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Anticancer activities of a chemically sulfated polysaccharide obtained from *Grifola frondosa* and its combination with 5-Fluorouracil against human gastric carcinoma cells

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Abstract

Chemically sulfated polysaccharide (S-GAP-P) was derived from water-insoluble polysaccharide of *Grifola frondosa* mycelia. In this research, we investigated the anticancer effects of S-GAP-P and its combination with 5-fluorouracil (5-FU) on human gastric carcinoma SGC-7901 cells. Results showed that S-GAP-P distinctly inhibited SGC-7901 cells growth in a dose-dependent manner and induced cell apoptosis evidenced by characteristic DNA ladder and sub-G_0/G_1 peak. Furthermore, the combination of S-GAP-P (10–50 μg/ml) with 1 μg/ml 5-FU resulted in a significant inhibition on SGC-7901 cells growth, meaning the beneficial interaction between the two drugs. All these results suggested that S-GAP-P has evident anticancer activity through apoptotic induction and could significantly accelerate the anticancer activity of 5-FU.

Keywords: Chemically sulfated polysaccharide (S-GAP-P); *Grifola frondosa*; Anticancer activity; 5-Fluorouracil (5-FU); SGC-7901 cells

1. Introduction

In the last decades, much attention has focused on the biological properties of polysaccharides and their chemical derivatives, especially sulfated derivatives (Han, Yao, Yang, Liu, & Gao, 2005; Lee, Bae, & Pyo, 2003; Yang, Du, Huang, Sun, & Liu, 2005; Xing et al., 2005). The roles of sulfated polysaccharides in biological systems were involved in several cellular processes, such as molecular recognition, cell development and differentiation, and cell–cell interaction. Many sulfated polysaccharides exerted potent antioxidant, anticoagulant, antithrombotic and antiviral activities (Han et al., 2005; Soeda, Ohmagari, Shimeno, & Nagamatsu, 1993; Urbinati, Bugatti, & Oreste, 2004; Xing et al., 2005). They are also known to inhibit some tumor development (Peng, Zhang, Zeng, & Kennedy, 2005; Yang et al., 2005).

Grifola frondosa, one traditional and edible mushroom in Southeast Asia, is commonly used in the treatment of various diseases, due to its considerable biological activities (Inoue, Kodama, & Nanba, 2002; Lee et al., 2003; Mayell, 2001). However the polysaccharides extracted with alkali from *G. frondosa* are mainly insoluble in water and less suitable for pharmaceutical study. Documents demonstrated that chemical sulfation of water-insoluble polysaccharide should, not only enhance the water solubility but also change the chain conformation, resulting in the alteration of their biological activities (Wang, Zhang, Li, Hou, & Zeng, 2004). For this reason, we had produced a sulfated derivate (S-GAP-P) of water-insoluble polysaccharide from *G. frondosa* mycelia by chemical modification.

In the present study, we carried out experiments to investigate the growth inhibitory effects of S-GAP-P alone and its combination with 5-fluorouracil (5-FU) against

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human gastric carcinoma SGC-7901 cells, and cell apoptosis was also detected.

2. Experiment

2.1. Microorganism and materials

The strain of *G. frondosa* GF9801 is preserved in Biopharmaceutics Laboratory of Southern Yangtze University (Wuxi, China). Medium RPMI1640 and fetal bovine serum (FBS) were purchased from Gibco-BRL, Life Technologies, Inc., USA. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), sodium dodecyl sulfate (SDS), ethidium bromide (EB), propidium iodide (PI), protease K and RNase A were purchased from Sigma Chemicals Co., USA. And 5-fluorouracil (5-FU) was from Shanghai Xundonghaipu Pharmaceutical Ltd., China. All other reagents were of analytical grade made in China.

