Effects of *Oplopanax horridus* on Human Colorectal Cancer Cells

XIAO-LI LI[1,2], SHI SUN[1,2], GUANG-JIAN DU[1,2], LIAN-WEN QI[1,2], STAINLEY WILLIAMS[1], CHONG-ZHI WANG[1,2] and CHUN-SU YUAN[1,2,3]

[1]Tang Center for Herbal Medicine Research,
[2]Department of Anesthesia and Critical Care, and
[3]Committee on Clinical Pharmacology and Pharmacogenomics,
The Pritzker School of Medicine, University of Chicago, Chicago, IL 60637, U.S.A.

Abstract. *Aim: In this study, we investigated the inhibitive effects of Oplopanax horridus extract (OhE) and its fractions (OhF1, OhF2, OhF3, OhF4 and OhF5) on the growth of human colorectal cancer cells and the possible mechanisms involved were investigated. Materials and Methods: The antiproliferative effects were evaluated by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) cell proliferation assay. Apoptotic effects and cell cycle distribution were analyzed by flow cytometry after staining with Annexin V/PI or PI/RNase. Results: After treatment for 48 h, OhE, OhF4 and OhF5 (10-100 μg/ml) inhibited proliferation of HCT-116, SW-480 and HT-29 cell lines, and cell growth decreased most with the treatment of OhF4. On the other hand, OhF1, OhF2 and OhF3 were not observed to have obvious suppressive effects on these cell lines at concentrations of 10-100 μg/ml. OhE, OhF4 and OhF5 (1-10 μg/ml) noticeably induced apoptosis time- and concentration-dependently compared to the control at the same time point. Treatment with OhE, OhF4 or OhF5 (1-10 μg/ml) for 24 h distinctly induced a G_2/M-phase arrest of the cell cycle in a dose-dependent manner. The trend of increasing cyclin A and cyclin B1 were similar to the increase of G_2/M phase cells in all treated groups. Conclusion: These results showed that OhE had potential antiproliferative effects on human colorectal cancer cells, and the active components are enriched in the OhF4 and OhF5 fractions. The anticancer mechanism of OhE, OhF4 and OhF5 might be attributed to the induction of apoptosis and the regulation of cell cycle transition.*

Correspondence to: Chun-Su Yuan, Ph.D., Tang Center for Herbal Medicine Research, and Department of Anesthesia & Critical Care, Pritzker School of Medicine, University of Chicago, 5841 South Maryland Avenue, MC 4028, Chicago, IL 60637, U.S.A. Tel: +1 7737021916, Fax: +1 7738340601, e-mail: CYuan@dacc.uchicago.edu

Key Words: Oplopanax horridus, human colorectal cancer, anti-proliferation, apoptosis, cell cycle, cyclin A, cyclin B1.

Colorectal cancer is the second-leading cause of cancer-related deaths in the United States among cancer that affects both men and women (1, 2), and it is the third most common cancer worldwide (3). Surgery is no longer the best treatment option for most patients with advanced colorectal cancer that has spread to other organs. Advances in chemotherapy that do a better job at shrinking both the primary colon tumor and the metastases are thought to be responsible for this change.

Although in recent years, chemotherapeutic options for colorectal carcinoma have been expanded, overall response rates are still too low, with high rates of toxicity (4-6). Treatment of colorectal cancer increasingly requires a multidisciplinary approach and multiple treatment options add to the complexity of clinical decision-making (7).

In recent years, plants have become increasingly important as a source of biologically active natural products (8, 9). Ethnopharmacological screening provides scientists with an alternative avenue to discover active components and compounds from numerous traditional medicines to treat diseases (10, 11). Our group seeks to determine the potential role of ginseng in the treatment of colorectal cancer (6, 12). We have reported the chemopreventive effects of the active fractions and major constitutes of American ginseng and Asian ginseng in human colorectal cancer cells, as well as the potential action mechanism (13-16).

Devil's Club, *Oplopanax horridus*, another member of the Araliaceae family related in taxonomy to well-known herbal medicines such as the Asian ginseng and American ginseng, is a deciduous shrub that grows in the Pacific Northwest, particularly in Alaska and British Columbia where it is most abundant (17, 18). This plant has been used internally by the indigenous people for the treatment of respiratory ailments and gastrointestinal complaints through drinking an aqueous

extract of the root or stem bark (19). Ethnobotanic data indicate that the extracts of the inner bark appear to have antipyretic, antitussive, antibacterial, and hypoglycemic properties (19, 20). One *in vitro* study showed that the extract of the root bark of *O. horridus* (OhE) was able to inhibit cell proliferation of human leukemia and breast cancer cell lines, K562, HL60, MCF-7 and MDA-MB-468 (18). Nerolidol, a constituent of OhE, was shown to inhibit azoxymethane-induced neoplasia of the large bowel in male F344 rats (21). However, the effect of OhE on human colorectal cancer and the related mechanisms have not yet been studied.

Thus, the present study was performed to investigate the effects of OhE and its fractions on the growth of human colorectal cancer cell lines and the possible mechanisms behind its actions.

Materials and Methods

Herbal materials and sample preparation. Air-dried root bark of *O. horridus* were supplied by Pacific Botanicals, LLC, which were gathered from Oregon, USA, and were authenticated by a botanist. The voucher specimens were deposited in the Tang Center for Herbal Medical Research at the University of Chicago.

The material was ground to powder and extracted with 70% ethanol for 4 h in a water-bath maintained at 90˚C. When cooled, the solution was filtered and the filtrate was collected. The residue was extracted with 70% ethanol once more and then filtered while the solution was cooled. The filtrates were combined and the solvent was evaporated under vacuum to obtain the *O. horridus* extract (OhE). The concentrated extract was submitted to column chromatography on macroporous resin HP-20 (75×24 cm i.d. Diaion, Sigama Aldrich) eluted with water, 30%, 50%, 70% and 100% ethanol. These fractions were evaporated under vacuum and then lyophilized to obtain fractions OhF1, OhF2, OhF3, OhF4 and OhF5, respectively.

