BreastDefend™ prevents breast-to-lung cancer metastases in an orthotopic animal model of triple-negative human breast cancer

JIAHUA JIANG1, ANITA THYAGARAJAN-SAHU1, JAGADISH LOGANATHAN1, ISAAC ELIAZ2, COLIN TERRY1, GEORGE E. SANDUSKY3 and DANIEL SLIVA1,4,5

1Cancer Research Laboratory, Methodist Research Institute, Indiana University Health, Indianapolis, IN 46202;
2Amitabha Medical Clinic and Healing Center, Sebastopol, CA; Departments of 3Pathology and 4Medicine, and
5Indiana University Simon Cancer Center, Indiana University School of Medicine, Indianapolis, IN, USA

Received May 2, 2012; Accepted June 27, 2012

DOI: 10.3892/or.2012.1936

Abstract. We have recently demonstrated that a natural dietary supplement BreastDefend (BD), which contains extracts from medicinal mushrooms (Coriolus versicolor, Ganoderma lucidum, Phellinus linteus), medicinal herbs (Scutellaria barbata, Astragalus membranaceus, Curcuma longa), and purified biologically active nutritional compounds (diindolylmethane and quercetin), inhibits proliferation and metastatic behavior of MDA-MB-231 invasive human breast cancer cells in vitro. In the present study, we evaluated whether BD suppresses growth and breast-to-lung cancer metastasis in an orthotopic model of human breast cancer cells implanted in mice. Oral application of BD (100 mg/kg of body weight for 4 weeks) by intragastric gavage did not affect body weight or activity of liver enzymes and did not show any sign of toxicity in liver, spleen, kidney, lung and heart tissues in mice. Moreover, BD significantly decreased the change in tumor volume over time compared to the control group (p=0.002). BD treatment also markedly decreased the incidence of breast-to-lung cancer metastasis from 67% (control) to 20% (BD) (p<0.05) and the number of metastases from 2.8 (0.0, 48.0) in the control group to 0.0 (0.0, 14.2) in the BD treatment group (p<0.05). Finally, anti-metastatic activity of BD in vivo was further confirmed by the downregulation of expression of PLAU (urokinase plasminogen activator, uPA) and CXCR4 (C-X-C chemokine receptor-4) genes in breast tumors. In conclusion, BD may be considered as a biological therapeutic agent against invasive breast cancers.

Introduction

The majority of newly diagnosed cancers are breast cancers and breast cancer is also a leading cause of cancer death in women globally (1). In spite of the early diagnosis, radiation and chemotherapy, breast cancer is the second leading cause of cancer death in the United States (2). One of the major reasons for such a high morbidity and mortality of breast cancer is the invasive behavior of breast cancer cells leading to cancer metastasis. Certain natural/dietary compounds, presented in vegetables and fruits, can affect various signaling pathways and molecular targets leading to their possible use in the combination therapy (3). The use of dietary supplements is highest among breast cancer survivors (4) suggesting that these supplements can prevent the exacerbation of comorbid conditions associated with breast cancer (5). Proper evaluation of toxicity and biological effects of polybotanical dietary supplements in cancer in general and in breast cancer in particular is of great importance.

BreastDefend™ (BD) is a polybotanical dietary supplement which inhibits growth and invasive behavior of highly metastatic human breast cancer cells in vitro (6). BD contains mycelium from Asian medicinal mushrooms (Coriolus versicolor, Ganoderma lucidum, and Phellinus linteus), which separately suppressed growth and inhibited invasiveness of breast cancer cells by different mechanisms (6-10). In addition, some of the natural agents in BD also demonstrated anticancer activities against breast cancer cells. For example, extracts or purified compounds from Scutellaria barbata, Astragalus membranaceus and Curcuma longa suppressed growth, induced oxidative stress and apoptosis, inhibited breast cancer cell invasiveness and prevented breast cancer metastases in mice (11-14). Quercetin, a flavonoid found in fruits, vegetables, leaves and grains inhibited proliferation of invasive breast cancer cells and its combination with other polyphenols further suppressed tumor growth and site-specific metastasis (15,16). Finally, 3,3’-diindolylmethane (DIM), the biologically active compound derived from the digestion of indole-3-carbinol, found in cruciferous vegetables such as broccoli, Brussels sprouts, cabbage and kale suppressed growth, migration and invasion of metastatic breast cancer cells (17,18).