2.2. Preparation and isolation of chemically sulfated polysaccharide

The mycelia of *G. frondosa* GF9801 were obtained through submerged cultivation (Cui et al., 2005), and water-insoluble polysaccharide (GAP-P) was extracted as described else (Shi, Xu, Ao, Sun, & Tao, 2003). Chemical sulfation of GAP-P was carried out using the chlorosulfonic acid/pyridine method (Yoshida, Yasuda, Mimura, & Kaneko, 1995). Briefly, 200 mg GAP-P sample was suspended in 20 ml anhydrous formanide, and the sulfation reagent was added. The mixture was maintained at 45 °C for 6 h, neutralized with 2.5 M NaOH, dialyzed against distilled water and precipitated with 95% ethanol. The precipitate was resolved in distilled water and centrifugated at 12,000 rpm. The resulting supernatant was loaded on to a column (2.6 cm × 13 cm) of DEAE Sepharose Fast Flow (Pharmacia Co., Sweden), followed by eluting at a flow rate of 0.25 ml/min successively with distilled water and a gradient of 0–2.0 M NaCl solution. Substance recovered from the main peak was concentrated and lyophilized to give a sulfated polysaccharide coded as S-GAP-P. FT-IR result of S-GAP-P showed that the sulfation reaction had actually occurred, and S-GAP-P was determined to be a sulfated glucan, with the average molecular weight of 28 kDa and the sulfur content of 16.4% (Shi et al., 2003).

2.3. Assay of cell growth

Human gastric carcinoma SGC-7901 cells were obtained from Shanghai Institute of Cell Biology (China). The cells were cultured in RPMI1640 medium containing 10% FBS in a humidified atmosphere of 5% CO_2 at 37 °C.

The cell growth was determined by the MTT cellular viability assay method (Cheng, 2000). 1×10^4 SGC-7901 cells were placed into 96-well microplates and cultured for 24 h prior to treatment. The cells were treated with various concentrations of S-GAP-P and/or 5-FU. At the end of treatment, 10 μl MTT solution (5 mg/ml) was added to each well and incubated for another 4 h. The medium was removed and 150 μl dimethylsulfoxide (DMSO) was added to the wells. Then the absorbance was measured at 570 nm using an ELISA reader (Bio-Tek EL × 800, USA). All determinations were conducted in triplicate. The inhibition of cell growth was calculated by following formula: inhibition (%) $= (1 - OD/OD_0) \times 100$, where OD and OD_0 indicated the absorbance of treated cells and untreated cells, respectively.

2.4. DNA agarose gel electrophoresis

The isolation of fragmented DNA was carried out according to the procedure of Brown, Sun, and Cohen (1993). SGC-7901 cells were treated with various concentrations of S-GAP-P and/or 5-FU for 48 h. 1×10^6 cells were harvested and suspended in 0.5 ml DNA lysis buffer (10 mM Tris–HCl, pH 8.0; 150 mM NaCl; 10 mM EDTA; 0.4%SDS; 100 μg/ml protease K), then incubated at 50 °C for 3 h. DNA was extracted with a mixed solvent system, which was made of phenol and equal volume of chloroform–isopropyl alcohol (24:1, v/v), then precipitated with 0.1 volume of sodium acetate buffer (3 M, pH 5.2) and 2.5 volume of ethanol at −20 °C overnight. After centrifugation, crude DNA was dried in air and dissolved in TE buffer (10 mM Tris–HCl, pH 8.0; 1 mM EDTA) containing 20 μg/ml RNase A. The mixture was incubated at 37 °C for 1 h. DNA dissolved in loading buffer were loaded on a 1.5% (w/v) agarose gel containing EB and subjected to electrophoresis at 75 V for 1–2 h. Separated DNA fragments were visualized at 254 nm.

2.5. Flow cytometric analysis

Apoptotic cells were detected by flow cytometry using propidium iodide (Nicoletti, Migliorati, Pagliacci, Grignani, & Riccardi, 1996). SGC-7901 cells were treated with various concentrations of S-GAP-P and/or 5-FU for 48 h. 1×10^6 cells were harvested and fixed in 70% ice-cold ethanol, then stored at −20 °C for 24 h. After centrifugation, the cells were suspended in 1 ml phosphate buffered saline (PBS; 0.01 M, pH 7.2) containing 0.1 mg/ml RNase A and 0.1 mg/ml PI, and incubated for 30 min in the dark. Then the cell suspensions were filtrated and analyzed by a FACScan flow cytometry system (Becton Dickinson, USA). Data from 10,000 cells of each sample were collected for evaluation. Sub-G_0/G_1 DNA content was taken as a basis for calculating the percentage of apoptotic cell.

2.6. Statistical analysis

The data obtained were expressed as mean ± SD and analyzed statistically by ANOVA method. Significance of any differences between groups were evaluated using Student's *t*-test.