Chemicals and reagents. All cell culture plasticware was purchased from Falcon Labware (Franklin Lakes, NJ, USA) and Techno Plastic Products (Trasadingen, Switzerland). Trypsin, McCoy's 5A medium, Leibovitz's L-15 medium, fetal bovine serum (FBS), and phosphate-buffered saline (PBS) were obtained from Mediatech, Inc. (Herndon, VA, USA). Penicillin G/streptomycin was obtained from Sigma (St. Louis, MO, USA). A MTS assay kit (CellTiter 96 Aqueous One Solution Cell Proliferation Assay) was purchased from Promega (Madison, WI, USA). Annexin V-FITC (fluorescein isothiocyanate) Apoptosis Detection Kit, FITC-conjugated Cyclin A Antibody Reagent Set and FITC-conjugated Cyclin B1 Antibody Reagent Set were obtained from BD Biosciences (Rockville, MD, USA). Propidium Iodide/RNase staining buffer was supplied from BD Biosciences Pharmingen (San Diego, CA, USA). Milli Q Water was supplied by a water purification system (US Filter, Palm Desert, CA, USA).

Cell culture. HCT-116, SW-480, and HT-29 human colorectal cancer cells (ATCC, Manassas, VA, USA) were routinely grown in a humidified atmosphere of 5% CO_2 at 37˚C in Leibovitz's L-15 medium (for SW-480) and in McCoy's 5A medium (for HCT-116 and HT-29), and supplemented with 10% FBS and 50 IU penicillin/streptomycin respectively. Cells were grown in a 25-ml flask and were routinely subcultured using 0.05% trypsin-EDTA solution. Cells were maintained at the culture conditions described above for all experiments.

Cell proliferation assay. The effect of tested samples on the viability of human colorectal cancer cells was determined by the MTS assay. The logarithmically growing HCT-116, SW-480 and HT-29 cells were plated into 96-well plates at approximately 1×10^4 cells/well and allowed to adhere for 24 h. Fresh culture media were changed prior to the addition of drugs. The cells were treated with OhE, OhF1, OhF2, OhF3, OhF4 or OhF5 (10, 30, 100 μg/ml) for 48 h. To determine the time- and concentration dependency of the drugs, HCT-116 cells were treated under the same conditions as described above and incubated with OhE, OhF4 or OhF5 (0.3, 1, 3, 10 μg/ml) for 24 h, 48 h or 72 h. Control cultures were incubated in a medium containing the vehicle alone. All experiments were performed in triplicate.

At the end of the sample exposure period, the used medium of each well was discarded and 100 μl fresh medium and 20 μl CellTiter 96 aqueous solution were added. The plate was returned to the incubator where it remained for 1-4 h in a humidified atmosphere of 37˚C. Subsequently, 60 μl of medium from each well were transferred to an ELISA 96-well plate, and the absorbance of the formazan product was measured at 490 nm. The blank control was recorded by measuring the absorbance at 490 nm with wells containing medium mixed with CellTiter 96 aqueous solution but no cells. Results were expressed as a percentage of the control (vehicle set at 100%).

Apoptosis analysis. HCT-116 cells were plated as 2×10^5 cells per 24-well tissue culture plate. The medium was replaced 24 h after seeding with fresh medium containing OhE, OhF4 or OhF5 (1, 3, 10 μg/ml). For apoptosis detection, floating cells in the medium and adherent cells were collected after 24 h, 48 h or 72 h of treatment. Using an Annexin V-FITC Apoptosis Detection Kit, cells were stained with Annexin V-FITC and PI according to the manufacturer's instructions. Untreated HCT-116 cells were used as the control for double staining. Cells were analyzed immediately by using a flow cytometer (Becton Dickinson, Mountain View, CA, USA). For each measurement, at least 20,000 cells were counted.

Cell cycle, cyclin A and cyclin B1 analysis. HCT-116 cells were treated under the same conditions as described above for the apoptosis analysis. To analyze the cell cycle distribution, cells were trypsinized after 24 h of exposure to these extracts, fixed gently with 80% ethanol at –20˚C for 2 h. They were then treated with 0.25% Triton X-100 for 5 min in an ice bath. The cells were resuspended in 300 μl of PBS containing 40 μg/ml PI and 0.1 mg/ml RNase, then 20 μl of cyclin A-FITC or cyclin B1-FITC were added to the cell suspension. Cells were incubated in a dark room for 20 min at room temperature and analyzed with a flow cytometer. For each measurement, at least 10,000 cells were counted.

Statistical analysis. Data were expressed as the mean±standard error (S.E.). Statistical significances between vehicle-treatment and drug-treatment were determined by one-way ANOVA and Student's *t*-test. A value of $p<0.05$ was considered statistically significant.

Results

Effects of OhE, OhF1, OhF2, OhF3, OhF4 and OhF5 on HCT-116, SW-480 and HT-29 cell proliferation. The antiproliferative effects of tested extracts on HCT-116, SW-480 and HT-29 human colorectal cancer cells are shown in Figure 1. After treatment for 48 h, significant suppression of proliferation of HCT-116 cells was observed with OhE, OhF4 and OhF5 at 10-100 μg/ml (all $p<0.01$). Proliferation of SW-480 cells was markedly suppressed with 30-100 μg/ml of OhE, OhF5 and 10-100 μg/ml of OhF4 (all $p<0.01$). Marked suppression of HT-29 cells was observed with 100 μg/ml of OhF5 and 30-100 μg/ml of OhF4 ($p<0.01$ or 0.05). OhF1, OhF2 and OhF3 were not observed to have distinct suppressive effects on these cell lines.

