in vivo *25*: 399-404 (2011)

Possible Reduction of Hepatoma Formation by Smmu 7721 Cells in SCID Mice and Metastasis Formation by B16F10 Melanoma Cells in C57BL/6 Mice by *Agaricus blazei* Murill Extract

MING-FANG WU[1,*], HSU-FENG LU[2,4,*], YU-MING HSU[1], MING-CHU TANG[1], HSUEH-CHIN CHEN[1], CHING-SUNG LEE[4], YI-YUAN YANG[5], MING-YANG YEH[3], HSIUNG-KWANG CHUNG[6,7], YI-PING HUANG[8], CHIH-CHUNG WU[9] and JING-GUNG CHUNG[10,11]

[1]*Animal Medicine Center, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.C.;*
[2]*Department of Clinical Pathology,* [3]*Office of Director, Cheng-Hsin General Hospital, Taipei, Taiwan, R.O.C.;*
[4]*Department of Restaurant, Hotel and Institutional Management, Fu-Jen Catholic University, New Taipei, Taiwan, R.O.C.;*
[5]*School of Medical Laboratory Science and Biotechnology, Taipei Medical University, Taipei, Taiwan, R.O.C.;*
[6]*Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan, R.O.C.;*
[7]*Department of Otorhinolaryngology-Head & Neck Surgery, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[8]*Department of Physiology, School of Medicine, China Medical University, Taichung 404, Taiwan, R.O.C.;*
[9]*Department of Nutrition and Health Sciences, Chang Jung Christian University, Tainan, Taiwan, R.O.C.;*
[10]*Department of Biological Science and Technology, China Medical University, Taichung, Taiwan, R.O.C.;*
[11]*Department of Biotechnology, Asia University, Wufeng, Taichung, Taiwan, R.O.C.*

Abstract. *Agaricus blazei Murill extract (ABM) has been reported to possess antitumor effects. In this study, the role of ABM in tumor growth and metastasis in vivo was evaluated in experimental Smmu 7721 hepatoma cells in severe combined immunodeficiency (SCID) mice and B16F10 melanoma cells lung metastasis in C57BL/6 mice. For the tumor growth model, the size of the liver tumor mass was about 10 mm to 20 mm in the control group. In comparison with the control group, the tumor mass seem to grow slowly with ABM treatment, especially at the high dose. For the tumor metastasis model, after a six-week treatment, the survival rates of B6 mice were 0%, 30%, 10% and 50% for control group, low, median and high concentration ABM treatment groups, respectively. The survival rate showed that pretreatment of C57BL/6 (B6) mice with ABM lengthened their lifespan after tumor cell inoculation, which supports the notion that ABM successfully reduced lung metastasis formation by B16F10 melanoma cells. The treatment effect was dependent on the concentration of ABM for tumor growth and metastasis in these models.*

Successful cancer treatment with chemotherapeutic agents is largely dependent on their ability to trigger cell death in tumor cells; therefore, novel inducers of apoptosis provide a new therapeutic approach for anticancer drug design (1-5). Several previous studies demonstrated that certain phytochemicals present in medicinal herbs exert anticancer activity by inducing apoptosis in cancer cells (1-12).

Since ancient times, mushrooms have been used as an important nutritional food and therapeutic item throughout the world on account of their composition (13). Mushroom extracts are common sources of immunological, hypocholesterlemic, antiviral, antibacterial, anti-carcinogenic, anti-inflammatory and antiparasitic activities (14). There are many reports on mushrooms containing more than one polysaccharide with antitumor activity. An interesting example is *Agaricus blazei*

*Both Authors contributed equally to this work.