3. Results and discussion

3.1. Growth inhibitory effects of S-GAP-P on SGC-7901 cells

A literature survey indicated that chemical sulfation of water-insoluble polysaccharide might lead to anticancer activity (Du et al., 2004; Lin, Zhang, Chen, & Jin, 2004;Wang et al., 2004; Williams et al., 1991). In the present study, the growth inhibitory effects of the chemically sulfated polysaccharide (S-GAP-P) against SGC-7901 cells were first examined. As shown in Table 1, S-GAP-P inhibited SGC-7901 cells growth in a dose-dependent manner. After 48 h treatment, S-GAP-P demonstrated distinct inhibition at the concentrations ranging from 50 to 500 μg/ml, and the IC_{50} for 48 h treatment was 264.28 μg/ml; whereas the growth inhibitory effects for 24 h treatment were not significant. It was reported that the introduction of sulfate groups to polysaccharide should change its physicochemical characterization and chain conformation, and the forces among sulfate groups result in a relatively expanded and stiff chain in aqueous solution; the relatively high chain stiffness and good water solubility of sulfated polysaccharides are beneficial to the enhancement of their antitumor effect (Lin et al., 2004; Wang et al., 2004).

Table 1
Growth inhibitory effects of S-GAP-P on SGC-7901 cells

S-GAP-P concentration (μg/ml)	Inhibition of cell growth (%)	
	24 h	48 h
10	2.88	4.92
20	3.56	13.37
50	5.07	25.35*
100	10.34	36.76*
250	18.80	48.84**
500	25.88*	59.62**

SGC-7901 cells were treated with various concentrations of S-GAP-P for 24 or 48 h, and the cell growth was determined by MTT assay. The data were expressed as mean ± SD of three experiments. Significant differences from the control were evaluated using Student's *t*-test: $*p < 0.05$, $**p < 0.01$.

3.2. Growth inhibitory effects of S-GAP-P combined with 5-FU on SGC-7901 cells

Combination therapy of two or more drugs is frequently used in cancer treatment and thought to have greater beneficial effects (Chopin, Slomianny, Hondermarck, & Le Bourhis, 2004; Kanzawa, Akiyama, Saijo, & Nishio, 2003; Notarbartolo et al., 2005). 5-Fluorouracil is one of the most effective anticancer drugs used in the chemotherapy against a variety of solid tumors including gastric cancer (Longley, Harkin, & Johnson, 2003), but continuous use of 5-FU is not always feasible because of cumulative toxicities and the development of drug resistance. Considering that some polysaccharides are believed to affect the sensitivities of many cancers to chemotherapy besides their biological activities (Wang, Wang, Huang, Wu, & Xu, 2002), it is interesting to research the combined treatment of S-GAP-P and 5-FU in SGC-7901 cells.

In the combination studies, we investigated the effects of various concentrations of S-GAP-P on SGC-7901 cell growth in the presence of 1 μg/ml 5-FU. As illustrated in Fig. 1, combined treatment with 10–50 μg/ml S-GAP-P and 1 μg/ml 5-FU resulted in a significant inhibition on SGC-7901 cells growth, compared with the controls or cells treated either drug on its own. Especially, S-GAP-P at 10 μg/ml significantly enhanced the 5-FU-induced cytotoxicity from 14.33% to 43.32%. On the other hand, the interaction between S-GAP-P at higher concentration (100–500 μg/ml) and 1 μg/ml 5-FU were not effective. The combination of 100–500 μg/ml S-GAP-P and 1 μg/ml 5-FU, only produced a little increase of the inhibitory effects on SGC-7901 cells, compared with the cells treated with S-GAP-P alone. Taking into account the fact that S-GAP-P at higher concentration exerted significant anticancer activity, we presumed that the stimulative effect between two drugs might be concealed and S-GAP-P