Since HCT-116 was found to be more sensitive to OhE, OhF4 and OhF5, we used this cell line and selected a lower concentration in further studies. After treatment with OhE, OhF4 or OhF5 for 24 h, 48 h or 72 h, the proliferation of HCT-116 cells was distinctly suppressed with time- and dose-dependence at concentrations between 1-10 μg/ml. As shown in Figure 2, in groups treated with 0.3 μg/ml of OhE, OhF4, and OhF5, cell proliferation did not decrease compared with the control at the same time points. The suppressive effects of OhF4 and OhF5 were significantly stronger ($p<0.05$ or 0.01) than that with OhE in the same time and as concentrations between 1-10 μg/ml. The antiproliferative effect of OhF4 was strongest among the three tested extracts, suggesting that OhF4 was the most potent antiproliferative fraction on HCT-116 cells.

Apoptotic effect of OhE, OhF4 and OhF5 on HCT-116 cells. To examine whether proliferation inhibition of treated cells was caused by the induction of apoptosis, flow cytometric analysis was used. The cytograms of bivariate Annexin V/PI analysis of HCT-116 cells after treatment with OhE, OhF4, and OhF5 for 72 h are shown in Figure 3A. Viable cells were negative for both PI and Annexin V (lower left quadrant); early apoptotic cells were positive for Annexin V and negative for PI (lower right quadrant); late apoptotic or necrotic cells displayed positivity for both Annexin V and PI (upper right quadrant); non-viable cells which underwent necrosis were positive for PI and negative for Annexin V (upper left quadrant). OhE (3 μg/ml, 10 μg/ml), OhF4 (1 μg/ml, 3 μg/ml and 10 μg/ml) and OhF5 (3 μg/ml, 10 μg/ml) induced early apoptosis, which is considered real apoptosis. Incubation with OhE, OhF4 or OhF5 at 10 μg/ml for 72 h, increased the percentage of early apoptotic cells by 52.15%, 62.15% and 47.15% respectively, compared with the corresponding control. The percentage of apoptotic cells (both early and late apoptosis) of tested groups for 24 h, 48 h and 72 h are shown in Figure 3B. OhE, OhF4 and OhF5 noticeably induced apoptosis time and concentration dependently. Compared to the control (apoptotic cells 6.1%), OhF4 as 1 μg/ml increased the percentage of apoptotic cells to 12.3% after 24 h treatment. On the other hand, even after treatment for 72 h, 1 μg/ml of OhE or OhF5 did not alter the percentage of apoptotic cells, which was essentially the same as that of the control. These results suggest that apoptotic induction should be one of the pathways through which OhE, OhF4 and OhF5 suppress cell growth. OhF4 was more effective than OhE or OhF5 on the induction of apoptosis at the same concentration and exposure time.

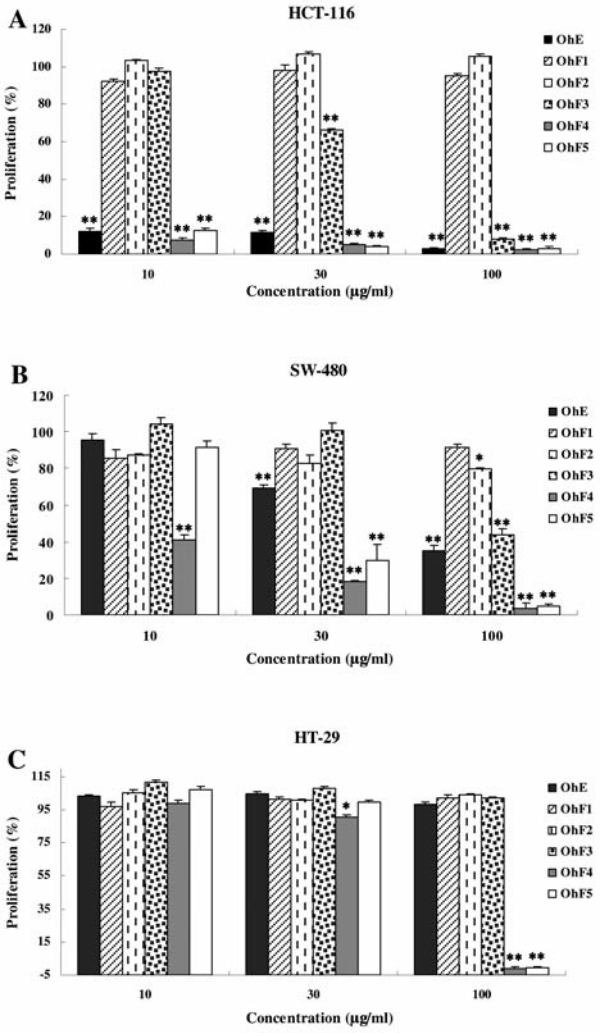

Figure 1. *Effects of Oplopanax horridus extract (OhE) and fractions of OhE column chromatographed over macroporous resin using a step gradient of water-ethanol (OhF1, OhF2, OhF3, OhF4, OhF5) on the proliferation of human colorectal cancer cells. Human colon cancer HCT-116 (A), SW-480 (B) and HT-29 (C) cells were incubated with different concentrations of test extracts (10, 30 and 100 μg/ml) for 48 h. Data are presented as the mean±standard error of the mean of triplicate experiments. *p<0.05, **p<0.01, vs. control.*

