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Novel Ginsenosides 25-OH-PPD and 25-OCH₃-PPD as Experimental Therapy for Pancreatic Cancer: Anticancer Activity and Mechanisms of Action

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Abstract

We recently isolated and characterized two novel ginsenosides, 25-OH-PPD and 25-OCH₃-PPD. We investigated whether these ginsenosides could represent safe and effective therapeutic agents for pancreatic cancer. In vitro and in vivo studies demonstrated that both compounds inhibited proliferation, caused cell cycle arrest, and induced apoptosis. They also both inhibited the growth of xenograft tumors without any host toxicity. Preliminary investigations into the mechanisms of action of the compounds suggest that their effects may be partially mediated by their inhibition of the *MDM2* oncogene and related pathways. The data presented here support further evaluation of the ginsenosides for pancreatic cancer therapy.

Keywords

Panax ginseng; *Panax notoginseng*; 25-OH-PPD; 25-OCH₃-PPD; ginsenoside; pancreatic cancer

1. Introduction

With a 5 year survival rate of only 5% in the United States, pancreatic cancer is the most deadly of all cancers [1]. Because pancreatic cancer does not typically present early symptoms, most patients are not diagnosed until the disease has metastasized. It is estimated that only 10% of pancreatic cancers are still localized at the time of diagnosis [2]. Given the highly metastatic nature of pancreatic cancer, treatment options are limited, and mostly palliative. Surgical resection offers the best option, increasing five year survival to 20% [2]. Chemotherapeutic agents such as 5-fluorouracil and gemcitabine, and radiation therapy, can also help increase

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survival. Nevertheless, the mortality rate remains high. Given the mortality rate and limited treatment options, there is an urgent need to find new approaches for treating pancreatic cancer.

Many novel anti-cancer agents have been derived from plants, and herbal medicines are becoming increasingly popular in the U.S. Ginseng (*Panax ginseng*) is a highly valued herbal medicine that has been used for thousands of years in China and other Asian countries for a variety of ailments. More recently, ginseng has been investigated in pre-clinical and clinical studies for the treatment of CNS, cardiovascular, endocrine and immune system disorders [3]. Particular interest in Western countries has been focused on *Panax ginseng*'s anti-cancer activities [4-6].

Although many classes of compounds can be extracted from the ginseng plant, and the related *Panax notoginseng*, it is the ginsenosides (saponins), that have shown the greatest activity. The ginsenosides target a variety of tissues, with numerous ginsenosides generating an assortment of potentially beneficial responses, ranging from increased immunosurveillance to anti-angiogenic effects to inhibition of transformation [7-11]. Studies have shown that the angi-suppressive effects of the ginsenosides (including Rg3) correlate with their anti-tumor efficacy [11,12]. Another ginsenoside, Rb₂, was shown to inhibit angiogenesis and metastasis of melanoma in mice [13]. In one clinical study, it was demonstrated that taking ginseng can decrease the risk of developing cancers of the lungs, oral cavity, and liver [14].

More than 60 ginsenosides have been isolated from *Panax ginseng* and related plants. We have been investigating the anti-cancer activities of ginsenosides for several years, and have discovered two highly active compounds, 25-OH-PPD (20(R)-dammarane-3 β ,12 β ,20,25-tetrol) from *P. ginseng*, and 25-OCH₃-PPD (20(S)-25-methoxyl-dammarane-3 β ,12 β ,20-triol) from *P. notoginseng*. These ginsenosides were the most potent anti-cancer agents found during a screen of ginsenosides and other natural products. We have previously established their activity against breast cancer [15,16]. The aim of the present study was to determine whether either of these two most effective ginsenosides might represent an urgently-needed novel therapeutic agent for pancreatic cancer. We examined the *in vitro* and *in vivo* anti-cancer effects of the compounds and accomplished a preliminary investigation into their mechanisms of action. The present study represents our continued efforts in searching for safe and effective natural product anticancer agents [17-19].