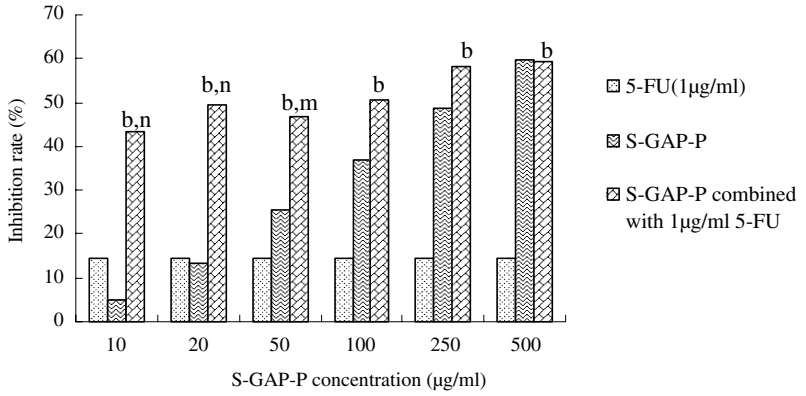

Fig. 1. Growth inhibitory effects of combined treatment with S-GAP-P and 5-FU on SGC-7901 cells. The cells were treated with S-GAP-P and/or 1 μg/ml 5-FU for 48 h, and the cell growth was determined by MTT assay. Significant differences between the combination and 1μg/ml 5-FU were evaluated using Student's *t*-test: a: $p < 0.05$, b: $p < 0.01$; significant differences between the combination and S-GAP-P at the same concentration were evaluated using Student's *t*-test: m: $p < 0.05$, n: $p < 0.01$.

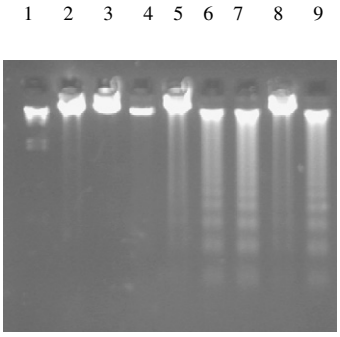

Fig. 2. Measurement of apoptosis by visualization of internucleosomal DNA ladders. DNA samples from SGC-7901 cells treated with S-GAP-P and/or 5-FU for 48 h were analyzed by agarose gel electrophoresis. Lane 1 was DNA marks, lane 2 represents result obtained with untreated cells, lanes 3–7 with cells treated with S-GAP-P at 10, 20, 50, 100 and 250 μg/ml, lane 8 for cells treated with 5-FU (1 μg/ml), lane 9 for cells treated with the combination of S-GAP-P (10 μg/ml) and 5-FU (1 μg/ml).

played the man role in the combined treatment. All of these results suggested that S-GAP-P at low concentration (10–20 μg/ml) could synergistically enhance anticancer activity of 1 μg/ml 5-FU.

3.3. Apoptotic induction in SGC-7901 cells by S-GAP-P and its combination

Programmed cell death (apoptosis) is a physiological and crucial process that is regarded as the preferred way to eliminate cancer cells (Mukhtar & Ahmad, 1999). Many polysaccharides have been found to induce apoptosis in cancer cells (Sogawa et al., 2000; Zhang & Huang, 2005). After treating SGC-7901 cells with S-GAP-P and its combination, DNA agarose gel electrophoresis and flow cytometric analysis were conducted.

As illustrated in Fig. 2, the treatment of S-GAP-P at high concentrations (100 and 250 μg/ml) resulted in the induction of DNA fragmentation, which is a characteristic feature of cell apoptosis (Brown et al., 1993). DNA fragmentation was also observed in SGC-7901 cells treated with the combination of S-GAP-P (10 μg/ml) with 5-FU (1 μg/ml), but not found in the cells treated with individual drug at that concentration.

Apoptotic induction was also confirmed by flow cytometric analysis of cell cycle. S-GAP-P has been shown to induce a dose-dependent accumulation of SGC-7901 cells in sub-G_0/G_1 peak indicating apoptotic phenomenon and