Correspondence to: Jing-Gung Chung, Department of Biological Science and Technology, China Medical University, No. 91, Hsueh-Shih Rd., Taichung 404, Taiwan, R.O.C. Tel: +886 422053366 ext. 2161, Fax: +886 422053764, e-mail: jgchung@mail.cmu.edu.tw and Ming-Yang Yeh, Office of Director, Cheng-Hsin General Hospital, No.45 Cheng Hsin St., Taipei 112, Taiwan, R.O.C. Tel: +886 228264400 ext 8009, Fax: +886 228214255, e-mail: ch1009@chgh.org.tw

Key Words: Agaricus blazei Murill, antitumor effects, Smmu 7721 hepatoma cells, B16F10 melanoma cells.

Murill (ABM), which is an edible mushroom, native to Brazil and cultivated in other areas including Taiwan, Japan, Korea, China and Indonesia. ABM possesses antitumor activity as shown by some reports (14-16), but there are only few reports regarding Smmu 7721 hepatoma cells in SCID mice and B16F10 melanoma cell lung metastasis in B6 mice. The aim of the present study was to examine whether ABM extract was effective against tumor-bearing mice and to determine whether the treatment effect was dependent on the concentration of ABM extract.

Materials and Methods

Experimental animals and housing conditions. Both C.B-17 SCID and C57BL/6 male mice, specific pathogen-free and 5 weeks old, were obtained from the National Taiwan University College of Medicine Animal Medicine Center (our own breeding colony). Animals were kept in polypropylene cages (5 animals/cage) covered with metallic grids in a room maintained under constant environmental conditions, with air filter tops in a filtered laminar air flow, with an ambient temperature of 20±2˚C, relative humidity 75±15%, and with a 12-h light-dark cycle. Mice were raised and cared for, given autoclaved water and fed laboratory pellet chow *ad libitum* following the animal procedures approved by the National Science Council of the Republic of China. Experiments were performed according to law, regulations and guidelines for animal experiments in Taiwan, which are in agreement with the Helsinki declaration.

ABM extracts and administration dose levels. ABM powder, obtained from S. Canaan Biotechnology Development Co. (Taipei, Taiwan, R.O.C.), at 22.5, 90 and 900 mg was separately suspended in 6 ml distilled water at 60˚C for 10 min, then cooled to room temperature and left for 5 h with 200 rpm stirring to form solutions of low, medium and high concentration. The ABM solution was filtered before use (17, 18).

Experimental design and treatment for hepatoma formation by Smmu 7721 cells in SCID mice. The mice were subcutaneously (*s.c.*) inoculated with Smmu 7721 cells (3×10^7 cells/mouse) in the dorsal area, while growing to 8 weeks old. After around 2-3 weeks (week 0) of inoculation, the mice with tumors of 1-3 mm in diameter were divided into 4 groups, each of 10 mice. Mice were fed with regular diet and double-distilled water. All four groups of mice were orally administered ABM extract daily as follows: 0 mg, 1.125 mg, 4.5 mg and 45 mg for control, low, medium and high concentration treatment groups, respectively. After 6 weeks' treatment, all the survivors were sacrificed under anesthesia by CO_2, and the liver tumor scored under gross examination. Tumor status was categorized into 4 scales: +, <5 mm; ++, 5- <10 mm; +++, 10 - <15 mm; ++++, >15 mm.

Experimental design and treatment for metastasis formation by B16F10 melanoma cells in C57BL/6 mice. Forty-five 8-week-old, C57BL/6 mice were inoculated with 5×10^4 B16F10 cells suspended in 0.1 ml PBS into the tail vein and were then divided into 5 groups (each group consisted of 10 mice except indicator group). Five mice acted as the indicator group and were killed around the 8th to 10th day to observe whether metastasis was present or not; from experience, it took about 10 days to display lung metastasis. Like normal control and indicator groups, all mice were fed with regular diet and double distilled water. After 10-day inoculation, the indicator group was assessed for black points of needle size on the surface lesions of lungs by visual inspection. Such finding in the indicator group, allowed the experiment to proceed. ABM extract (0.3 ml) was daily administered orally for 6 weeks to another 3 experimental groups (low, median and high concentration treatment group) of mice. Lung tissues were collected and tumor lesions were scored immediately after the animals were died. At the end of the experiments (after 6 weeks' treatment), the survival rate was assessed by counting the surviving mice and all the survivors were sacrificed under anesthesia by CO_2. The number of melanotic nodules on each lung was counted and scored under gross examination. Tumor status was categorized into 4 scales: +, 1-12 tumor masses; ++, 13-24; +++, 25-36; ++++, 36 tumor masses.