2. Materials and methods

2.1. Reagents

The identities of ginseng and notoginseng compounds 25-OH-PPD and 25-OCH₃-PPD were established previously [15,16]. All chemicals and solvents were of the highest analytical grade available. Cell culture media, fetal bovine serum (FBS); phosphate-buffered saline (PBS), HEPES buffer, sodium pyruvate, penicillin-streptomycin and other cell culture supplies were obtained from the Comprehensive Cancer Center Media Preparation Shared Facility (University of Alabama at Birmingham). The anti-human MDM2 (SMP14), p21 (C-19), Bcl-2 (100), Bax (N-20), E2F1 (KH95), p27(C-19), CDK2 (M2), CDK4 (H-22), CDK6 (C-21), Cyclin D1 (DCS-6), cdc25c, and cdc2 antibodies were from Santa Cruz Biotechnologies (Santa Cruz, CA), and the Caspase-3 (9662), Caspase-8 (9746) and Caspase-9 (9502) antibodies were from Cell Signaling Technology, Inc (Danvers, MA). The anti-human p53 (Ab-6) antibody was from EMD Chemicals, Inc (Gibbstown, NJ).

2.2. Cell culture

Panc-1 cells were cultured in RPMI 1640 medium containing 10mM HEPES, 1.5 mg/mL sodium bicarbonate, 25 μ g/mL gentamicin, and 0.25 μ g/mL amphotericin B. The HPAC human

pancreatic cancer cells were cultured in 1:1 mixture of DMEM and Ham's F12 medium containing 15mM HEPES, 0.002mg/mL insulin, 0.005mg/mL transferrin, 40ng/mL hydrocortisone and 10ng/mL epidermal growth factor. Panc-1 and HPAC cells were obtained from the American Type Tissue Culture collection (Rockville, MD). All culture media contained 10% FBS and 1% penicillin-streptomycin.

2.3. Cell survival assay

The effects of the test compounds on human pancreatic cancer cell growth, expressed as the percentage of cell survival, were determined using the MTT assay. The cells were grown in 96-well plates at $4-5 \times 10^3$ cells per well and exposed to various concentrations of the test compounds (0, 1, 10, 25, 50, 100 μ M) for 72 h. The absorbance at 570 nm was recorded using an OPTImax microplate reader (Molecular Devices; Sunnyvale, CA). The cell survival percentages were calculated by dividing the mean OD of compound-containing wells by that of control wells.

2.4. Animals

Pathogen-free male athymic nude mice (nu/nu, 4-6 weeks) were purchased from Frederick Cancer Research and Development Center (Frederick, MD). All animals were fed with commercial diet and provided water *ad libitum*. All animal studies were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee.

2.5. Xenograft models and treatment

The Panc-1 xenograft model was established using methods described previously [20]. In brief, cultured cells were washed with and re-suspended in serum-free medium. Portions of the suspension (5×10^6 cells in 0.2 mL/mouse) were mixed in a 3:1 ratio with Matrigel basement membrane matrix (Becton Dickinson Labware, Bedford, MA) and injected into the left inguinal area of the mice. The mice were monitored by measuring tumor growth and body weight, and by general clinical observation. Tumor-bearing mice were randomly divided into multiple treatment and control groups. The test compounds were dissolved in PEG400:ethanol:saline (57.1%: 14.3%: 28.6%, v/v/v), and given by i.p. injection, and were administered at doses of 1 mg/kg/d, 5 mg/kg/d and 10 mg/kg/d, 5 days/wk for 6 weeks. The control group received the vehicle only. All animals were monitored for activity, physical condition, body weight, and tumor growth. Tumor size was determined by caliper measurement of two perpendicular diameters of the implant every other day. Tumor weight (in g) was estimated by use of the formula $1/2a \times b^2$, where "a" is the long diameter and "b" is the short diameter (in cm).

2.6. Cell proliferation

The effects of the test compounds on cell proliferation were determined by BrdUrd incorporation assay (Oncogene, La Jolla, CA), following the manufacturer's protocol. Cells were seeded in 96-well plates (8×10^3 to 1.2×10^4 cells per well) and incubated with various concentrations of test compounds (0-50 μ M) for 24 h. BrdUrd was added to the medium 10 h before termination of the experiment. The BrdUrd incorporated into cells was determined by anti-BrdUrd antibody, and absorbance was measured at dual wavelengths of 450/540 nm with an OPTImax microplate reader (Molecular Devices; Sunnyvale, CA).

2.7. Cell cycle measurements

For determination of the effects on the cell cycle, $2-3 \times 10^5$ cells were exposed to the test compounds (0, 1, 10, 25 μ M) and incubated for 24 h prior to analysis. Cells were trypsinized, washed with PBS, and fixed in 1.5 mL of 95% ethanol at 4°C overnight, followed by incubation with RNase and staining with propidium iodide (Sigma). The DNA content was determined by flow cytometry.