In the present study, we evaluated toxicity and anti-cancer activities of BD in an animal model of breast-to-lung cancer metastasis with triple-negative highly invasive human breast cancer cells MDA-MB-231 implanted in the mammary pad of nude mice. Here, we show that BD is not toxic and its oral application significantly suppresses time-dependent increase in tumor sizes and inhibits breast-to-lung cancer metastasis.
In addition, BD inhibits expression of pro-metastatic genes PLAU and CXCR4, in breast cancer xenografts. Our results confirm that BD is not toxic and inhibits growth and metastasis of invasive human breast cancer cells in vivo.

Materials and methods

Cell culture and reagents. Highly invasive human breast cancer cells (MDA-MB-231) were obtained from ATCC (Manassas, VA). MDA-MB-231 cells were maintained in DMEM medium supplemented with penicillin (50 U/ml), streptomycin (50 U/ml), and 10% fetal bovine serum (FBS). Media and supplements came from Gibco BRL (Grand Island, NY). FBS was obtained from Hyclone (Logan, UT). BreastDefend (BD) was supplied by EcoNugenics®, Inc. (Santa Rosa, CA). BD contains the following active weight components: herb and active nutritional blend 57.56%, [Quercetin (98% bioflavonoids), Turmeric rhizome extract (Curcuma longa) complex with enhanced bioavailability (BCM-95®), Scutellaria barbata herb extract, and Astragalus membranaceus root extract], mushroom mycelium blend 30.3% (Coriolus versicolor, Ganoderma lucidum, Phellinus linteus), and 3.3-dioxygenmethane blend 12.12%. BD is manufactured consistent with the FDA Good Manufacturing Practices (GMP) regulation for dietary supplements as defined in 21 CFR§111, for batch to batch consistency and quality controls. BD stock solution was prepared by dissolving BD in dimethylsulfoxide (DMSO) at a concentration 25 mg/ml and stored at 40°C.

Toxicology studies. Toxicity of BD was evaluated in the 6-week old female nude mice (Harlan, Indianapolis, IN, USA). The mice were acclimatized for 1 week, and BD was applied by intragastrical gavage 5 times/week for additional 33 days at the following doses: 0, 100, 200 and 400 mg/kg of body weight, n=10 per group. The body weight was evaluated three times per week. At the end of the experiment animals were euthanized by CO2 inhalation. Blood was collected and a gross pathology examination performed. Liver, spleen, kidney, lung and heart were harvested, fixed in 10% neutral buffered formalin at 4°C for 24 h followed tissue processing overnight, and then embedded in paraffin. Five-micrometer sections were stained with hematoxylin and eosin (H&E). In addition, gross necropsy did not show any sign of toxicity this change in body weight overtime was significant (p<0.001). In addition, gross necropsy did not show any sign of toxicity and weights of liver, spleen, kidney, lung and heart were not different between the treatment and control groups (not shown).

Materials and methods

Quantitative RT-PCR. The quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the ABI PRISM 7900HT Fast Real-Time PCR system (Applied Biosystems) according to the manufacturer's instructions. Total RNA was isolated from tumors with RNAeasy (Qiagen, Valencia, CA). The RNA samples were reverse transcribed into cDNA (RT-PCR) using random hexamer primers and TaqMan reverse transcription kit (Applied Biosystems). The cDNA (100 ng per sample) was subjected to qPCR analysis in quadruplicate using forward and reverse primers, TaqMan Universal Master Mix, and probe (10 µl per reaction) in fast optical 96-well plates. The data were analyzed using the ABI PRISM 7900 relative quantification (DDCt) study software (Applied Biosystems). In this study we have used primers for PLAU, CXCR4, EZR, HRAS, S100A4, CDK91A, and HTATIP2 genes with β-actin gene as internal control (Applied Biosystems). The gene expressions levels are normalized to β-actin and are presented as arbitrary fold changes compared between control and treated groups.

Statistical analysis. Toxicology analyses of plasma were summarized using median (min, max) and compared across groups using Kruskal-Wallis tests and Mann-Whitney U tests with significance level adjusted using the Bonferroni correction. The changes in body weight and tumor volume over time were tested using a random effects mixed model. Metastasis incidence was summarized using percentage of animals with metastases and compared between control and BD treatment groups using Fisher's exact test. Metastasis multiplicity and qRT-PCR data were summarized using median (min, max) and compared between control and BD treatment groups using Wilcoxon rank sum test.