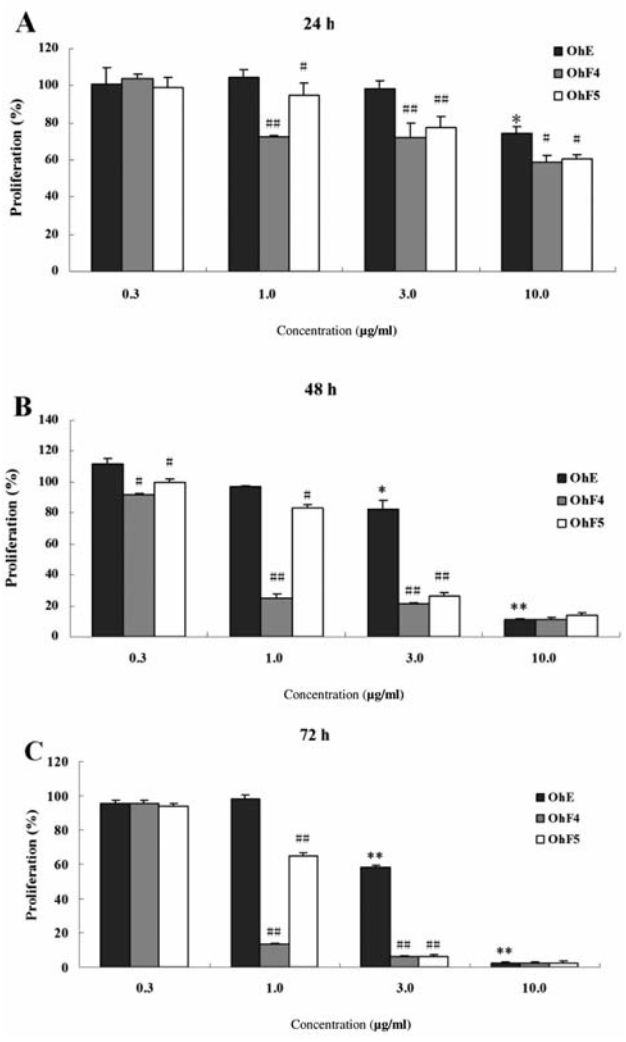

Figure 2. *Effects of Oplopanax horridus extract (OhE) and fractions of OhE column-chromatographed over macroporous resin eluted with 70% and 100% ethanol (OhF4, OhF5) on the proliferation of HCT-116 cells. HCT-116 cells were treated with 0.3-10 μg/ml OhE, OhF4 and OhF5 for 24 h (A), 48 h (B) and 72 h (C). Data are presented as the mean±standard error of mean of triplicate experiments. *p<0.05, **p<0.01, vs. control; #p<0.05, ##p<0.01, vs. corresponding OhE groups.*

Effects of OhE, OhF4 and OhF5 on the cell cycle distribution in HCT-116 cells. To examine whether the decrease of proliferation in treated cells is a consequence of the cell cycle being arrested at a specific phase, the cell cycle of treated HCT-116 cells by flow cytometry was analyzed. As shown in Figure 4, treatment with OhE (10 μg/ml), OhF4 (1 μg/ml, 3 μg/ml), and OhF5 (3 μg/ml, 10 μg/ml) for 24 h induced G_2/M-phase arrest of the cell cycle in a dose-dependent manner. For the untreated control, the fraction of cells in different cell cycle phases were 30.0% (G_1-phase), 47.7% (S-phase) and 22.3% (G_2/M-phase). Following exposure to OhF4 at 1 μg/ml and 3 μg/ml, the percentage of HCT-116 cells at the G_2/M-phase increased by 20.7% and 29.6%, respectively; At the same time, the percentage of cells in the G_1-phase was markedly reduced in OhF4-treated groups. Since viable cells were difficult to obtain, the cell cycle profile of the 10 μg/ml OhF4 treatment group was not evaluated. The percentages of each cell cycle phase with various treatments are shown in Table I. More cells treated with OhF4 were arrested in the G_2/M-phase compared to those treated with OhE or OhF5 at the same concentration.

Effects of OhE, OhF4 and OhF5 on expression of cyclin A and cyclin B1. The changes of cyclin A and cyclin B1 expression in HCT-116 cells treated with OhE, OhF4 or OhF5 are shown in Figures 5 and 6. After incubation with OhF4 at 1 μg/ml and 3 μg/ml for 24 h, the fraction of cyclin A-positive cells increased by 19.1% and 34.4% respectively, and the fraction of cyclin B1-positive cells increased by 13.3% and 14.9%, compared with the corresponding control group. Since viable cells were difficult to obtain, the assay of 10 μg/ml of the OhF4 treatment group was not evaluated. Following exposure to OhE (1 μg/ml, 3 μg/ml, 10 μg/ml), and OhF5 (1 μg/ml, 3 μg/ml, 10 μg/ml), the percentage of cyclin A- and cyclin B1-positive cells also increased dose-dependently. But at the same concentration, OhF4 approved to be more effective than OhE and OhF5 on cyclin A and cyclin B1 expression.

Discussion

Previous studies have shown that OhE possesses antiproliferative activity on several human cancer cell lines, including leukemia, breast cancer, and ovarian cancer (22). Recently, our group observed the chemical composition and antiproliferative effects of *O. horridus* stem and berry extracts on human colorectal cancer cells (23). The effect of the root extract of *O. horridus* or OhE on colorectal cancer cells has however not been studied. The present study was performed to evaluate the effects of the different water fractions of OhE on the growth of human colorectal cancer cells, and identify the potential active fractions and action mechanisms.

Botanical extracts are comprised of complicated mixtures of natural components which may possess biological activities (24). To identify the active constituents, bioassay guided fractionation is an essential step (25). Using chromatographic techniques, the active components can be enriched in certain fractions, and subsequent isolation can identify bioactive natural compounds (26).