2.8. Detection of apoptosis

Cells in early and late stages of apoptosis were detected using an Annexin V-FITC apoptosis detection kit from BioVision (Mountain View, CA), according to the manufacturer's protocol. In brief, $2-3 \times 10^5$ cells were exposed to the test compounds (0, 1, 10, 25, 50 μ M) and incubated for 48 h prior to analysis. The samples were analyzed using a Becton Dickinson FACSCalibur instrument (Ex = 488nm; Em = 530 nm). Cells that were positive for Annexin V-FITC alone (early apoptosis) and Annexin V-FITC and PI (late apoptosis) were counted.

2.9. Western blot analysis

The protein levels were assessed using methods described previously [20]. In these *in vitro* studies, cells were exposed to various concentrations of the compounds for 24 h, then whole cell lysates were examined for protein expression.

2.10. Luciferase assay for *MDM2* promoter activity

Panc-1 cells were co-transfected with full-length or deleted *MDM2* P2 promoter luciferase vectors and a *Renilla* luciferase reporter for 12 h followed by incubation with the ginsenosides for 24 h. The luciferase activity of the promoter reporters was quantified using the Dual-Luciferase Reporter Assay System (Promega), and the reporter activity was normalized to the corresponding *Renilla* luciferase reporter activity.

2.11. Statistical analysis

The data was expressed as means and standard deviations, and the significance of differences was analyzed by ANOVA or student's t-test using SPSS 13.0 (Chicago, IL).

3. Results

3.1. 25-OH-PPD and 25-OCH₃-PPD decrease pancreatic cancer growth in vitro

Ginsenosides 25-OH-PPD and 25-OCH₃-PPD were isolated and purified as described previously [15,16] (Fig. 1). The compounds were evaluated for their effects on pancreatic cancer cell viability *in vitro* by use of the MTT assay. The IC₂₀, IC₅₀, and IC₈₀ values were calculated following incubation with various concentrations of the compounds (Table 1). 25-OCH₃-PPD had lower IC₅₀ values for both HPAC and Panc-1 cells, although 25-OH-PPD had a lower IC₈₀ value in the Panc-1 cells compared to 25-OCH₃-PPD.

3.2. 25-OCH₃-PPD and 25-OH-PPD inhibit the growth of xenograft pancreatic tumors

To determine whether the compounds were effective against *in vivo* tumors, we evaluated the anti-tumor effects of 25-OCH₃-PPD and 25-OH-PPD against the Panc-1 xenograft model. The compounds both decreased the growth of the pancreatic xenograft tumors. While 25-OH-PPD did not exert any appreciable effect at 1 mg/kg, the 5 mg/kg dose led to approximately 14% tumor growth inhibition, and the 10 mg/kg dose led to significant ($p=.024$) 52% tumor growth inhibition (Fig. 2A). 25-OCH₃-PPD exhibited more potent effects than 25-OH-PPD. Even the 1 mg/kg dose of the *notoginseng* compound led to impressive, but non-significant, tumor growth inhibition by about 16%. The higher 5 mg/kg and 10 mg/kg doses led to approximately 30% and 55% ($p=0.042$ and $p=0.010$, respectively) inhibition of tumor growth, respectively (Fig. 2B). Nevertheless, while 25-OCH₃-PPD was more effective at lower doses than 25-OH-PPD, both compounds had similar levels of efficacy at the 10 mg/kg dose, and neither led to any significant toxicity in the mice at any of the doses used, as monitored by changes in body weight (Fig. 2C and D). Moreover, while there was not a significant anti-tumor effect at the lower doses (1 mg/kg or 5 mg/kg) of 25-OH-PPD or the 1 mg/kg dose of 25-OCH₃-PPD, the anti-tumor effects of the compound appeared to follow a trend toward dose-dependence. It is

possible that by further optimizing the dosing frequency or formulation, it might be possible to achieve significant anti-cancer activity at lower doses of either of the compounds.

3.3. The ginsenosides inhibit cell proliferation

To begin assessing the mechanism(s) by which the compounds exert their effects against pancreatic cancer cells, we first examined their effects on cell proliferation. 25-OCH₃-PPD and 25-OH-PPD both demonstrated significant anti-proliferative effects in Panc-1 and HPAC cell lines at the 50 μ M concentration (Fig. 3A and B). Panc-1 cells were more sensitive to ginsenoside treatment at the highest concentration (50 μ M) than HPAC cells, however, the HPAC cells were more sensitive to the lower concentrations of the ginsenosides, with the 25 μ M concentration of both compounds leading to a significant decrease in proliferation.