Results

Toxicity of BD in vivo. Our recent study demonstrated cytostatic effect of BD on human breast cancer cells MDA-MB-231 (6). Although BD was not toxic for these cells in vitro, systemic toxicity in animals needs to be evaluated. To evaluate the toxicity of BD in vivo, female nude mice were orally gavaged by 0, 100, 200 and 400 mg BD per kg of body weight for 33 days as described in Materials and methods. A seen in Fig. 1A, all groups demonstrated increase in body weight but the increase in 400 mg/kg group was decreased by 5% to control group and this change in body weight overtime was significant (p<0.001).

In addition, gross necropsy did not show any sign of toxicity and weights of liver, spleen, kidney, lung and heart were not different between the treatment and control groups (not shown). H&E staining of control and highest BD dose treatment group (400 mg/kg) of liver, spleen, kidney, lung and heart also did not demonstrate any abnormalities (Fig. 1B). Although the liver enzyme profiles in plasma (ALT, AST and ALP) were not...
changed by BD treatment (0-400 mg/kg), the levels of albumin and total protein were decreased at the 200 and 400 mg BD per kg groups (Table I). Therefore, 100 mg BD/kg dose was decided to be used in our experiments.

**BD suppresses tumor growth and prevents breast-to-lung cancer metastasis in an orthotopic model of human breast cancer.**

Based on our data demonstrating that 100 mg/kg of BD is not toxic in vivo, we have employed an animal orthotopic model of human breast cancer. MDA-MB-231 cells were inoculated into mammary fat pad of female nude mice. When the forming tumors reached the size ~20-30 mm³, the mice were divided into the control group (water) and the treatment group (BD 100 mg/kg of body weight/3 times/week). There were no changes in the tumor volumes for the first 2 weeks of the treatment. After that the tumors in the BD treatment group were smaller, and we detected a significant difference in the change in tumor volume over time between control and BD treatment groups (p=0.002) (Fig. 2A).

Since we used an orthotopic model of breast cancer where tumors form from the human breast cancer cells and metastasize to lungs, we evaluated the incidence of metastasis and metastasis multiplicity number in the control and BD treatment groups. In the control group we detect breast-to-lung cancer metastases in 10 of 15 animals (67%) whereas in the BD group only 3 of 15 animals (20%) developed breast to lung cancer metastasis (Fig. 2B). Therefore, the 100 mg/kg of BD significantly decreased incidence of breast-to-lung cancer metastasis by 70% (p=0.025) demonstrating preventive effect of BD against breast-to-lung cancer metastasis (Table II). Moreover, BD also significantly suppressed the amount of lung metastases from 2.8 (0.0, 48) to 0 (0.0, 14.2) (Table II).

**Effect of BD on the gene expression in tumors.** We have recently demonstrated that BD inhibits invasive behavior and expression of uPA and CXCR4 in MDA-MB-231 cells (6). Because cell

---

**Figure 1.** Toxicity of BD. Female nude mice were gavaged with BD (0, 100, 200 and 400 mg/kg of body weight, n=10 per group) 5 times/week for 4 weeks. (A) The body weight was evaluated three times per week. *p<0.001 change in body weight overtime control vs BD 400 mg/kg. (B) Liver, spleen, kidney, lung and heart were harvested and stained with H&E as described in Materials and methods. The pictures are representative from control and high dose BD treatment (400 mg/kg) groups.

**Figure 2.** Effect of BD on the tumor size. MDA-MB-231 cells were injected into mammary fat pad into female nude mice and treated with water (control) or 100 mg BD/kg of body weight 3 times/week for daily for 33 days (n=15 mice per group) as described in Materials and methods. (A) Tumor volumes: data are the mean ± SD and the changes in tumor volumes were statistically evaluated by a random effects mixed model (p=0.002). (B) Breast tumors were induced as described above. At the end of experiments, mice were sacrificed, lungs harvested and stained with H&E as described in Materials and methods. Representative picture of control and BD treatment are shown. The data analysis is in Table II.
inhibited liver metastasis (30), and suppressed pulmonary metastasis of melanoma cancer. Although we observed a modest but significant inhibition of breast-to-lung cancer metastases in an animal model of metastatic human breast cancer. We hypothesized that the inhibition of breast-to-lung cancer metastasis by BD is associated with the downregulation of expression of uPA and CXCR4 in primary tumors. To evaluate the effect of BD on the expression of PLAU (uPA protein) and CXCR4 genes in breast tumors, we isolated RNA and performed quantitative RT-PCR in control and BD treated mice as described in Materials and methods. In agreement with our in vitro study (6), BD treatment significantly downregulated expression of PLAU (p=0.026) and CXCR4 (p=0.002) in breast tumors (Fig. 3). Moreover, PLAU and CXCR4 expression in primary tumors was significantly increased in animals with breast-to-lung cancer metastasis (Table III). In addition, we have evaluated expression of other genes associated with breast-to-lung cancer metastasis: ezrin (EZR) (19), HRAS (20), S100A4 (21), CDKN1A (protein p21) (22) and HTATIP2 (protein TIP30) (23). However, BD treatment did not change expression of these genes in the primary tumors (Fig. 3).

