Liu ES, Li Y, So HL, Cho CC, Sheng HP, et al. Protective effect of polysaccharides-enriched fraction from Angelica sinensis on hepatic injury. *Life Sci* 2001;69:637–46.
- [7] Ye YN, Liu ES, Shin VY, Koo MW, Li Y, Wei EQ, et al. A mechanistic study of proliferation induced by Angelica sinensis in a normal gastric epithelial cell line. *Biochem Pharmacol* 2001;61:1439–48.
- [8] Ye YN, So HL, Liu ES, Shin VY, Cho CH. Effect of polysaccharides from Angelica sinensis on gastric ulcer healing. *Life Sci* 2003;72:925–32.
- [9] Schwartzmann G, Ratain MJ, Cragg GM, Wong JE, Saijo N, Parkinson DR, et al. Anticancer drug discovery and development throughout the world. *J Clin Oncol* 2002;20:47S–59.
- [10] Zhao KJ, Dong TT, Tu PF, Song ZH, Lo CK, Tsim KW. Molecular genetic and chemical assessment of radix Angelica (Danggui) in China. *J Agric Food Chem* 2003;51:2576–83.
- [11] Chen QC, Lee J, Jin W, Youn U, Kim H, Lee IS, et al. Cytotoxic constituents from angelicae sinensis radix. *Arch Pharm Res* 2007;30:565–9.
- [12] Li JM, Zhao YH, Zhong GC, Long ZJ, Zhou P, Yuan M. Synthesis of ferulic acid derivatives and their inhibitory effect on platelet aggregation. *Yao Xue Xue Bao* 2011;46:305–10.
- [13] Yin ZZ, Zhang LY, Xu LN. The effect of Dang-Gui (Angelica sinensis) and its ingredient ferulic acid on rat platelet aggregation and release of 5-HT (author's translation). *Yao Xue Xue Bao* 1980;15:321–6.
- [14] Zhong YH, Han G, Zhou YF, Peng M, Xie CH, Zhou FX, et al. Protection of Angelica sinensis against radiation-induced pulmonary fibrosis in mice. *Zhonghua Yu Fang Yi Xue Za Zhi* 2007;41:105–9.
- [15] Xie CH, Zhang MS, Zhou YF, Han G, Cao Z, Zhou FX, et al. Chinese medicine Angelica sinensis suppresses radiation-induced expression of TNF-alpha and TGF-beta1 in mice. *Oncol Rep* 2006;15:1429–36.
- [16] Han YH, Cao X, Lin B, Lin F, Kolluri SK, Stebbins J, et al. Regulation of Nur77 nuclear export by c-Jun N-terminal kinase and Akt. *Oncogene* 2006;25:2974–86.
- [17] Cheng YL, Chang WL, Lee SC, Liu YG, Chen CJ, Lin SZ, et al. Acetone extract of Angelica sinensis inhibits proliferation of human cancer cells via inducing cell cycle arrest and apoptosis. *Life Sci* 2004;75:1579–94.
- [18] Lee WH, Jin JS, Tsai WC, Chen YT, Chang WL, Yao CW, et al. Biological inhibitory effects of the Chinese herb danggui on brain astrocytoma. *Pathobiology* 2006;73:141–8.
- [19] Choy YM, Leung KN, Cho CS, Wong CK, Pang PK. Immunopharmacological studies of low molecular weight polysaccharide from Angelica sinensis. *Am J Chin Med* 1994;22:137–45.
- [20] Cao W, Li XQ, Liu L, Wang M, Fan HT, Li C, et al. Structural analysis of water-soluble glucans from the root of Angelica sinensis (Oliv.) Diels. *Carbohydr Res* 2006;341:1870–7.
- [21] Cao W, Li XQ, Wang X, Li T, Chen X, Liu SB, et al. Characterizations and anti-tumor activities of three acidic polysaccharides from Angelica sinensis (Oliv.) Diels. *Int J Biol Macromol* 2010;46:115–22.
- [22] Tsai NM, Chen YL, Lee CC, Lin PC, Cheng YL, Chang WL, et al. The natural compound n-butylidenephthalide derived from Angelica sinensis inhibits malignant brain tumor growth in vitro and in vivo. *J Neurochem* 2006;99:1251–62.
- [23] Mullady EL, Millett WP, Yoo HD, Weiskopf AS, Chen J, Ditullio D, et al. A phthalide with in vitro growth inhibitory activity from an oidioidendron strain. *J Nat Prod* 2004;67:2086–9.
- [24] Yoshikawa K, Kokudo N, Hashimoto T, Yamamoto K, Inose T, Kimura T. Novel phthalide compounds from Sparassis crispa (Hanabiratake), Hanabiratakelide A-C, exhibiting anti-cancer related activity. *Biol Pharm Bull* 2010;33:1355–9.