In this study, we observed, for the first time, that OhE and its fractions possessed antiproliferative activities on selected human colorectal cancer cell lines, *i.e.* HCT-116, SW-480 and HT-29 cells. The MTS results showed that OhE, OhF4 and OhF5 was able to significantly inhibit cell proliferation in a dose-dependent manner in HCT-116 and SW-480 cells at concentrations between 10-100 μg/ml. The proliferative

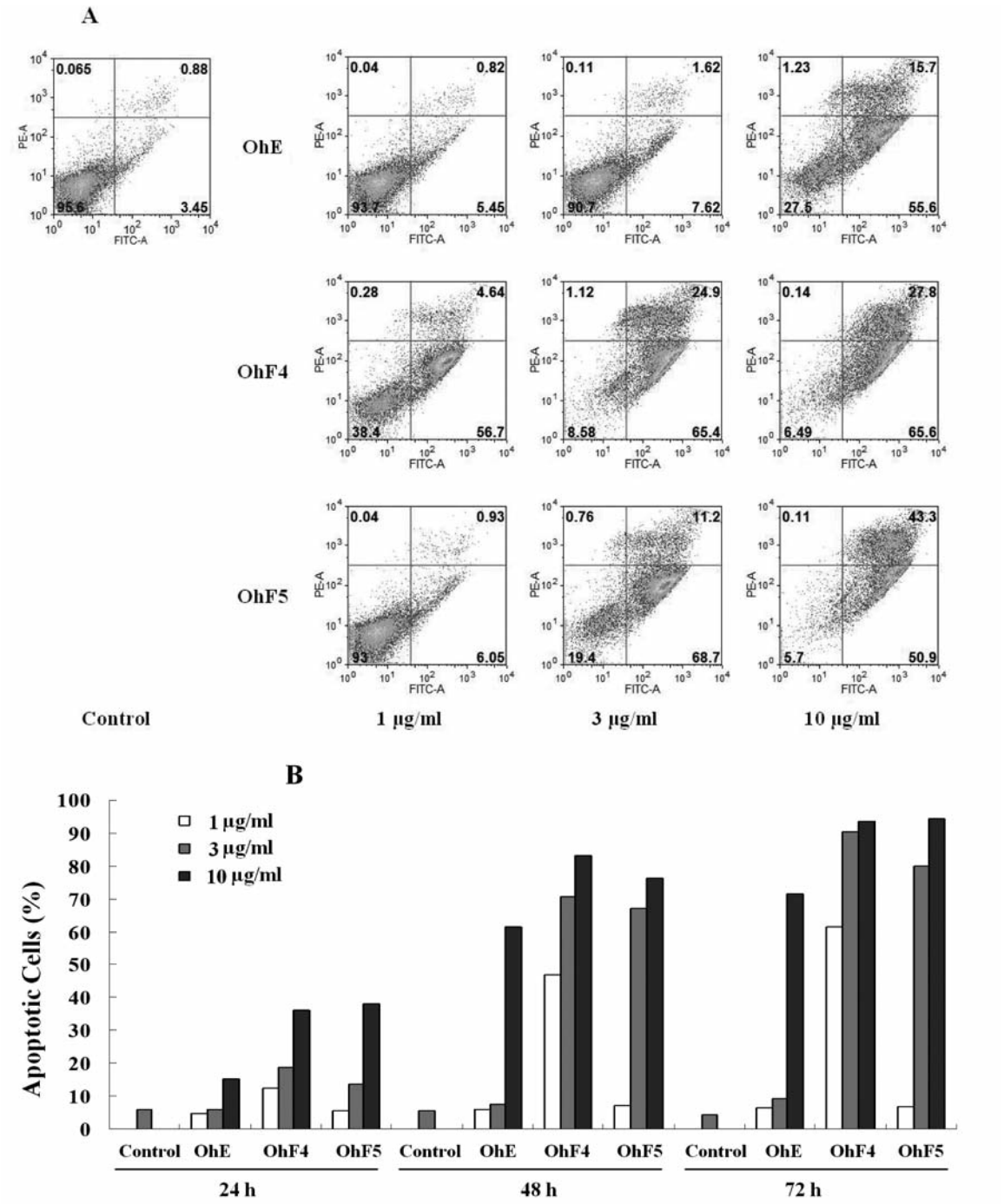

Figure 3. *Effects of Oplopanax horridus extract (OhE) and fractions (OhF4, OhF5) on apoptosis. HCT-116 cells were treated with 0.3-10 μg/ml OhE, OhF4 and OhF5 for 24 h, 48 h and 72 h. A: The representative cytogram of cells treated for 72 h. B: The percentage of apoptotic cells in each experimental group.*

inhibition by OhE and its fractions, OhF4 and OhF5, was more pronounced on HCT-116 cells than on SW-480 cells. However, suppression on cell growth was not observed in OhE-treated HT-29 cells, suggesting different colorectal cancer cells may have different responses to OhE treatment. Based on the above results, a further study was conducted in HCT-116 cells treated with OhE, OhF4 and OhF5 at even lower concentrations than 10 μg/ml, and our data showed that at 1-3 μg/ml for 24 h, 48 h and 72 h treatments, significant antiproliferative effects were maintained. At all observed time points, OhF4 showed the