Results

ABM extract appears to affect hepatoma formation by Smmu 7721 cells in SCID mice. For the tumor growth model, inoculation of Smmu 7721 tumor cells into SCID mice induced tumor of 1-3 mm in diameter after around 2-3 weeks. Hepatoma was successfully induced in 40 mice in this study. After six weeks' treatment, all 40 mice had survived. Naked eye observation clearly revealed a tumor mass in the liver (Figure 1). All 10 mice of the control group survived and were scored +++ or ++++. This means that the size of the tumor mass elevated sharply and was ~10 mm to 15 mm during the 6 weeks of development, indicating that tumor mass multiplied by 10-fold. Only 3/10 mice were scored + on high treatment with ABM. Seven and two mice were scored +++ due to no or low-dose treatment with ABM (Table I). The oral administration of ABM had no effect on inhibiting tumor growth on account of the increase of tumor size. But compared with the control group, the tumor mass seemed to grow more slowly with ABM treatment. We may conclude that tumor growth may be dependent on ABM dose.

ABM extract appears to affect metastasis formation by B16F10 melanoma cells in C57BL/6 mice. For the tumor metastasis model, all five mice of the indicator group formed metastases at around 10 days. Inoculation of B16F10 melanoma tumor cells into C57BL/6 mice induced metastasis in all 45 mice in this study and much more rapid tumor growth was shown in all the 4 groups, especially indicator and control groups. After six weeks' treatment, the survival rates were 0%, 30%, 10% and 50% for the control group, low, medium and high concentration treatment groups, respectively. All mice of the control group survived less than 4 weeks and were scored ++++. The average survival of the control group was almost 3 weeks (not including 10 days' induction) and far shorter than those of the experimental groups. Five out of ten mice in the high concentration treatment group survived, but three and one mice survived in the low and medium groups, respectively. The survival rate shows that pretreatment of C57BL/6 mice