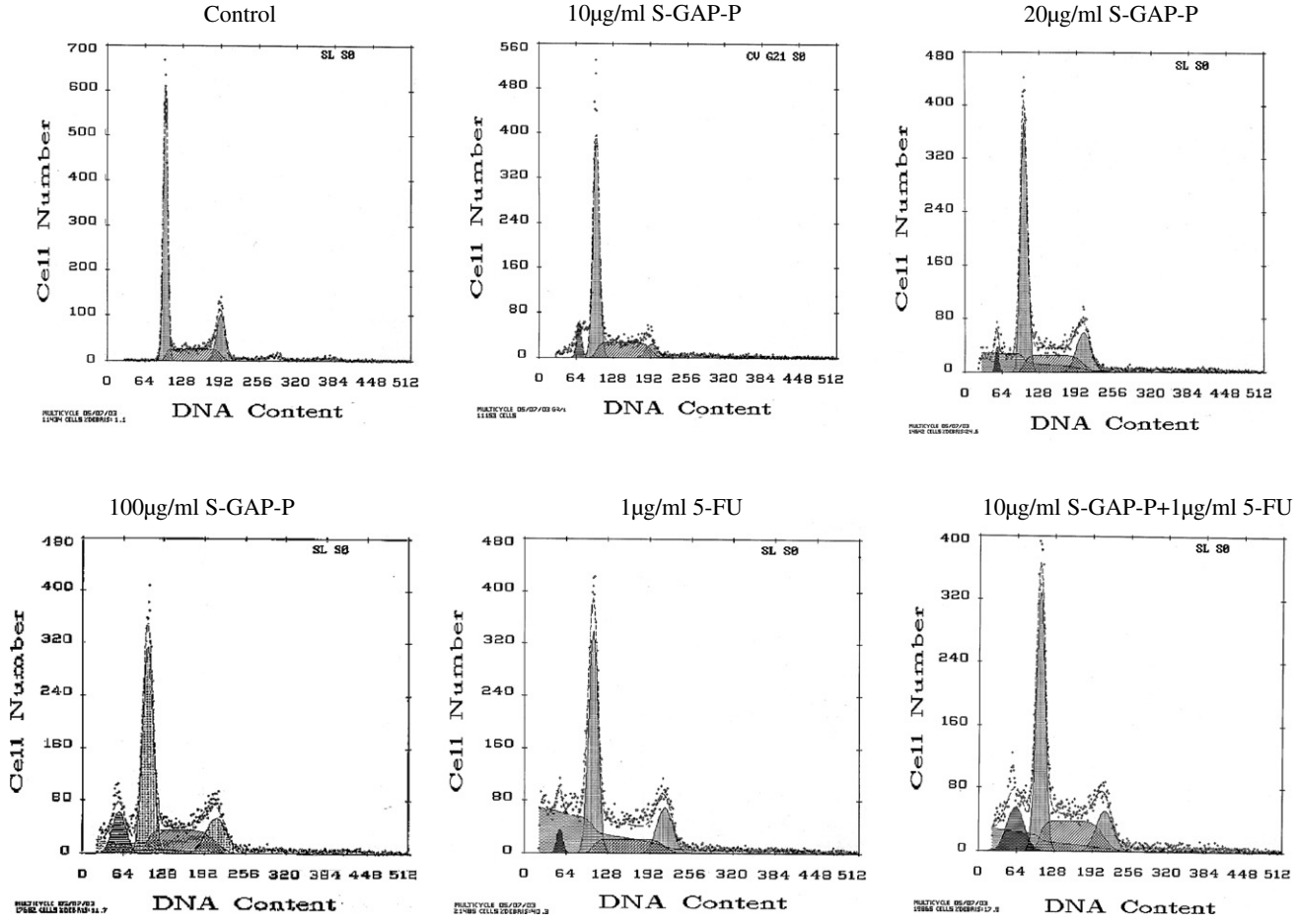

Fig. 3. Flow cytometric analysis of human gastric carcinoma cells. SGC-7901 cells were treated with S-GAP-P and/or 5-FU for 48 h. Then the cells were fixed with 70% ethanol, stained with PI and analyzed by flow cytometry.

Table 2
Effect of S-GAP-P and its combination with 5-FU on SGC-7901 cell apoptosis

Treatment	% Apoptotic population
Control	1.2 ± 0.05
S-GAP-P (10 μg/ml)	2.1 ± 0.16
S-GAP-P (20 μg/ml)	6.8 ± 0.52*
S-GAP-P (100 μg/ml)	13.9 ± 0.71**
5-FU (1 μg/ml)	5.9 ± 0.28
S-GAP-P (10 μg/ml) + 5-FU (1 μg/ml)	14.3 ± 0.65**,b,n

SGC-7901 cells were treated with S-GAP-P and/or 5-FU for 48 h. The cells were then fixed with 70% ethanol, stained with PI and analyzed by flow cytometry. The data were expressed as mean ± SD of three experiments. Significant differences from the control were evaluated using Student's *t*-test: $*p < 0.05$, $**p < 0.01$; significant differences between the combination and 1μg/ml 5-FU were evaluated using Student's *t*-test: -a: $p < 0.05$, b: $p < 0.01$; significant differences between the combination and 10 μg/ml S-GAP-P were evaluated using Student's *t*-test: -m: $p < 0.05$, n: $p < 0.01$.

a progressive loss of G_0/G_1 phase (Fig. 3), and the apoptotic content in SGC-7901 cells exposed to 10, 20 and 100 μg/ml S-GAP-P were 2.1%, 6.8% and 13.9%, respectively (Table 2). These results suggested that S-GAP-P inhibited the growth of gastric carcinoma cells through apoptotic induction. Furthermore, the combination of 10 μg/ml S-GAP-P and 1 μg/ml 5-FU performed a significant higher apoptosis of SGC-7901 cells, compared with the controls or cells treated with either drug on its own. This result further substantiated the beneficial interaction between the two drugs. The beneficial effects might attribute to the sensitivity improvement of SGC-7901 cells to 5-FU (Park et al., 2004; Terashima, Irinoda, Fujiwara, Nakaya, & Takagane, 2002).