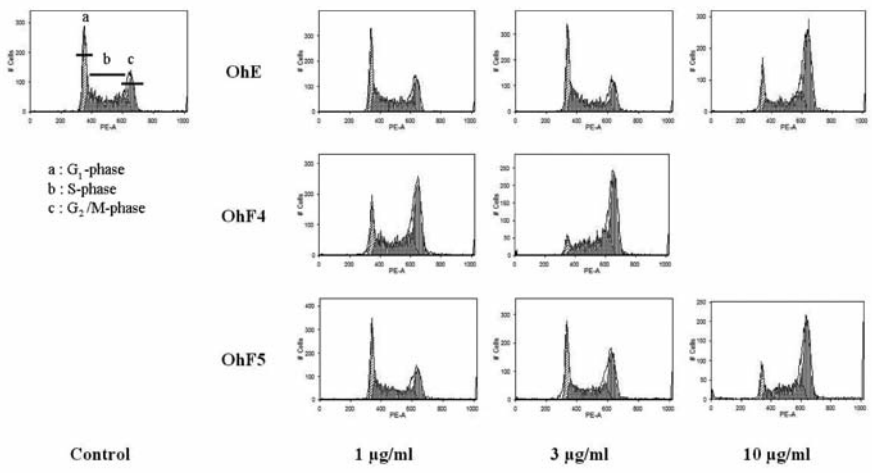

Figure 4. *Effects of Oplopanax horridus extract (OhE) and fractions (OhF4, OhF5) on cell cycle distribution. HCT-116 cells were treated with 0.3-10 μg/ml OhE, OhF4 and OhF5 for 24 h.*

Table I. *Percentage of HCT-116 cells in G_1-, S- and G_2/M-phase.*

	Control	OhE			OhF4		OhF5		
		1 μg/ml	3 μg/ml	10 μg/ml	1 μg/ml	3 μg/ml	1 μg/ml	3 μg/ml	10 μg/ml
G_1	30.0	25.8	26.3	11.8	14.5	6.3	26.6	25.3	10.0
S	47.7	50.5	49.9	35.4	42.5	41.8	46.9	38.5	36.9
G_2/M	22.3	23.7	23.8	52.8	43.0	51.9	26.5	36.2	53.1

strongest antiproliferative effect among the tested samples. These results further pointed out that the active components in OhE may be enriched in the fractions of OhF4 and OhF5 since both of these fractions produced more inhibition of HCT-116 cell growth.

Both OhF4 and OhF5 are hydrophobic fractions, which were eluted with 70% and 100% ethanol from the Dianion HP20 column. In a previous report (17), several polyacetylenes were isolated from the hydrophobic fraction of OhE, in which they are the major constituents. Combined with the bioassay data in this study, the active constituents in OhF4 and OhF5 could be polyacetylenes.

In the apoptotic analysis by flow cytometry, the percentage of apoptotic cells, including early and late apoptosis, was much higher in OhF4- and OhF5-treated HCT-116 cells than that in OhE-treated cells at the same concentration and treatment time. This observation not only strengthened the evidence of the activity of the active fractions, but also provided the potential mechanism by which OhE and its fractions induced colon cancer cell growth inhibition.

Cell cycle progression, a series of events in a eukaryotic cell leading to its replication, is halted at the transition from the G_1 to the S-phase or from the G_2- to the M-phase after DNA damage (27, 28). The analysis of the cell cycle distribution in this study demonstrated that the G_2/M-phase was arrested in HCT-116 cells in response to OhE and its fractions, OhF4 and OhF5, suggesting that G_2/M arrest is the target cell cycle checkpoint by treatment with OhE. This supported the evidence that the suppression of the cell cycle transition is involved in the OhE-induced antitumor action in human colorectal cancer cells.

To further confirm the molecule involved in OhE-induced cell cycle arrest, we performed analysis of the expression of cyclin A and cyclin B1. Cyclin A, a member of Cyclin-dependent kinase 2 (CDK2) which are a family of proteins involved in the progress of cells through the cell cycle, binds to CDK2 and is required for the cell to progress through the S-phase (29); in contrast, cyclin B1 plays an important role in the control of the G_2-M transition of the cell cycle and is the marker of the G_2/M-phase (30). The present results showed the high expression of cyclin A and cyclin B1 in HCT-116 cells after exposure to OhE, OhF4 and OhF5, indicating that OhE facilitated the cell's progression through the S-phase into the G_2/M-phase and led to the accumulation of cells in the G_2/M-phase. This may partially explain the observed arrest of HCT-116 cells in the G_2/M-phase.

The present study is the first to systemically evaluate the potential antiproliferative activity of OhE on human colorectal cancer cells. We further identified that the active components

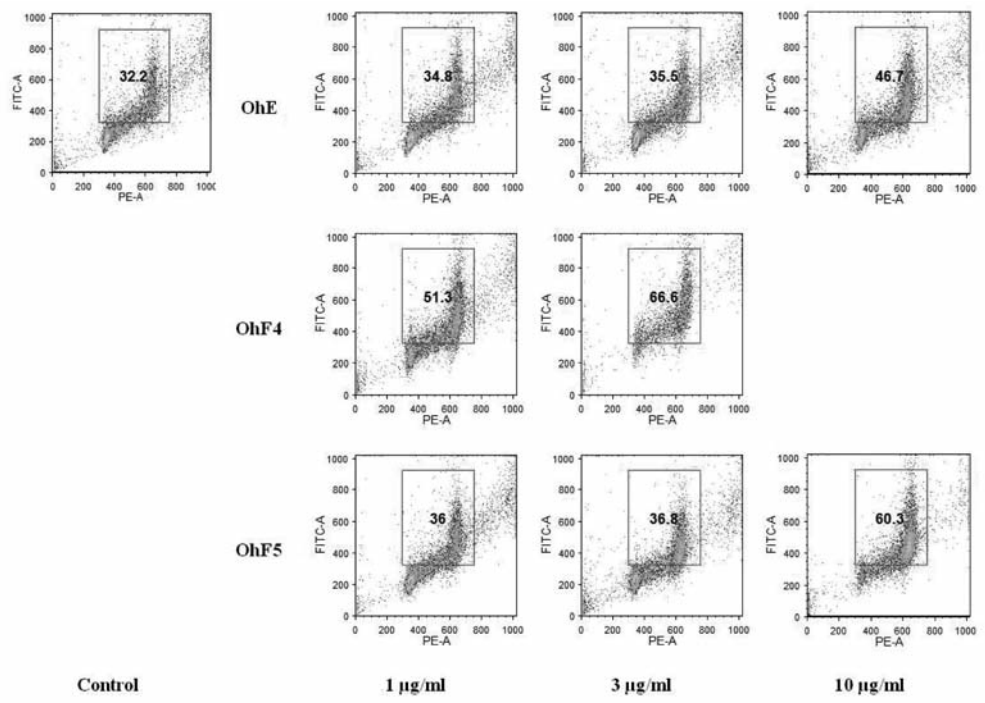

Figure 5. *Effects of Oplopanax horridus extract (OhE) and fractions (OhF4, OhF5) on the expression of cyclin A. HCT-116 cells were treated with 0.3-10 µg/ml OhE, OhF4 and OhF5 for 24 h. The percentage of cyclin A-positive cells is shown in the gate.*