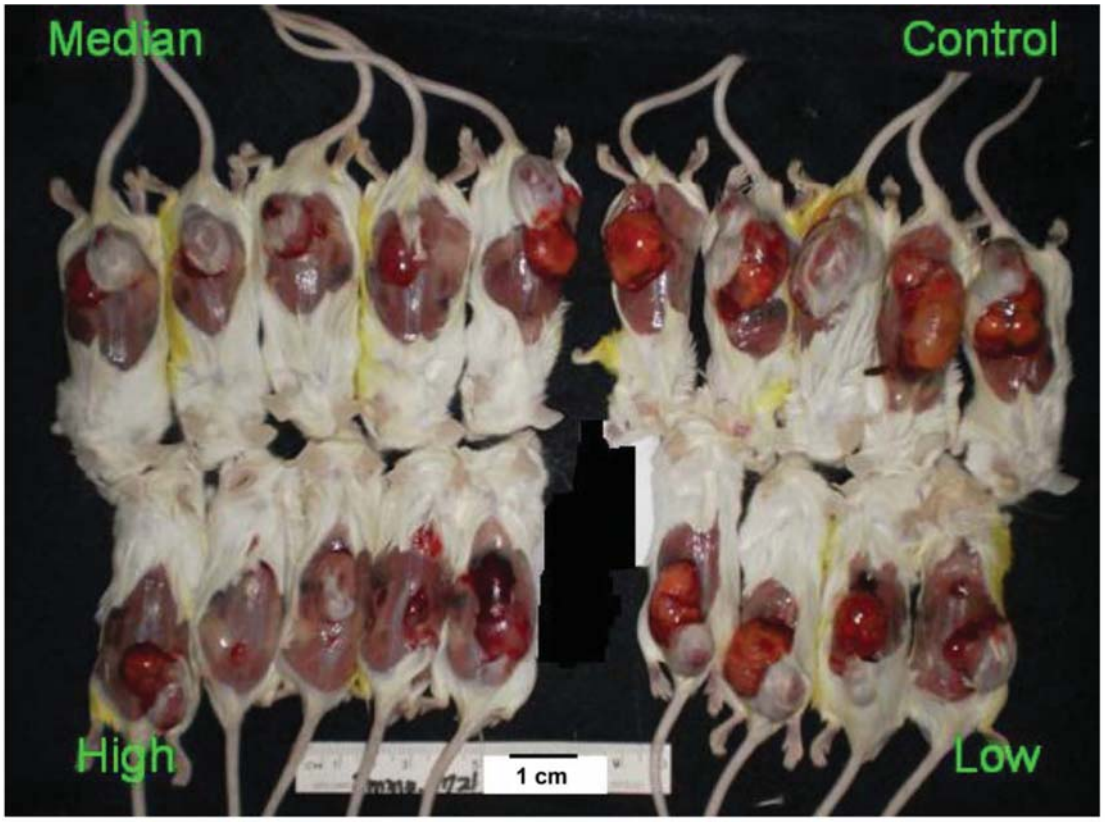

Figure 1. *After inoculation by Smmu 7721 cells to initialize hepatoma, mice of experimental groups were orally administered different doses of ABM extract. After 6 weeks' treatment, all the survivors were sacrificed, and the size of liver tumor was assessed. High-dose treatment by ABM can evidently reduce the tumor growth by comparison with control group.*