4. Conclusion

The chemically sulfated polysaccharide (S-GAP-P) was derived from water-insoluble polysaccharide of *Grifola frondosa* mycelia by chlorosulfonic acid/pyridine method. S-GAP-P has shown evident growth inhibition on human gastric carcinoma SGC-7901 cells through apoptotic induction, and could significantly enhance the anticancer activity of 5-FU. The use of S-GAP-P as an adjuvant drug in combination with chemotherapeutic agents may potentially represent an interesting approach for cancer treatment.

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References

Brown, D. G., Sun, X. M., & Cohen, G. M. (1993). Dexamethasone-induced apoptosis involves cleavage of DNA to large fragments prior to internucleosomal fragmentation. *Journal of Biological Chemistry, 268*, 3037–3039.

Cheng, B. L. (2000). *The culture technology of animal cells*. Guangzhou: South China University of Technology Press, pp. 131–132.

Chopin, V., Slomianny, C., Hondermarck, H., & Le Bourhis, X. (2004). Synergistic induction of apoptosis in breast cancer cells by co-treatment with butyrate and TNF-alpha, TRAIL, or anti-Fas agonist antibody involves enhancement of death receptors' signaling and requires $P21^{waf1}$. *Experimental Cell Research, 298*, 560–573.

Cui, F.J., Li, Y., Xu, Z.H., Xu, H.Y., Sun, K., & Tao, W.Y. (2005). Optimization of the medium composition for production of mycelial biomass and exo-polymer by *Grifola frondosa* GF9801 using response surface methodology. Bioresource Technology, available on line.

Du, Y. G., Gu, G. F., Hua, Y. X., Wei, G. H., Ye, X. S., & Yu, G. L. (2004). Synthesis and antitumor activities of glucan derivatives. *Tetrahedron, 60*, 6345–6351.

Han, F., Yao, W. B., Yang, X. B., Liu, X. N., & Gao, X. D. (2005). Experimental study on anticoagulant and antiplatelet aggregation activity of a chemically sulfated marine polysaccharide YCP. *International Journal of Biological Macromolecules, 36*, 201–207.

Inoue, A., Kodama, N., & Nanba, H. (2002). Effect of maitake (Grifola frondosa) D-fraction on the control of the Tlymph node Th-1/Th-2 proportion. *Biological and Pharmaceutical Bulletin, 25*, 536–540.

Kanzawa, F., Akiyama, Y., Saijo, N., & Nishio, K. (2003). In vitro effects of combinations of *cis*-amminedichloro (2-methylpyridine) platinum (II) (ZD0473) with other novel anticancer drugs on the growth of SBC-3, a human small cell lung cancer cell line. *Lung Cancer, 40*, 325–332.

Lee, B. C., Bae, J. T., & Pyo, H. B. B. P. (2003). Biological activities of the polysaccharides produced from submerged culture of the edible Basidiomycete *Grifola frondosa*. *Enzyme and Microbial Technology, 32*, 574–581.

Lin, Y. L., Zhang, L. N., Chen, L., & Jin, Y. (2004). Molecular mass and antitumor activities of sulfated derivatives of β-glucan from *Poria cocos* mycelia. *International Journal of Biological Macromolecules, 34*, 231–236.

Longley, D. B., Harkin, D. P., & Johnson, G. P. (2003). 5-Fluorouracil: mechanisms of action and clinical strategies. *Natural Review Cancer, 3*, 330–338.

Mayell, M. (2001). Maitake extracts and their therapeutic potential. *Alternative Medical Review, 6*, 48–60.

Mukhtar, H., & Ahmad, N. (1999). Cancer chemoprevention: future holds in multiple agents. *Toxicology and Applied Pharmacology, 158*, 207–210.