The compounds also altered the expression of a variety of cell proliferation/cell cycle progression-related proteins in both Panc-1 and HPAC cells (Fig. 3C). Treatment with either ginsenoside led to decreased MDM2, E2F1, Cdk2, Cdk4, Cdk6, cdc25c, Cyclin D, and Cyclin E expression. In contrast, exposure to 25-OH-PPD or 25-OCH₃-PPD increased the expression of p21 and p27 in both cell lines.

3.4. Cell cycle arrest in the G1 phase is induced by ginsenosides

In both pancreatic cancer cell lines, 25-OCH₃-PPD and 25-OH-PPD significantly induced cell cycle arrest in the G1 phase, with initial effects beginning at the 10 μ M concentration (Fig. 4). The effects of the compounds were consistently greater in the Panc-1 cell line than in HPAC cells, suggesting that effects on cell cycle arrest may be cell-line-dependent.

3.5. Induction of apoptosis by ginsenosides 25-OH-PPD and 25-OCH₃-PPD

Because the pancreatic cancer cells lines exhibited differences in their sensitivity to the compounds in terms of proliferation and cell cycle arrest, we also examined whether the ginsenosides affected the level of cellular apoptosis. Both 25-OH-PPD and 25-OCH₃-PPD induced apoptosis in the pancreatic cancer cell lines in a dose-dependent manner. However, the Panc-1 cells were more sensitive to both compounds, with significant induction of apoptosis occurring at even the 1 μ M concentration. Additionally, the *notoginseng*-derived compound was slightly more active, producing an approximately 6-fold increase in apoptosis at 50 μ M in both the HPAC and Panc-1 cell lines (Fig. 5A and B).

Further demonstrating their ability to induce apoptosis in human pancreatic cancer cells, we observed that both compounds led to activation and induction of apoptosis-related proteins (Fig. 5C). Exposure to either 25-OH-PPD or 25-OCH₃-PPD increased the expression of both cleaved PARP and cleaved caspases -3, -8 and -9. However, the effects on caspase activation were only noted in the HPAC cells, suggesting that there may be cell-line specific effects.

3.6. The ginsenosides inhibit transcription of the MDM2 oncogene

Given the involvement of MDM2 in the regulation of many of the affected proteins [21], and our previous observation of MDM2 inhibition in prostate cancer by these ginsenosides [22, 23], we wanted to further explore the effects of the ginsenosides on MDM2 in pancreatic cancer cells. We used a luciferase assay to monitor *MDM2* P2 promoter activity, and observed that there was a dose-dependent decrease in *MDM2* transcription in Panc-1 cells exposed to 25-OCH₃-PPD (Fig. 6). The greatest effect occurred with the shortest promoter construct (-132 to +33). There are several important transcription factor binding sites within this section, indicating that one of these (ETS, AP1, MEF2 or NFAT) may be affected by the ginsenoside.

Discussion

Traditional cancer chemotherapy frequently uses cytotoxic agents to destroy cancer cells at the expense of normal host tissue. Numerous ginsenosides have been discovered to have anti-cancer properties, while demonstrating little toxicity to normal cells [3-14,22,23]. Since so many ginsenosides have been identified, and because each of them varies in its potency and effects, it is important to investigate the activities and mechanisms of action of the compounds, and to determine which are the most promising candidates for clinical development. We have previously studied the activity of a variety of natural products, including numerous ginseng compounds [15-19,22-25]. In previous studies, we found that while Rh2 and PPD (ginsenosides structurally-related to 25-OCH₃-PPD and 25-OH-PPD) decreased proliferation and viability in prostate cancer cells, they were also more cytotoxic to normal (immortalized) fibroblasts than 25-OCH₃-PPD [22]. The two ginsenosides investigated during the present study were found to be both more effective and less toxic (to normal cells) than any of the previously investigated ginsenosides. The differences in activity and toxicity among the different compounds may be attributed to several factors, including differences in cell specificity, in compound stability in cells, and in mechanisms of action. For example, the anti-cancer activity of Rg3 has frequently been attributed to its anti-angiogenic effects, explaining why the compound exerts lesser effects on cancer cells than other cytotoxic compounds, and also why the compound has low toxicity to normal cells [26].

