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 seemed 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, hypocholesterolemic, 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.
Murrill (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 CO₂. 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.
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), all the surviving mice were sacrificed, and the size of liver tumors was scored.

### 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.

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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.

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

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 mouse 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).
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


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