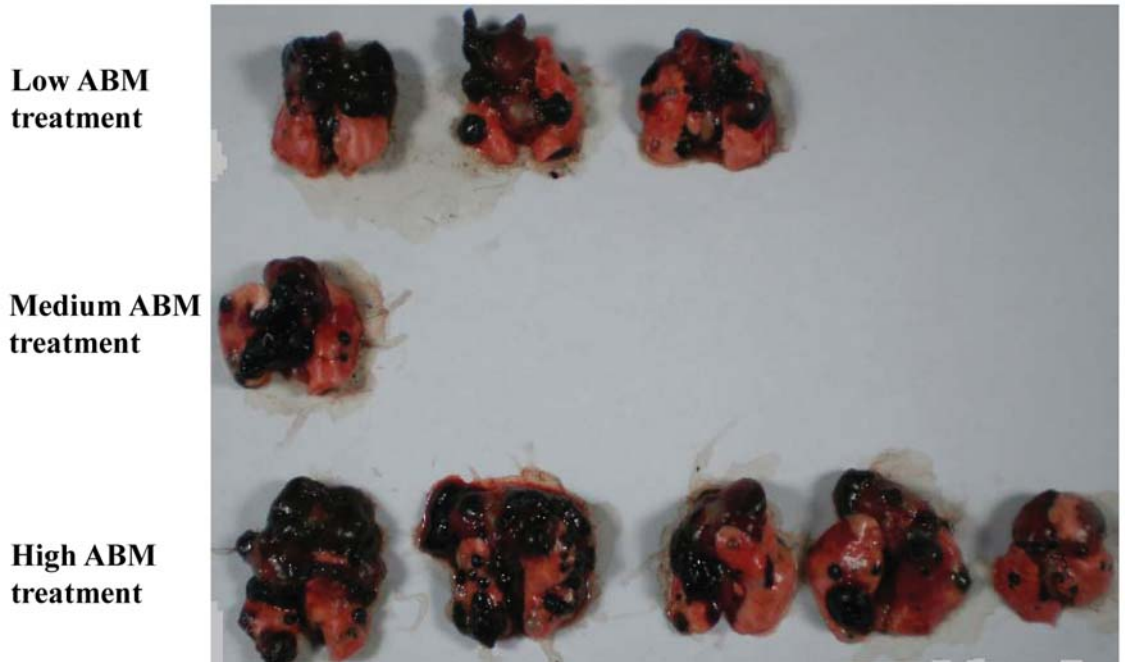

Figure 2. *At the end of the experiments (after 6 weeks' treatment), the surviving nine mice were sacrificed to collect lung fresh samples. The size of tumor mass huge variation. The figure does not include the control group on account of all the mice of the control group were dead in 4 weeks.*

Table I. *Tumor mass from control and ABM-treated mice inoculated with Smmu 7721 hepatoma cells. All mice were inoculated with Smmu 7721 cells. After 2-3 weeks, mice of experimental groups were orally administered different doses of ABM. After 6 weeks' treatment, all the survivors were sacrificed, and the size of liver tumors was scored.*

	Score			
Treatment	+	++	+++	++++
Control			3	7
Low		2	6	2
Medium		5	5	
High	3	5	2	

Tumor status was categorized into 4 scales: +, tumor mass <5 mm; ++, 5- <10 mm; +++, 10- <15 mm; ++++, > 15 mm.

Table II. *The tumor mass from control and ABM treatment of mice inoculated with Smmu 7721 hepatoma cells. ABM extract (0.3 ml) was daily administered orally for 6 weeks to three experimental groups of mice. At the end of the experiments, all the survivors were sacrificed and the number of melanotic nodules on lungs was counted and scored under gross examination.*

	Score			
Treatment	+	++	+++	++++
Control				10
Low			3	7
Median			1	9
High		3	2	5

Tumor status was categorized into 4 scales: +, 1-12 tumor masses; ++, 13-24; +++, 25-36; ++++, 36 tumor masses.

with ABM extract significantly lengthened their lifespan after tumor cell inoculation, which supports the notion that ABM may reduce metastasis formation by B16F10 melanoma cells. At the end of the experiments (after 6 weeks' treatment), the surviving 9 mice were sacrificed to collect fresh lung samples (Figure 2). Treatment effect was dependent on the concentration of ABM. In previous research, to assess the effect of ABM extract on the growth rate, solid tumor volumes were measured every two or three days. Nevertheless it is different to evaluate treatment effect by tumor volume for this study because many metastases in the lung were only needle-point size. Hence, the score was for assessment in place of tumor volume. Table II shows the score of all 40 mice and apparently indicates that there may be significant differences between the control group and the experimental groups. The 9 surviving mice were all scored as ++ or +++. Mice with ++++ score were unable to survive although they accepted treatment by ABM. Treatment with ABM dramatically increased lifespan and appeared to inhibit tumor growth in the experimental groups.

Discussion

The medicinal use of mushrooms has a very long tradition in Asian countries, whereas their use in the Western hemisphere has only been increasing in recent decades. Mushrooms constitute at least 14,000, and perhaps as many as 22,000, known species. The number of mushroom species on the earth is estimated to be 140,000, suggesting that only 10% are known. Assuming that the proportion of useful mushrooms among the undiscovered and unexamined mushrooms will be only 5%, this implies that 7,000 as yet undiscovered species will be of possible benefit to mankind (19). Even among the known species, the proportion of mushrooms that have been extensively investigated is very low.