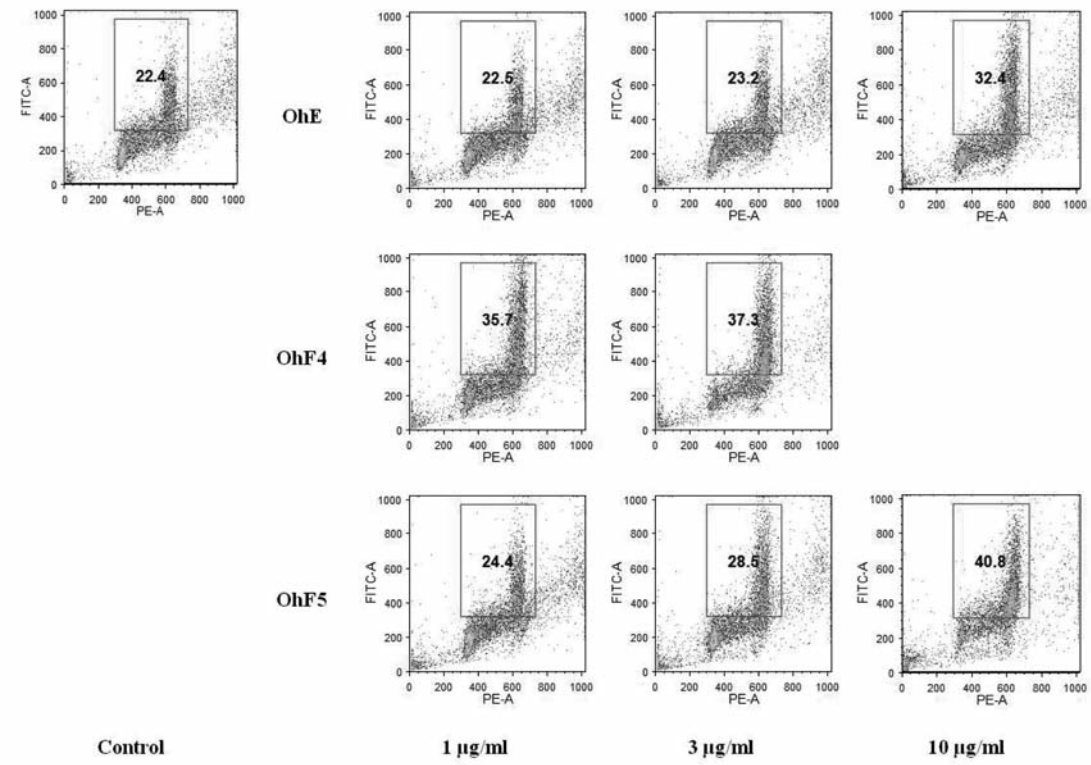

Figure 6. *Effects of Oplopanax horridus extract (OhE) and fractions (OhF4, OhF5) on the expression of cyclin B1. HCT-116 cells were treated with 0.3-10 µg/ml OhE, OhF4 and OhF5 for 24 h. The percentage of cyclin B1-positive cells is shown in the gate.*

are enriched in the fractions eluted by 70-100% ethanol from OhE. The anticancer mechanism of OhE might be attributed to its induction of cell apoptosis and arrest of cells in the G_2/M-phase, in which the stimulation of OhE on the expression of cyclin A and cyclin B1 plays critical roles. From a therapeutic perspective, our results demonstrate that *Oplopanax horridus* may have a role to play as an alternative medicine for the treatment of human colorectal cancer. Bioassay-guided fractionation may lead to the isolation of active compounds from OhE.

Acknowledgements

This study was supported in part by the NIH/NCCAM grants AT003255, AT003441, AT004418 and 5P30DK042086.