- [25] Zheng GQ, Kenney PM, Zhang J, Lam LK. Chemoprevention of benzo[a]pyrene-induced forestomach cancer in mice by natural phthalides from celery seed oil. *Nutr Cancer* 1993;19:77–86.
- [26] Kan WL, Cho CH, Rudd JA, Lin G. Study of the anti-proliferative effects and synergy of phthalides from Angelica sinensis on colon cancer cells. *J Ethnopharmacol* 2008;120:36–43.
- [27] Yu YL, Yu SL, Su KJ, Wei CW, Jian MH, Lin PC, et al. Extended O6-methylguanine methyltransferase promoter hypermethylation following n-butylidenephthalide combined with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) on inhibition of human hepatocellular carcinoma cell growth. *J Agric Food Chem* 2010;58:1630–8.
- [28] Bonham M, Arnold H, Montgomery B, Nelson PS. Molecular effects of the herbal compound PC-SPEs: identification of activity pathways in prostate carcinoma. *Cancer Res* 2002;62:3920–4.
- [29] Giese A, Bjerkvig R, Berens ME, Westphal M. Cost of migration: invasion of malignant gliomas and implications for treatment. *J Clin Oncol* 2003;21:1624–36.
- [30] Hu H, Ahn NS, Yang X, Lee YS, Kang KS. Ganoderma lucidum extract induces cell cycle arrest and apoptosis in MCF-7 human breast cancer cell. *Int J Cancer* 2002;102:250–3.
- [31] Kao ST, Yeh CC, Hsieh CC, Yang MD, Lee MR, Liu HS, et al. The Chinese medicine Bu-Zhong-Yi-Qi-Tang inhibited proliferation of hepatoma cell lines by inducing apoptosis via G0/G1 arrest. *Life Sci* 2001;69:1485–96.
- [32] Lee SM, Li ML, Tse YC, Leung SC, Lee MM, Tsui SK, et al. Paeoniae Radix, a Chinese herbal extract, inhibit hepatoma cells growth by inducing apoptosis in a p53 independent pathway. *Life Sci* 2002;71:2267–77.
- [33] Yano H, Mizoguchi A, Fukuda K, Haramaki M, Ogasawara S, Momosaki S, et al. The herbal medicine sho-saiko-to inhibits proliferation of cancer cell lines by inducing

- apoptosis and arrest at the G0/G1 phase. *Cancer Res* 1994;54:448–54.
- [34] Tsai NM, Lin SZ, Lee CC, Chen SP, Su HC, Chang WL, et al. The antitumor effects of *Angelica sinensis* on malignant brain tumors in vitro and in vivo. *Clin Cancer Res* 2005;11:3475–84.
- [35] Ross BD, Zhao YJ, Neal ER, Stegman LD, Ercolani M, Ben-Yoseph O, et al. Contributions of cell kill and posttreatment tumor growth rates to the repopulation of intracerebral 9L tumors after chemotherapy: an MRI study. *Proc Natl Acad Sci U S A* 1998;95:7012–7.
- [36] Bardon S, Foussard V, Fournel S, Loubat A. Monoterpenes inhibit proliferation of human colon cancer cells by modulating cell cycle-related protein expression. *Cancer Lett* 2002;181:187–94.
- [37] Bello L, Carrabba G, Giussani C, Lucini V, Cerutti F, Scaglione F, et al. Low-dose chemotherapy combined with an antiangiogenic drug reduces human glioma growth in vivo. *Cancer Res* 2001;61:7501–6.
- [38] Blacklock JB, Wright DC, Dedrick RL, Blasberg RG, Lutz RJ, Doppman JL, et al. Drug streaming during intra-arterial chemotherapy. *J Neurosurg* 1986;64:284–91.
- [39] Cobb MA, Husain M, Andersen BJ, al-Mefty O. Significance of proliferating cell nuclear antigen in predicting recurrence of intracranial meningioma. *J Neurosurg* 1996;84:85–90.
- [40] Elliott PJ, Hayward NJ, Huff MR, Nagle TL, Black KL, Bartus RT. Unlocking the blood-brain barrier: a role for RMP-7 in brain tumor therapy. *Exp Neurol* 1996;141:214–24.
- [41] Mahaley Jr MS, Mettlin C, Natarajan N, Laws Jr ER, Peace BB. National survey of patterns of care for brain-tumor patients. *J Neurosurg* 1989;71:826–36.
- [42] Santarius T, Kirsch M, Rossi ML, Black PM. Molecular aspects of neuro-oncology. *Clin Neurol Neurosurg* 1997;99:184–95.