Mushrooms need antibacterial and antifungal compounds to survive in their natural environment. It is therefore not surprising that antimicrobial compounds with more or less activity can be isolated from many mushrooms and that they could be of benefit for human (20). Experience from Asian and Eastern Europe an countries shows that mushrooms could play an important role in prevention and treatment of cancer. Antitumor effects of several extracts and isolated compounds have been demonstrated in tumor cell systems and in animal assays (21-28). So-called 'immunomodulators' (biological response modifier, immunopotentiators and immunostimulants) are the most important medicinal drugs derived from mushrooms, used especially in Taiwan, Japan, China, Korea and other East Asian countries today. Some polysaccharides or polysaccharide–protein complexes from mushrooms are able to stimulate the non-specific immune system and to exert antitumor activity through the stimulation of the host's defense mechanism (23-26). Among so many kinds of mushrooms, ABM is interesting because it has components including polysaccharides or polysaccharide–protein complexes, which may play a pivotal role as immunomodulators (27-29).

Direct intratumoral injection of ABM can induce apoptosis and cell cycle arrest of tumor cells *in vitro* (27). Animal studies indicated that ABM had antitumor activity in tumor-bearing mice (28). ABM components appear to stimulate the host immune response by enhancement of macrophages, natural killer (NK) cells and cytolytic T lymphocyte activities, which participate in the antitumor response in the sarcoma 180 and Meth A fibrosarcoma-bearing mice systems (28). Substances occurring in the powdered ABM meal may stimulate the hepatic detoxifying enzymatic system or antioxidant free radical-scavenging activities (30). ABM fed in dry powdered form to Wistar rats at 10% of the diet exhibited significant chemopreventive influence on the promoting phase of chemical hepatocarcinogenesis (29). It

was observed that the NK cell activity was maintained at a significant level in gynecological cancer patient groups undergoing chemotherapy when ABM was orally consumed. NK cells display dramatic effects on the reduction of tumor growth, as well as on the inhibition of metastatic tumors (31).

Even though there are nearly 40 articles directly associated with ABM in animal studies, this study is the first to evaluate the reduction of hepatoma formation by Smmu 7721 cells in SCID mice and metastasis formation by B16F10 melanoma cells in C57BL/6 mice by ABM extract.

In conclusion, the present study suggests that the *Agaricus blazei* Murill solution may cause antitumor and antimetastatic effects *in vivo* in a dose-dependent manner. Further studies in order to indentify the active components of ABM and their mechanism of action are warranted.

Acknowledgements

This study was supported in part by the Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH100-TD-B-111-004) and in part by the Cheng-Hsin General Hospital.

References

1 Su CC, Yang JS, Lu CC, Chiang JH, Wu CL, Lin JJ, Lai KC, Hsia TC, Lu HF, Fan MJ and Chung JG: Curcumin inhibits human lung large cell carcinoma cancer tumour growth in a murine xenograft model. Phytother Res *24(2)*: 189-192, 2010.

2 Lu HF, Chie YJ, Yang MS, Lee CS, Fu JJ, Yang JS, Tan TW, Wu SH, Ma YS, Ip SW and Chung JG: Apigenin induces caspase-dependent apoptosis in human lung cancer A549 cells through Bax- and Bcl-2-triggered mitochondrial pathway. Int J Oncol *36(6)*: 1477-1484, 2010.

3 Lin HJ, Su CC, Lu HF, Yang JS, Hsu SC, Ip SW, Wu JJ, Li YC, Ho CC, Wu CC and Chung JG: Curcumin blocks migration and invasion of mouse-rat hybrid retina ganglion cells (N18) through the inhibition of MMP-2, -9, FAK, Rho A and Rock-1 gene expression. Oncol Rep *23(3)*: 665-670, 2010.

4 Lu HF, Chen YS, Yang JS, Chen JC, Lu KW, Chiu TH, Liu KC, Yeh CC, Chen GW, Lin HJ and Chung JG: Gypenosides induced G_0/G_1 arrest *via* inhibition of cyclin E and induction of apoptosis *via* activation of caspases-3 and -9 in human lung cancer A-549 cells. In Vivo *22(2)*: 215-221, 2008.