The MDM2 protein may be a major target of 25-OCH₃-PPD and 25-OH-PPD. MDM2 is best known for its well-characterized regulation of the p53 tumor suppressor [27], making it a key molecule for the regulation of cell cycle progression, cell proliferation, and apoptosis. Downregulation of MDM2 could be responsible for the observed anti-cancer effects. Confirming the effects of the ginsenosides on MDM2, we observed a decrease in MDM2 transcription in cells following exposure to 25-OCH₃-PPD (Fig. 6).

Although MDM2 was initially identified for its role in regulating p53, the oncoprotein also functions independently of p53 to regulate the cell cycle through interactions and regulation of proteins such as p21 and E2F1 [21]. This could explain the effects in Panc-1 (p53 mut) cells. MDM2 is overexpressed in many different cancers, and efforts to down-regulate MDM2 have led to decreases in tumor growth and improved tumor responses to traditional chemotherapy in rodent models, indicating that MDM2 is an attractive target for therapy [20,28]. Further investigation is needed to examine the mechanism(s) of action of these ginsenosides, focusing on their effects on MDM2, particularly the p53-independent functions of MDM2, and the importance of these effects on the anti-cancer activity of the compounds.

While the effects of the compounds on proliferation likely contribute to their anti-cancer activity, it appears that the pancreatic cancer cells are more sensitive to apoptosis induced by the compounds. The IC₅₀ values reflect overall changes in cell viability, which include changes in both proliferation and apoptosis. Thus, while no major changes in proliferation are observed for either cell line at concentrations lower than 25 μ M, it is possible to see changes in apoptosis at lower concentrations (beginning at 1 μ M). This explains the relatively low IC₂₀ values. Additionally, it is interesting that the Panc-1 cells are more sensitive to the effects of the compounds on apoptosis, while the HPAC cells are more sensitive to their effects on proliferation. Further investigation is needed to ascertain whether this is due to the differences in p53 status between the cell lines.

In conclusion, both of the ginsenosides, 25-OH-PPD and 25-OCH₃-PPD, might represent novel therapeutic agents for pancreatic cancer. Both compounds exerted potent *in vitro* and *in vivo* anti-cancer activities, and led to down-regulation of important oncogenic and pro-growth/pro-proliferation proteins. A dose of 10 mg/kg of either compound inhibited the growth of Panc-1

xenograft tumors by more than 50%, with no appreciable toxicity to the mice. This indicates that not only might the ginseng and notoginseng compounds represent potential therapeutic agents, but that they may also be safe enough to be used to prevent primary or metastatic tumors. Our present data provide support for the further development of the compounds for pancreatic cancer.

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References

1. American Cancer Society. Cancer Facts and Figures 2008. Atlanta, Ga: American Cancer Society; 2008 [10/1/08]. Also available online at: http://www.cancer.org/downloads/STT/Cancer_Statistics_2008
2. American Cancer Society. Learn About Pancreatic Cancer. Atlanta, GA: American Cancer Society; 2008 [10/1/08]. Available online at: http://www.cancer.org/docroot/CRI/content/CRI_2_4_4X_Surgery_34.asp?nav=cricri
3. Xiang YZ, Shang HC, Gao XM, Zhang BL. A comparison of the ancient use of ginseng in traditional Chinese medicine with modern pharmacological experiments and clinical trials. *Phytother Res* 2008;22:851–858. [PubMed: 18567057]
4. Hofseth LJ, Wargovich MJ. Inflammation, cancer, and targets of ginseng. *J Nutr* 2007;137:183S–185S. [PubMed: 17182823]
5. Helms S. Cancer prevention and therapeutics: Panax ginseng. *Altern Med Rev* 2004;9:259–274. [PubMed: 15387718]
6. Chang YS, Seo EK, Gyllenhaal C, Block KI. Panax ginseng: a role in cancer therapy? *Integr Cancer Ther* 2003;2:13–33. [PubMed: 12941165]
7. Attele AS, Wu JA, Yuan CS. Ginseng pharmacology: multiple constituents and multiple actions. *Biochem Pharmacol* 1999;58:1685–1693. [PubMed: 10571242]
8. Li TSC. Asian and American ginseng: a review. *Hort Technology* 1995;5:27–34.
9. Kitts DD, Hu C. Efficacy and safety of ginseng. *Pub Health Nut* 2000;4:473–485.
10. Xie JT, Mehendale SR, Li X, Quigg R, Wang X, Wang CZ, Wu JA, Aung HH, Rue PA, Bell GI, Yuan CS. Anti-diabetic effect of ginsenoside Re in ob/ob mice. *Biochim Biophys Acta* 2005;1740:319–325. [PubMed: 15949698]
11. Shibata S. Chemistry and cancer preventing activities of ginseng saponins and some related triterpenoid compounds. *J Korean Med Sci* 2001;16:S28–37. [PubMed: 11748374]
12. Leung KW, Yung KK, Mak NK, Yue PY, Luo HB, Chen YK, Fan TP, Yeung HW, Ng TB, Wong RN. Angiomodulatory and neurological effects of ginsenosides. *Curr Med Chem* 2007;14:1371–1380. [PubMed: 17504218]
13. Sato K, Mochizuki M, Saiki I, Yoo YC, Samaukawa K, Azuma I. Inhibition of tumor angiogenesis and metastasis by a saponin of Panax ginseng, ginsenoside-Rb2. *Biol Pharmaceut Bull* 1994;17:635–639.
14. Yun TK, Choi SY. Preventive effect of ginseng intake against various human cancers: a case-control study on 1987 pairs. *Cancer Epidemiol Biomarkers Prev* 1995;4:401–408. [PubMed: 7655337]