References

1 Joseph DA, Rim SH and Seeff LC: Use of colorectal cancer tests – United States, 2002, 2004, and 2006. MMWR Morb Mortal Wkly Rep *57*: 253-258, 2008.
2 Rychahou PG, Kang J, Gulhati P, Doan HQ, Chen LA, Xiao SY, Chung DH and Evers BM: Akt2 overexpression plays a critical role in the establishment of colorectal cancer metastasis. Proc Natl Acad Sci USA *105*: 20315-20320, 2008.
3 Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB and Maisonneuve P: Smoking and colorectal cancer: a meta-analysis. JAMA *300*: 2765-2778, 2008.
4 Delval L and Klastersky J: Optic neuropathy in cancer patients. Report of a case possibly related to 5 fluorouracil toxicity and review of the literature. J Neurooncol *60*: 165-169, 2002.
5 Henriette Tanja L, Guchelaar HJ and Gelderblom H: Pharmacogenetics in chemotherapy of colorectal cancer. Best Pract Res Clin Gastroenterol *23*: 257-273, 2009.
6 Viale PH and Yamamoto DS: Cardiovascular toxicity associated with cancer treatment. Clin J Oncol Nurs *12*: 627-638, 2008.
7 Chau I and Cunningham D: Treatment in advanced colorectal cancer: what, when and how? Br J Cancer *100*: 1704-1719, 2009.
8 Jackson LN, Zhou Y, Qiu S, Wang Q and Evers BM: Alternative medicine products as a novel treatment strategy for inflammatory bowel disease. Am J Chin Med *36*: 953-965, 2008.
9 Efferth T, Fu YJ, Zu YG, Schwarz G, Konkimalla VS and Wink M: Molecular target-guided tumor therapy with natural products derived from traditional Chinese medicine. Curr Med Chem *14*: 2024-2032, 2007.
10 Lee HJ, Lee EO, Lee JH, Lee KS, Kim KH, Kim SH and Lu J: *In vivo* anti-cancer activity of Korean *Angelica gigas* and its major pyranocoumarin decursin. Am J Chin Med *37*: 127-142, 2009.
11 Wang CZ and Yuan CS: Potential role of ginseng in the treatment of colorectal cancer. Am J Chin Med *36*: 1019-1028, 2008.
12 Wang CZ, Zhang B, Song WX, Wang A, Ni M, Luo X, Aung HH, Xie JT, Tong R, He TC and Yuan CS: Steamed American ginseng berry: ginsenoside analyses and anticancer activities. J Agric Food Chem *54*: 9936-9942, 2006.
13 Fishbein AB, Wang CZ, Li XL, Mehendale SR, Sun S, Aung HH and Yuan CS: Asian ginseng enhances the anti-proliferative effect of 5-fluorouracil on human colorectal cancer: comparison between white and red ginseng. Arch Pharm Res *32*: 505-513, 2009.
14 Li XL, Wang CZ, Mehendale SR, Sun S, Wang Q and Yuan CS: Panaxadiol, a purified ginseng component, enhances the anti-cancer effects of 5-fluorouracil in human colorectal cancer cells. Cancer Chemother Pharmacol, 2009.
15 Luo X, Wang CZ, Chen J, Song WX, Luo J, Tang N, He BC, Kang Q, Wang Y, Du W, He TC and Yuan CS: Characterization of gene expression regulated by American ginseng and ginsenoside Rg3 in human colorectal cancer cells. Int J Oncol *32*: 975-983, 2008.
16 Wang CZ, Li XL, Wang QF, Mehendale SR, Fishbein AB, Han AH, Sun S and Yuan CS: The mitochondrial pathway is involved in American ginseng-induced apoptosis of SW-480 colon cancer cells. Oncol Rep *21*: 577-584, 2009.
17 Kobaisy M, Abramowski Z, Lermer L, Saxena G, Hancock RE, Towers GH, Doxsee D and Stokes RW: Antimycobacterial polyynes of Devil's Club (*Oplopanax horridus*), a North American native medicinal plant. J Nat Prod *60*: 1210-1213, 1997.
18 Tai J, Cheung S, Cheah S, Chan E and Hasman D: *In vitro* anti-proliferative and antioxidant studies on Devil's Club *Oplopanax horridus*. J Ethnopharmacol *108*: 228-235, 2006.
19 Bloxton J, DerMarderosian A and Gibbs R: Bioactive constituents of Alaskan devil's root (*Oplopanax horridus*, Araliaceae). Econ Bot *56*: 285-287, 2002.
20 Smith GW: Arctic pharmacognosia II. Devil's Club, *Oplopanax horridus*. J Ethnopharmacol *7*: 313-320, 1983.
21 Wattenberg LW: Inhibition of azoxymethane-induced neoplasia of the large bowel by 3-hydroxy-3,7,11-trimethyl-1,6,10-dodecatriene (nerolidol). Carcinogenesis *12*: 151-152, 1991.
22 Tai J, Cheung S, Chan E and Hasman D: Inhibition of human ovarian cancer cell lines by Devil's club *Oplopanax horridus*. J Ethnopharmacol, 2009.
23 Wang CZ, Xie JT, Fishbein A, Aung HH, He H, Mehendale SR, He TC, Du W and Yuan CS: Antiproliferative effects of different plant parts of Panax notoginseng on SW480 human colorectal cancer cells. Phytother Res *23*: 6-13, 2009.
24 Ang-Lee MK, Moss J and Yuan CS: Herbal medicines and perioperative care. JAMA *286*: 208-216, 2001.
25 Newton SM, Lau C, Gurcha SS, Besra GS and Wright CW: The evaluation of forty-three plant species for *in vitro* antimycobacterial activities; isolation of active constituents from *Psoralea corylifolia* and *Sanguinaria canadensis*. J Ethnopharmacol *79*: 57-67, 2002.
26 Brahmachari G, Mondal S, Gangopadhyay A, Gorai D, Mukhopadhyay B, Saha S and Brahmachari AK: Swertia (Gentianaceae): chemical and pharmacological aspects. Chem Biodivers *1*: 1627-1651, 2004.
27 Hartwell LH and Weinert TA: Checkpoints: controls that ensure the order of cell cycle events. Science *246*: 629-634, 1989.
28 Shi P, Huang Z and Chen G: Rhein induces apoptosis and cell cycle arrest in human hepatocellular carcinoma BEL-7402 cells. Am J Chin Med *36*: 805-813, 2008.
29 Lim YJ, Rhee JC, Bae YM and Chun WJ: Celecoxib attenuates 5-fluorouracil-induced apoptosis in HCT-15 and HT-29 human colon cancer cells. World J Gastroenterol *13*: 1947-1952, 2007.
30 Hassan KA, El-Naggar AK, Soria JC, Liu D, Hong WK and Mao L: Clinical significance of cyclin B1 protein expression in squamous cell carcinoma of the tongue. Clin Cancer Res *7*: 2458-2462, 2001.

Received August 17, 2009
Revised December 29, 2009
Accepted January 5, 2010