The objective of the present study was to examine the combined effects of dietary supplements consisting of 

**ABSTRACT**

combined inhibition of invasive behavior of metastatic breast cancer cells by *Ganoderma lucidum* and green tea.  

**INTRODUCTION**

Breast cancer is the leading cause of cancer death among women 20-39 years old and contributed to one third of newly diagnosed cancer cases of breast cancer in the United States in 2006. The other leading risk factors include obesity, menopause, and hormone replacement therapy. Breast cancer is associated with the dietary behavior, suggesting the preventative effect of nutrients against cancer. Although thousands of a variety of phytochemicals representing the importance of different nutritional factors are known for cancer prevention. However, while important, these studies are highly variable because they are usually focused only on one nutritional product. For example, a consumption of green tea was correlated to the prevention of a variety of cancers, and an inverse correlation between mushroom intake and the risk of gastric cancer was described.

Extracts from an edible mushroom *Ganoderma lucidum* has been used in traditional Chinese medicine for the prevention or treatment of a variety of diseases. *G. lucidum* is commonly consumed in the form of tea, powder or extract as a dietary supplement. Furthermore, in vitro studies demonstrated inhibition of biomarkers, cell viability, and lung and colorectal cancer in mice by *G. lucidum*. The objective of the present study was to evaluate the combined effects of dietary supplements consisting of *G. lucidum* and green tea on the growth and invasive behavior of breast cancer cells. The inhibition of cell invasion (migration, adhesion, motility) is mediated through the MMP-9/plasminogen activator (uPA) and green tea leaf extract (GTE, Tegreen97), containing 97% polyphenols (38% EGCG) were obtained from Pharmanex LLC (Provo, UT). These extracts were combined for evaluation of the anti-cancer effect of each extract.

**MATERIALS AND METHODS**

**Materials**

*G. lucidum* extract (GLE, ReishiBio®, containing 13.5% polysaccharides and 0.5% triterpenes, 100 µg/ml), green tea extract (GTE, Tegreen97, containing 38% EGCG, 0.5% flavonoids and 5% polyphenols, 100 µg/ml) were obtained from Phamarincept (Provo, UT). Hyclone (Logan, UT).

**Cell culture**

The human breast cancer cell line MDA-MB-231 was obtained from ATCC (Manassas, VA) and were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS) and antibiotics. The cells were subcultured at 1:10 split ratio. Cells were seeded at a density of 4500 cells/ml and treated at 37 °C. Cell viability was determined by Trypan blue staining and cell number was counted by a hemocytometer. Cell viability was determined by Trypan blue staining and cell number was counted by a hemocytometer. Cell viability was determined by Trypan blue staining and cell number was counted by a hemocytometer. Cell viability was determined by Trypan blue staining and cell number was counted by a hemocytometer. Cell viability was determined by Trypan blue staining and cell number was counted by a hemocytometer. 

**Cell proliferation**

MDA-MB-231 cells were cultured in 24-well plates and treated with 10, 20, and 50 µg/ml of GLE or GTE, or their combination (GLE/GTE) for 24, 48, and 72 hours. Cell proliferation was determined by the BrdU incorporation assay. Cells were incubated with BrdU (Roche Molecular Biochemicals; Indianapolis, IN, USA.), 10 µl/well, for 24 h, followed by cell lysis and detection of incorporation using a fluorimetric assay (Roche Molecular Biochemicals). Data were analyzed as described in Materials and Methods. The results were expressed as the mean ± SD of three independent experiments.

**Cell adhesion, migration, and invasion assays.**

Colony formation (anchorage-independent growth). Cell adhesion, migration, and invasion assays were performed using Transwell chambers. 2 × 10^5 cells were treated with 100 µg/ml GLE or GTE for 24, 48, and 72 hours. Proliferation was measured as described in Materials and Methods. The results were expressed as the mean ± SD of three independent experiments.

**Sarcoma, and lung and colon cancers in mice by *G. lucidum*. The effect on growth was evaluated by the inhibition of cell proliferation (anchorage-dependent growth) and colony formation of MDA-MB-231 cells treated with (A) GTE (0 - 20 µg/ml) for 24, 48, and 72 hours, (B) GLE (0 – 125 µg/ml) and GTE (0 – 60 µg/ml) for 24, 48, and 72 hours, (C) GLE (0 – 30 µg/ml) and GTE (0 – 30 µg/ml) for 24, 48, and 72 hours. Figure 1 C shows the combined effect of GLE and GTE on cell proliferation and invasion. The results were expressed as the mean ± SD of three independent experiments.

**SUMMARY**

• GTE increases anti-cancer effect of GLE of anchorage-dependent (cell proliferation) as well as anchorage-independent (colon formation) of breast cancer cells through the down-regulation of expression of uPA.

• The combination of GTE and GTE synergistically inhibits invasive behavior of breast cancer cells through the suppression of secretion of uPA from breast cancer cells.

• The combination of *G. lucidum* and green tea extracts can be considered for breast cancer chemoprevention and/or treatment.

**Acknowledgement**

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**References**