15. Wang W, Zhao Y, Rayburn ER, Hill DL, Wang H, Zhang R. In vitro anti-cancer activity and structure-activity relationships of natural products isolated from fruits of *Panax ginseng*. *Cancer Chemother Pharmacol* 2007;59:589–601. [PubMed: 16924497]
16. Zhao Y, Wang W, Han L, Rayburn ER, Hill DL, Wang H, Zhang R. Isolation, structural determination, and evaluation of the biological activity of 20(S)-25-methoxyl-dammarane-3 β , 12 β , 20-triol [20(S)-25-OCH₃-PPD], a novel natural product from *Panax notoginseng*. *Med Chem* 2007;3:51–60. [PubMed: 17266624]
17. Chen T, Li M, Zhang R, Wang H. Dihydroartemisinin Induces Apoptosis and Sensitizes Human Ovarian Cancer Cells to Carboplatin Therapy. *J Cell Mol Med*. 2008 May 2;Epub ahead of print
18. Hou J, Wang D, Zhang R, Wang H. Experimental Therapy of Hepatoma with Artemisinin and Its Derivatives: In vitro and In vivo Activity, Chemosensitization, and Mechanisms of Action. *Clin Cancer Res* 2008;14:5519–5530. [PubMed: 18765544]
19. Hao M, Zhao Y, Chen P, Huang H, Liu H, Jiang H, Zhang R, Wang H. Structure-activity relationship and substrate-dependent phenomena in effects of ginsenosides on activities of drug-metabolizing P450 enzymes. *Plos ONE* 2008;3:e2697. [PubMed: 18628990]10 pages
20. Wang H, Yu D, Agrawal S, Zhang R. Experimental therapy of human prostate cancer by inhibiting MDM2 expression with novel mixed-backbone antisense oligonucleotides: in vitro and in vivo activities and mechanisms. *Prostate* 2003;54:194–205. [PubMed: 12518324]
21. Zhang Z, Zhang R. p53-Independent Activities of MDM2 and Their Relevance to Cancer Therapy. *Curr Cancer Drug Targets* 2005;5:9–20. [PubMed: 15720185]
22. Wang W, Wang H, Rayburn ER, Zhao Y, Hill DL, Zhang R. 20(S)-25-methoxyl-dammarane-3 β , 12 β , 20-triol, a novel natural product for prostate cancer therapy: activity in vitro and in vivo and mechanisms of action. *Br J Cancer* 2008;98:792–802. [PubMed: 18253123]
23. Wang W, Rayburn ER, Hao M, Zhao Y, Hill DL, Zhang R, Wang H. Experimental therapy of prostate cancer with novel natural product anti-cancer ginsenosides. *Prostate* 2008;68:809–819. [PubMed: 18324646]
24. Li M, Zhang Z, Hill DL, Chen X, Wang H, Zhang R. Genistein, a dietary isoflavone, down-regulates the MDM2 oncogene at both transcriptional and posttranslational levels. *Cancer Res* 2005;65:8200–8208. [PubMed: 16166295]
25. Li M, Zhang Z, Hill DL, Wang H, Zhang R. Curcumin, a dietary component, has anticancer, chemosensitization, and radiosensitization effects by down-regulating the MDM2 oncogene through the PI3K/mTOR/ETS2 pathway. *Cancer Res* 2007;67:1988–1996. [PubMed: 17332326]
26. Yue PY, Wong DY, Leung PY, Mak NK, Yeung HW, Liu L, Cai Z, Jiang ZH, Fan TP, Wong RN. The angiosuppressive effects of 20(R)-ginsenoside Rg3. *Biochem Pharmacol* 2006;72:437–445. [PubMed: 16793023]
27. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* 1992;69:1237–1245. [PubMed: 1535557]
28. Rayburn ER, Zhang R. Antisense, RNAi, and gene silencing strategies for therapy: mission possible or impossible? *Drug Discov Today* 2008;13:513–521. [PubMed: 18549978]