**Discussion**

Since cancer metastases are the major reason for the mortality of cancer patients, prevention of metastases will significantly extend life of cancer patients. Here we showed that dietary supplement BD markedly prevented breast-to-lung cancer metastases in an animal model of metastatic human breast cancer. Although we observed a modest but significant inhibition in tumor volume over time between control and BD treatment groups, this effect was not so dramatic. Nevertheless, BD treatment prevented breast-to-lung cancer metastases by 70%. The in vivo systemic effect of BD, after an oral application of BD, can protect against breast-to-lung cancer metastasis as we are demonstrating here.

BD is a polybotanical compound and some of its isolated components suppressed cancer metastases in vivo. For example, dietary administration of curcumin decreased the incidence of breast-to-lung cancer metastasis in nude mice (24). Apigenin, biologically active compound from Scutellaria barbata, prevented hepatocyte growth factor induced lung metastasis of breast cancer cells (25). Protein-bound polysaccharide isolated from Coriolus versicolor, PSK (Krestin) inhibited lung metastasis in mice (26), and when combined with chemotherapy, PSK significantly prolonged survival of patients with metastatic gastric cancer (27). Isolated polysaccharides or an extract from Phellinus linteus suppressed pulmonary metastasis of melanoma cells in mice (28,29). In addition, triterpenoid fraction from Ganoderma lucidum inhibited liver metastasis (30), and G. lucidum in diet or i.p. injection of isolated ganoderic acid T suppressed lung metastasis, respectively (31,32). Finally, an oral administration of DIM markedly inhibited lung metastasis of murine cancer cells in mice (33). Therefore, in agreement with our in vitro study with BD (6) combined anti-metastatic effects of isolated components in BD could result in the prevention of breast-to-lung cancer metastasis in vivo.

### Table I. Levels of liver enzymes, albumin and total protein in plasma after BD treatment.

| Treatment | ALT (U/L) | AST (U/L) | ALP (U/L) | Albumin (g/L) | Total protein (g/L) |
|-----------|-----------|-----------|-----------|---------------|--------------------|
| Control   | 104 (32, 310) | 236 (189, 867) | 157 (149, 191) | 1.6 (1.3, 1.7) | 5.3 (4.9, 5.6) |
| BD (100 mg/kg) | 64 (15, 215) | 232 (155, 396) | 165 (145, 464) | 1.7 (1.6, 1.8) | 5.4 (5.2, 5.6) |

Data summarized using median (min, max) and compared across groups using Kruskal-Wallis tests. Comparisons of each group with its respective control performed using Mann-Whitney U tests with significance level adjusted using the Bonferroni correction. *A significant difference with control.

### Table II. BD prevents breast-to-lung cancer metastasis.

| Treatment | Metastasis incidence | Metastasis multiplicity |
|-----------|----------------------|------------------------|
|           | (animals with metastases, %) | (metastases per animal) |
| Control   | 10/15 (67)          | 2.8 (0.0, 48.0)        |
| BD (100 mg/kg) | 3/15 (20)*         | 0.0 (0.0, 14.2)*       |

Metastasis incidence is summarized using percentage of animals with metastases and compared between control and BD treatment groups using Fisher's exact test; *p=0.025. Metastasis multiplicity are summarized using median (min, max) and compared between control and BD treatment groups using Wilcoxon rank sum test; *p=0.033.