Nicoletti, I., Migliorati, G., Pagliacci, M. C., Grignani, F., & Riccardi, C. (1996). A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. *Journal of Immunological Methods, 139*, 271–279.

Notarbartolo, M., Poma, P., Perri, D., Dusonchet, L., Cervello, M., & D'Alessandro, N. (2005). Antitumor effects of curcumin, alone or in combination with cisplatin or doxorubicin, on human hepatic cancer cells. Analysis of their possible relationship to changes in NF-kB activation levels and in IAP gene expression. *Cancer Letter, 224*, 53–65.

Park, J. S., Yoon, S. Y., Kim, J. M., Yeom, Y., Kim, Y. S., & Kim, N. S. (2004). Identification of novel genes associated with the response to 5-FU treatment in gastric cancer cell lines using a cDNA microarray. *Cancer Letter, 214*, 19–33.

Peng, Y. F., Zhang, L. N., Zeng, F. B., & Kennedy, J. F. (2005). Structure and antitumor activities of the water-soluble polysaccharides from *Ganoderma tsugae* mycelium. *Carbohydrate Polymer, 59*, 385–392.

Shi, B. J., Xu, Z. H., Ao, Z. H., Sun, Z. H., & Tao, W. Y. (2003). Preparation and analysis of *Grifola frodosa* polysaccharide sulfate. *Journal of Wuxi University of Light Industry, 22*, 75–78.

Soeda, S., Ohmagari, Y., Shimeno, H., & Nagamatsu, A. (1993). Preparation of oversulfated fucoidan fragments and evaluation of their antithrombotic activities. *Thrombosis Research, 72*, 247–256.

Sogawa, K., Yamada, T., Sumida, T., Hamakawa, H., Kuwabara, H., & Matsuda, M. (2000). Induction of apoptosis and inhibition of DNA topoisomerase-I in K-562 cells by a marine microalgal polysaccharide. *Life Science, 66*, 227–231.

Terashima, M., Irinoda, T., Fujiwara, H., Nakaya, T., & Takagane, A. (2002). Roles of thymidylate synthase and dipyrimidine dehydrogenase in tumor progression and sensitivity to 5-fluorouracil in human gastric cancer. *Anticancer Research, 22*, 761–768.

Urbinati, C., Bugatti, A., & Oreste, P. (2004). Chemically sulfated Escherichia coli K5 polysaccharide derivatives as extracellular HIV-1 Tat protein antagonists. *FEBS Letter, 568*, 171–177.

Wang, Y., Wang, Z. W., Huang, Z. S., Wu, Y. N., & Xu, Z. D. (2002). Synergistic effect of Aloe polysaccharides in the combination with chemotherapeutics for cancer treatment. *Chinese Medical Clinic Pharmacology, 13*, 89–91.

Wang, Y. F., Zhang, L. N., Li, Y. Q., Hou, X. H., & Zeng, F. B. (2004). Correlation of structure to antitumor activities of five derivatives of a β-glucan from *Poria cocos* sclerotium. *Carbohydrate Research, 339*, 2567–2574.

Williams, D. L., Pretus, H. A., McNamee, R. B., Jones, E. L., Ensley, H. E., Browder, W., et al. (1991). Development, physicochemical characterization and preclinical efficacy evaluation of a water soluble glucan sulfate derived from *Saccharomyces cerevisiae*. *Immunopharmacology, 22*, 139–156.

Xing, R., Liu, S., Yu, H. H., Guo, Z. Y., Li, Z., & Li, P. C. (2005). Preparation of high-molecular weight and high-sulfate content chitosans and their potential antioxidant activity in vitro. *Carbohydrate Polymer, 61*, 148–154.

Yang, J. H., Du, Y. M., Huang, R. H., Sun, L. P., & Liu, H. (2005). Chemical modification and antitumour activity of Chinese lacquer polysaccharide from *lac* tree Rhus vernicifera. *Carbohydrate Polymer, 59*, 101–107.

Yoshida, T., Yasuda, Y., Mimura, T., & Kaneko, Y. (1995). Synthesis of curdlan sulfates having inhibitory effects *in vitro* against AIDS viruses HIV-1 and HIV-2. *Carbohydrate Research, 276*, 425–436.

Zhang, C. X., & Huang, K. X. (2005). Apoptosis induction on HL-60 cells of a novel polysaccharide from the mucus of the loach, *Misgurnus anguillicaudatus*. *Journal of Ethnopharmacology, 99*, 385–390.