5 Lu CC, Yang JS, Huang AC, Hsia TC, Chou ST, Kuo CL, Lu HF, Lee TH, Wood WG and Chung JG: Chrysophanol induces necrosis through the production of ROS and alteration of ATP levels in J5 human liver cancer cells. Mol Nutr Food Res *54(7)*: 967-976, 2010.

6 Lu HF, Lai TY, Hsia TC, Tang YJ, Yang JS, Chiang JH, Lu CC, Liu CM, Wang HL and Chung JG: Danthron induces DNA damage and inhibits DNA repair gene expressions in GBM 8401 human brain glioblastoma multiforms cells. Neurochem Res *35(7)*: 1105-1110, 2010.

7 Lu HF, Wang HL, Chuang YY, Tang YJ, Yang JS, Ma YS, Chiang JH, Lu CC, Yang JL, Lai TY, Wu CC and Chung JG: Danthron induced apoptosis through mitochondria- and caspase-3-dependent pathways in human brain glioblastoma multiforms GBM 8401 cells. Neurochem Res *35(3)*: 390-398, 2010.

8 Huang WW, Chiu YJ, Fan MJ, Lu HF, Yeh HF, Li KH, Chen PY, Chung JG and Yang JS: Kaempferol induced apoptosis *via* endoplasmic reticulum stress and mitochondria-dependent pathway in human osteosarcoma U-2 OS cells. Mol Nutr Food Res *54(11)*: 1585-1595, 2010.

9 Lu HF, Lai KC, Hsu SC, Lin HJ, Yang MD, Chen YL, Fan MJ, Yang JS, Cheng PY, Kuo CL and Chung JG: Curcumin induces apoptosis through FAS and FADD, in caspase-3-dependent and -independent pathways in the N18 mouse–rat hybrid retina ganglion cells. Oncol Rep *22(1)*: 97-104, 2009.

10 Lu HF, Lai KC, Hsu SC, Lin HJ, Kuo CL, Liao CL, Yang JS and Chung JG: Involvement of matrix metalloproteinases on the inhibition of cells invasion and migration by emodin in human neuroblastoma SH-SY5Y cells. Neurochem Res *34(9)*: 1575-1583, 2009.

11 Lu HF, Yang JS, Lai KC, Hsu SC, Hsueh SC, Chen YL, Chiang JH, Lu CC, Lo C, Yang MD and Chung JG: Curcumin-induced DNA damage and inhibited DNA repair genes expressions in mouse–rat hybrid retina ganglion cells (N18). Neurochem Res *34(8)*: 1491-1497, 2009.

12 Chiang JH, Yang JS, Ma CY, Yang MD, Huang HY, Hsia TC, Kuo HM, Wu PP, Lee TH and Chung JG: Danthron, an anthraquinone derivative, induces DNA damage and caspase cascade-mediated apoptosis in SNU-1 human gastric cancer cells through mitochondrial permeability transition pores and Bax-triggered pathways. Chem Res Toxicol *24(1)*: 20-29, 2011.

13 Murakawa K, Fukunaga K, Tanouchi M, Hosokawa M, Hossain Z and Takahashi K: Therapy of myeloma *in vivo* using marine phospholipid in combination with *Agaricus blazei* Murill as an immune respond activator. J Oleo Sci *56(4)*: 179-188, 2007.

14 Hetland G, Johnson E, Lyberg T, Bernardshaw S, Tryggestad AM and Grinde B: Effects of the medicinal mushroom *Agaricus blazei* Murill on immunity, infection and cancer. Scand J Immunol *68(4)*: 363-370, 2008.

15 Talcott JA, Clark JA and Lee IP: Measuring perceived effects of drinking an extract of basidiomycetes *Agaricus blazei* Murill: a survey of Japanese consumers with cancer. BMC Complement Altern Med *7*: 32, 2007.

16 Yu CH, Kan SF, Shu CH, Lu TJ, Sun-Hwang L and Wang PS: Inhibitory mechanisms of *Agaricus blazei* Murill on the growth of prostate cancer *in vitro* and *in vivo*. J Nutr Biochem *20(10)*: 753-764, 2009.