### Table III. PLAU and CXCR4 expression based on metastasis status.

| Gene | No metastases | Metastases |
|------|---------------|------------|
|      | n=17          | n=13       |
| PLAU | 0.4 (0.3, 0.9) | 1.1 (0.8, 2.2)* |
| CXCR4| 0.1 (0.0, 0.6) | 1.7 (0.9, 2.3)* |

Expression of PLAU and CXCR4 was determined by qRT-PCR as in Materials and methods. The results are expressed as the relative expression ratios of specific mRNA to β-actin. Data are summarized using median (min, max) and compared between groups using Wilcoxon rank sum test; *p<0.001.
Urokinase plasminogen activator (uPA; PLA2 gene) is one of the major proteins involved in the invasive behavior (adhesion, migration and invasion) of cancer cells and cancer metastasis (34,35). Moreover, mice with knockout PLA2 gene demonstrated slower growth and fewer metastases of human xenografted breast cancer cells in immunodeficient mice (36). Overexpression of the chemokine receptor CXCR4 was originally detected in human breast cancer cells, malignant breast tumors and metastases (37). Inhibiting CXCR4 expression in breast cancer cells, using different strategies (e.g., siRNA silencing, phenotypic CXCR4 knockout, peptide inhibitor of protein kinase-α), also suppressed breast cancer metastasis (38-40). Therefore, targeting PLA2 and CXCR4 expression with natural compounds should result in the suppression of breast to lung cancer metastasis. Indeed, here we show that 70% of the inhibition of breast-to-lung cancer metastases is associated with the downregulation of expression of PLA2 and CXCR4 in primary tumors in mice treated with BD. Moreover, the relative expression of PLA2 and CXCR4 in primary tumors is a predictive marker of breast-to-lung cancer metastasis because we have identified significant increase of these genes in animals with metastases.

As mentioned above, the effect of BD on the growth of primary tumors was significant albeit modest. However, we observed a dominant effect in the inhibition of breast-to-lung cancer metastases. Since BD mainly prevents metastases, combination therapy with other agents directly targeting tumor growth and/or surgical tumor resection could be considered for the alternative treatment of invasive breast cancers.

In our study we induced primary tumors and breast-to-lung cancer metastasis with MDA-MB-231 cells. These cells are characterized as basal-like/triple-negative breast cancer cells; lacking expression of estrogen receptor-α (ER), progesterone receptor (PR) and ErbB2/neu (HER2), which represent a highly aggressive breast cancer subtype, that is resistant to treatment and is associated with poor prognosis (41). Therefore, BD inhibits breast-to-lung cancer metastasis in a model based on breast cancer cells which do not respond to the targeted receptor treatments (e.g., trastumazab and hormonal treatments) (42), suggesting BD as a natural dietary agent for the treatment of triple-negative therapy resistant breast cancer cells.

In conclusion, our data demonstrate that the novel dietary supplement BreastDefend (BD): i) is not toxic in vivo at the...
concentration 100 mg/kg of body weight, and ii) inhibits growth of breast tumors and breast-to-lung cancer metastases in mice. Our data suggest that BD specifically targets expression of PLAU and CXCR4 in triple-negative and highly aggressive breast tumors in vivo. In conclusion, BD may be considered as novel polybotanical preparation for the prevention and alternative therapy of metastatic breast cancer.