- [43] Chen YL, Jian MH, Lin CC, Kang JC, Chen SP, Lin PC, et al. The induction of orphan nuclear receptor Nur77 expression by n-butylphenylthalide as pharmaceuticals on hepatocellular carcinoma cell therapy. *Mol Pharmacol* 2008;74:1046–58.
- [44] Lin PC, Chen YL, Chiu SC, Yu YL, Chen SP, Chien MH, et al. Orphan nuclear receptor, Nur-77 was a possible target gene of butylidenephthalide chemotherapy on glioblastoma multiform brain tumor. *J Neurochem* 2008;106:1017–26.
- [45] Lin PC, Lin SZ, Chen YL, Chang JS, Ho LI, Liu PY, et al. Butylidenephthalide suppresses human telomerase reverse transcriptase (TERT) in human glioblastomas. *Ann Surg Oncol*; 2011.
- [46] Li H, Kolluri SK, Gu J, Dawson MI, Cao X, Hobbs PD, et al. Cytochrome c release and apoptosis induced by mitochondrial targeting of nuclear orphan receptor TR3. *Science* 2000;289:1159–64.
- [47] Williams GT, Lau LF. Activation of the inducible orphan receptor gene nur77 by serum growth factors: dissociation of immediate-early and delayed-early responses. *Mol Cell Biol* 1993;13:6124–36.
- [48] Winoto A, Littman DR. Nuclear hormone receptors in T lymphocytes. *Cell* 2002;109(Suppl):S57–66.
- [49] Woronicz JD, Calnan B, Ngo V, Winoto A. Requirement for the orphan steroid receptor Nur77 in apoptosis of T-cell hybridomas. *Nature* 1994;367:277–81.
- [50] Li QX, Ke N, Sundaram R, Wong-Staal F. NR4A1, 2, 3 – an orphan nuclear hormone receptor family involved in cell apoptosis and carcinogenesis. *Histol Histopathol* 2006;21:533–40.
- [51] Lin B, Kolluri SK, Lin F, Liu W, Han YH, Cao X, et al. Conversion of Bcl-2 from protector to killer by interaction with nuclear orphan receptor Nur77/TR3. *Cell* 2004;116:527–40.
- [52] Wilson AJ, Arango D, Mariadason JM, Heerdt BG, Augenlicht LH. TR3/Nur77 in colon cancer cell apoptosis. *Cancer Res* 2003;63:5401–7.
- [53] Youn HD, Sun L, Prywes R, Liu JO. Apoptosis of T cells mediated by Ca²⁺-induced release of the transcription factor MEF2. *Science* 1999;286:790–3.
- [54] Chintharlapalli S, Burghardt R, Papineni S, Ramaiah S, Yoon K, Safe S. Activation of Nur77 by selected 1,1-Bis(3'-indolyl)-1-(p-substituted phenyl)methanes induces apoptosis through nuclear pathways. *J Biol Chem* 2005;280:24903–14.
- [55] Rajpal A, Cho YA, Yelent B, Koza-Taylor PH, Li D, Chen E, et al. Transcriptional activation of known and novel apoptotic pathways by Nur77 orphan steroid receptor. *EMBO J* 2003;22:6526–36.
- [56] Weih F, Ryseck RP, Chen L, Bravo R. Apoptosis of nur77/N10-transgenic thymocytes involves the Fas/Fas ligand pathway. *Proc Natl Acad Sci U S A* 1996;93:5533–8.
- [57] Liu S, Wu Q, Ye XF, Cai JH, Huang ZW, Su WJ. Induction of apoptosis by TPA and VP-16 is through translocation of TR3. *World J Gastroenterol* 2002;8:446–50.
- [58] Kim H, Lee JE, Kim BY, Cho EJ, Kim ST, Youn HD. Menin represses JunD transcriptional activity in protein kinase C theta-mediated Nur77 expression. *Exp Mol Med* 2005;37:466–75.
- [59] Flores I, Benetti R, Blasco MA. Telomerase regulation and stem cell behaviour. *Curr Opin Cell Biol* 2006;18:254–60.
- [60] Wu KJ, Grandori C, Amacker M, Simon-Vermot N, Polack A, Lingner J, et al. Direct activation of TERT transcription by c-MYC. *Nat Genet* 1999;21:220–4.
- [61] Kyo S, Takakura M, Taira T, Kanaya T, Itoh H, Yutsudo M, et al. Sp1 cooperates with c-Myc to activate transcription of the human telomerase reverse transcriptase gene (hTERT). *Nucleic Acids Res* 2000;28:669–77.
- [62] Coates SS, Lehnert BE, Sharma S, Kindell SM, Gary RK. Beryllium induces premature senescence in human fibroblasts. *J Pharmacol Exp Ther* 2007;322:70–9.