17 Talorete TP, Isoda H and Maekawa T: *Agaricus blazei* (class *Basidiomycotina*) aqueous extract enhances the expression of c-Jun protein in MCF7 cells. J Agric Food Chem *50(18)*: 5162-5166, 2002.

18 Jin CY, Moon DO, Choi YH, Lee JD and Kim GY: Bcl-2 and caspase-3 are major regulators in *Agaricus blazei*-induced human leukemia U937 cell apoptosis through dephoshorylation of Akt. Biol Pharm Bull *30(8)*: 1432-1437, 2007.

19 Hawksworth DL: Mushrooms: the extent of the unexplored potential. Int J Med Mushrooms *3(5)*: 333-337, 2001.

20 Lindequist U, Teuscher E and Narbe G: Neue Wirkstoffe aus Basidiomyceten. Z Phytother *11*: 139-149, 1990.

21 Kahlos K, Kangas L and Hiltunen R: Antitumor activity of some compounds and fractions from an *n*-hexane extract of *Inonotus obliquus in vitro*. Acta Pharm Fennica *96*: 33-40, 1987.

22 Burczyk J, Gawron A, Slotwinska M, Smietana B and Terminska K: Antimitotic activity of aqueous extracts of *Inonotus obliquus*. Boll Chim Farm *135(5)*: 306-309, 1996.

23 Chihara G, Maeda Y, Hamuro J, Sasaki T and Fukuoka F: Inhibition of mouse sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.) sing. Nature *222(5194)*: 687-688, 1969.

24 Mizuno T: The extraction and development of antitumor-active polysaccharides from medicinal mushrooms in Japan (review). Int J Med Mushrooms *1*: 9-30, 1999.

25 Wasser SP and Weis AL: Medicinal properties of substances occurring in higher *Basidiomycetes* mushrooms: current perspectives (review). Int J Med Mushrooms *1*: 31-62, 1999.

26 Reshetnikov SV, Wasser SP and Tan KK: Higher basidiomycetes as a source of antitumor and immunostimulating polysaccharides (review). Int J Med Mushrooms *3*: 361-394, 2001.

27 Fujimiya Y, Suzuki Y, Oshiman K, Kobori H, Moriguchi K, Nakashima H, Matumoto Y, Takahara S, Ebina T and Katakura R: Selective tumoricidal effect of soluble proteoglucan extracted from the basidiomycete, *Agaricus blazei* Murill, mediated *via* natural killer cell activation and apoptosis. Cancer Immunol Immunother *46(3)*: 147-159, 1998.

28 Ito H, Shimura K, Itoh H and Kawade M: Antitumor effects of a new polysaccharide-protein complex (ATOM) prepared from *Agaricus blazei* (Iwade strain 101) Himematsutake and its mechanisms in tumor-bearing mice. Anticancer Res *17(1A)*: 277-284, 1997.

29 Pinheiro F, Faria RR, de Camargo JL, Spinardi-Barbisan AL, da Eira AF and Barbisan LF: Chemoprevention of preneoplastic liver foci development by dietary mushroom *Agaricus blazei* Murill in the rat. Food Chem Toxicol *41(11)*: 1543-1550, 2003.

30 Liu F, Ooi VE and Fung MC: Analysis of immunomodulating cytokine mRNAs in the mouse induced by mushroom polysaccharides. Life Sci *64(12)*: 1005-1011, 1999.

31 Soiffer RJ, Murray C, Shapiro C, Collins H, Chartier S, Lazo S and Ritz J: Expansion and manipulation of natural killer cells in patients with metastatic cancer by low-dose continuous infusion and intermittent bolus administration of interleukin 2. Clin Cancer Res *2(3)*: 493-499, 1996.

Received December 13, 2010
Revised February 22, 2011
Accepted February 24, 2011