Acknowledgements

This study was supported by a research grant from EcoNugenics, Inc., Santa Rosa, CA. The authors would like to thank Barry Wilk for his contribution to this study and Saravanan Kanababasi for the help with qRT-PCR.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin 61: 69-90, 2011.
2. Jemal A, Siegel R, Xu J and Ward E: Cancer statistics. CA Cancer J Clin 60: 277-300, 2010.
3. Aggarwal BB and Shishodia S: Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol 71: 1391-421, 2006.
4. Velicer CM and Ulrich CM: Vitamin and mineral supplement use among US adults after cancer diagnosis: a systematic review. J Clin Oncol 26: 665-673, 2008.
5. Miller MF, Bellizzi KM, Sutfan ML, Ambus AH, Goldstein MS and Ballard-Barbash R: Dietary supplement use in individuals living with cancer and other chronic conditions: a population-based study. J Am Diet Assoc 108: 483-494, 2008.
6. Jiang J, Wojnowski R, Jedinak A and Sliva D: Suppression of proliferation and invasive behavior of human metastatic breast cancer cells by dietary supplement BreastDefend. Integr Cancer Ther 10: 192-200, 2011.
7. Ho CY, Kim CF, Leung KN, Fung KP, Tse TF, Chan H and Lau CB: Differential anti-tumor activity of Coriolus versicolor (Yunzhi) extract through p53- and/or Bcl-2-dependent apoptotic pathway in human breast cancer cells. Cancer Biol Ther 4: 638-644, 2005.
8. Jiang J, Slivova V and Sliva D: Ganoderma lucidum inhibits proliferation of human breast cancer cells by downregulation of estrogen receptor and NF-kappaB signaling. Int J Oncol 29: 695-703, 2006.
9. Thayagaraju J, Jiang J, Hopf A, Adamec J and Sliva D: Inhibition of oxidative stress-induced invasiveness of cancer cells by Ganoderma lucidum is mediated through the suppression of interleukin-8 secretion. Int J Mol Med 18: 657-664, 2006.
10. Sliva D: Suppression of cancer invasiveness by dietary compounds. Mimi Rev Med Chem 8: 677-688, 2008.
11. Bui-Xuan NH, Tang PM, Wong CK and Fung KP: Photo-activated phorbophore-a, an active component of Scutellaria barbata, enhances apoptosis via the suppression of ERK-mediated autophagy in the estrogen receptor-negative human breast adenocarcinoma cells MDA-MB-231. J Ethnopharmacol 131: 95-103, 2010.
12. Ye MN and Chen HF: Effects of Astragalus injection on proliferation of basal-like breast cancer cell line MDA-MB-468. Zhong Xi Yi Jie He Xue Bao 6: 399-404, 2006.
13. Shao ZM, Shen ZZ, Liu CH, Sartippour MR, Go VL, Heber D and Nguyen M: Curcumin exerts multiple suppressive effects on human breast carcinoma cells. Int J Cancer 98: 234-240, 2002.
14. Bachmeier B, Nerlich AG, Iancu CM, Cilli M, Schleicher E, Venet R, DellEva R, et al.: The chemopreventive polyphenol Curcumin prevents hemagogenous breast cancer metastases in immunodeficient mice. Cell Physiol Biochem 19: 137-152, 2007.
15. Schlachterman A, Valle F, Wall KM, Azios NG, Castillo L, Morell L, Washington AV, et al.: Combined resveratrol, quercetin, and catechin treatment reduces breast tumor growth in a nude mouse model. Transl Oncol 1: 19-27, 2008.
16. Castillo-Piched L, Martinis-Montemayor MM, Martinez JE, Wu CW, Ishihara Y, Iijima H and Matsunaga K: Inhibition of mammary tumor growth and metastases to bone and liver by dietary grape polyphenols. Clin Exp Metastasis 26: 505-516, 2009.
17. Hsu EL, Chen N, Westbrook A, Wang F, Zhang R, Taylor RT and Hankinson O: CXCR4 and CXCL12 down-regulation: a novel mechanism for the chemoprotection of 3,3'-diindolylmethane for hormone and ovarian cancers. Cancer Lett 265: 113-123, 2008.
18. Ahmad A, Kong D, Wang Z, Sarkar SH, Banerjee S and Sarkar FH: Down-regulation of uPA and uPAR by 3,3'-diindolylmethane contributes to the inhibition of cell growth and migration of breast cancer cells. J Cell Biochem 108: 916-925, 2009.
19. Elliott BE, Meens JA, Sen Gupta SK, Louvard D and Arpin M: The membrane cytoskeletal crosslinker integrin is required for metastasis of breast carcinoma cells. Breast Cancer Res 7: R365-R373, 2005.
20. Gelmann EP, Thompson EW and Sommers CL: Invasive and metastatic properties of MCF-7 cells and rAS-h-transfected MCF-7 cell lines. Int J Cancer 50: 665-669, 1992.
21. Xue G, Plieh D, Venet R, Xu C and Neillson EG: The gatekeeper effect of epithelial-mesenchymal transition regulates the frequency of breast cancer metastasis. Cancer Res 63: 3386-3394, 2003.