The abbreviations used are

25-OH-PPD

20(R)-dammarane-3 β ,12 β ,20,25-tetrol

25-OCH₃-PPD

20(S)-25-methoxyl-dammarane-3 β ,12 β ,20-triol

PBS

phosphate-buffered saline

MTT

3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

BrdUrd

bromodeoxyuridine

Figure 1.
Structures of (A) 25-OH-PPD and (B) 25-OCH₃-PPD.

Figure 2.

In vivo activity of the ginsenosides. Ginsenosides 25-OH-PPD and 25-OCH₃-PPD were administered by i.p. injection to nude mice bearing Panc-1 xenograft tumors at doses of 1 mg/kg/d, 5 mg/kg/d and 10 mg/kg/d, 5 days/wk for 6 weeks. The effects of (A) 25-OCH₃-PPD and (B) 25-OH-PPD on tumor growth were monitored, and changes in body weight following (C) 25-OCH₃-PPD or (D) 25-OH-PPD treatment were examined as a surrogate endpoint for toxicity.

Figure 3.

In vitro anti-proliferative effects of the compounds on pancreatic cancer cells. Panc-1 (A) or HPAC (B) cells were exposed to various concentrations of 25-OH-PPD or 25-OCH₃-PPD for 24 h followed by the BrdUrd cell proliferation assay. The effects of the compounds on the expression of various proliferation-related proteins were assessed in the human pancreatic cancer cells. Panc-1 and HPAC cells were exposed to various concentrations of 25-OH-PPD or 25-OCH₃-PPD for 24 h, and the targets were detected by immunoblotting with specific antibodies (C). * $p < 0.01$, # $p < 0.05$

Figure 4.

Effect of ginseng compounds on cell cycle progression of pancreatic cancer cells. Panc-1 and HPAC cells were exposed to various concentrations of the compounds for 24 h followed by cell cycle distribution assay. All assays were performed in triplicate. * $p < 0.01$, # $p < 0.05$

Figure 5.

Induction of apoptosis in pancreatic cancer cells. Panc-1 (A) or HPAC (B) cells were exposed to various concentrations of the ginsenosides for 48 h, followed by apoptosis assays. The effects of the compounds on the expression of apoptosis-related proteins were assessed after a 24 h exposure to the compounds. Targets were detected by immunoblotting with specific antibodies (C). * $p < 0.01$, # $p < 0.05$

Figure 6. Panc-1 cells were co-transfected with full-length or deleted *MDM2* P2 promoter luciferase vectors (A) and a *Renilla* luciferase reporter for 12 h followed by incubation with the ginsenosides for 24 h. The luciferase activity of the full-length promoter following exposure to different concentrations of 25-OCH₃-PPD (B) and the effect of 10 μ M 25-OCH₃-PPD on the various *MDM2* deletions (C) was quantified using the Dual-Luciferase Reporter Assay System. The reporter activity was normalized to the corresponding *Renilla* luciferase reporter activity. * p <0.01, # p <0.05

Table 1Cytotoxicity of 25-OCH₃-PPD and 25-OH-PPD to pancreatic cancer cells

Cell line	Inhibitory Conc. (μM)	25-OH-PPD	25-OCH ₃ -PPD
Panc-1	IC20	10.9 ± 1.8	1.2 ± 0.1
	IC50	21.2 ± 1.1	7.8 ± 0.4
	IC80	41.3 ± 1.4	51.6 ± 0.7
HPAC	IC20	9.8 ± 2.2	0.9 ± 0.3
	IC50	22.5 ± 1.3	5.8 ± 0.1
	IC80	51.5 ± 0.8	36.6 ± 0.1