22. Vanzulli SI, Soldati R, Meiss R, Colombo LM, Molinolo AA and Lanari C: Estrogen or anti-progestin treatment induces complete regression of pulmonary and axillary metastases in an experimental model of breast cancer progression. Carcinogenesis 26: 1055-1063, 2005.
23. Zhao J, Ni H, Ma Y, Dong L, Dai J, Zhao F, Yan X, et al.: TIP30/CC3 expression in breast carcinoma: relation to metastasis, clinicopathologic parameters, and P53 expression. Hum Pathol 38: 293-298, 2007.
24. Aggarwal BB, Shishodia S, Takada Y, Banerjee S, Newman RA, Bueso-Ramos CE and Price JE: Curcumin suppresses the paxil-taxed-induced nuclear factor-kappaB pathway in breast cancer cells and inhibits lung metastasis of breast cancer in nude mice. Clin Cancer Res 11: 7490-7498, 2005.
25. Lee WJ, Chen WK, Wang CJ, Lin WL and Tseng TH: Apigenin inhibits HGF-promoted invasive growth and metastasis involving blocking p33/Akt pathway and beta 4 integrin function. Toxicol Appl Pharmacol 226: 178-191, 2008.
26. Ishihara Y, Iijima H and Matsunaka K: Contribution of cytokines on the suppression of lung metastasis. Biotherapy 11: 267-275, 1998.
27. Maehara Y, Tsujitani S, Saeki H, Oki E, Yoshinaga K, Emi Y, Morita M, et al.: Biological mechanism and clinical effect of protein-bound polysaccharide K (KRESTIN®): review of development and future perspectives. Surg Today 42: 8-28, 2012.
28. Han SB, Lee CW, Jeon YJ, Hong ND, Yoo ID, Yang KH and Kim HM: The inhibitory effect of polysaccharides isolated from Phellinus linteus on tumor growth and metastasis. Immunopharmacology 41: 157-164, 1999.
29. Lee HJ, Lee HJ, Lim ES, Ahn KS, Shim BS, Kim HM, Gong SJ, et al.: Cambodian Phellinus linteus inhibits experimental metastasis of melanoma cells in mice via regulation of urokinase type plasminogen activator (uPA) gene and uPA receptor. Biologics 26: 27-31, 2005.
30. Kimura Y, Taniguchi M and Baba K: Antitumor and antimetastatic effects on liver of triterpenoid fractions of Phellinus linteus. Biotechnol Biochem 72: 1399-1408, 2008.
31. Nonaka Y, Ishibashi H, Nakai M, Shibata H, Kiso Y and Abe S: Effects of the antlered form of Ganoderma lucidum on tumor growth and metastasis in cyclophosphamide-treated mice. Biosci Biotechnol Biochem 72: 1399-1408, 2008.
32. Chen NH, Liu JW and Zhong JJ: Ganoderic acid T inhibits tumor invasion in vitro and in vivo through inhibition of MPP expression. Pharmacol Rep 62: 150-162, 2010.
33. Kim EJ, Shin M, Park H, Hong JE, Shin HK, Kim J, Kwon DY, et al.: Oral administration of 3,3'-diindolylmethane inhibits lung metastasis of 4T1 murine mammary carcinoma cells in BALB/c mice. J Nutr 139: 2373-2379, 2009.
34. Sato Y, Jedina K, Kawagishi I, Harvey K and Slivova V: Phellinus linteus suppresses growth, angiogenesis and invasive behaviour of breast cancer cells through the inhibition of AKT signalling. Br J Cancer 98: 1348-1356, 2008.
35. Han B, Nakamuro M, Mori I, Nakamura Y and Kakudo K: Urokinase-type plasminogen activator (uPA) is a growth of an experimental human breast cancer using a combined uPA gene-disrupted and immunodeficient xenograft model. Cancer Res 61: 532-537, 2001.
37. Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, et al: Involvement of chemokine receptors in breast cancer metastasis. Nature 410: 50-56, 2001.

38. Liang Z, Yoon Y, Votaw J, Goodman MM, Williams L and Shim H: Silencing of CXCR4 blocks breast cancer metastasis. Cancer Res 65: 967-671, 2005.

39. Ma WF, Du J, Fu LP, Fang R, Chen HY and Cai SH: Phenotypic knockout of CXCR4 by a novel recombinant protein TAT/54R/KDEL inhibits tumors metastasis. Mol Cancer Res 7: 1613-1621, 2009.

40. Kim J, Thorne SH, Sun L, Huang B and Mochly-Rosen D: Sustained inhibition of PKCα reduces intravasation and lung seeding during mammary tumor metastasis in an in vivo mouse model. Oncogene 30: 323-333, 2011.

41. Gircz O, Calvo V, Pero SC, Krag DN, Sparano JA and Kenny PA: GRB7 is required for triple-negative breast cancer cell invasion and survival. Breast Cancer Res Treat: Oct 18, 2011 (Epub ahead of print).

42. Cleator S, Heller W and Coombes RC: Triple-negative breast cancer: therapeutic options. Lancet Oncol 8: 235-244, 2007.