

Chemopreventive Properties of Mushrooms Against Breast Cancer and Prostate Cancer

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Previous research from our laboratory has found mushrooms, including white button mushrooms, containing phytochemicals that can suppress aromatase/estrogen biosynthesis. Aromatase is the enzyme that converts androgen to estrogen. An abnormal expression of aromatase in breast tissue is considered to be a risk factor for breast cancer. In our laboratory, we have found that of the seven vegetable extracts tested, mushroom extract was the most effective in inhibiting the activity of human placental aromatase activity. Cell culture experiments were performed to further evaluate the anti-aromatase and anti-breast-cancer activity of mushrooms. Our laboratory has prepared one breast cancer cell line, MCF7aro. This cell line is ER positive/aromatase positive and demonstrates increased cell proliferation in the presence of testosterone. The addition of mushroom extract decreased the advantage gained by the addition of testosterone to a similar level as seen with 4-OHA, a known aromatase inhibitor. Furthermore, mushroom extract was found not to affect the proliferation of MCF-10A, a noncancer cell line. These findings suggest that the inhibitory effect of white button mushroom extract is through a specific anti-aromatase action, not a cytotoxic effect.

To better understand the cancer protective effects of mushrooms, our laboratory decided to characterize the anti-aromatase chemicals and to investigate the *in vivo* action of mushroom extract. Three sets of animal experiments have been conducted, and

the results suggest that the oral intake of mushroom extracts might slow down MCF-7aro-derived tumor growth in nude mice. Histological examination of the tumors revealed that the levels of apoptosis between tumors from the control and mushroom-extract-fed animals were similar, once again indicating that the tumor-suppressing effect of mushroom extract is not through a cytotoxic effect. These results significantly indicate that these phytochemicals in mushroom are orally active and maintain their activity after ingestion. Preliminary studies from this laboratory have found more than one chemical in mushrooms that can inhibit aromatase, and some of them may be fatty acid derivatives. The exact nature of the active chemicals is not yet determined.

A series of *in vitro* and *in vivo* experiments have also been carried out to demonstrate that white button mushrooms can be a chemopreventing agent against prostate cancer. A 20% methanol extract of white button mushrooms has been found to contain phytochemicals that suppress steroid 5α -reductase and aromatase. Steroid 5α -reductase converts testosterone to dihydrotestosterone (DHT) and has been shown to play an important role in the development of prostate cancer and benign prostate hyperplasia. The use of steroid 5α -reductase inhibitors has been found to decrease the incidence of prostate cancer. Cell culture experiments involving cells treated with mushroom extract for 10 days have been carried out. Through these experiments, we have revealed that white button mushroom extract has the ability to

suppress the growth of hormone-resistant prostate cancer cells such as PC-3 and DU145 as well as hormone-dependent LNCaP cells in a dose-dependent manner. The mushroom extract was found not to affect the proliferation of normal prostate epithelial cells.

We have also carried out *in vivo* chemoprevention studies using prostate cancer cell-implanted male athymic nude mouse models. There were two groups of mice gavaged with two different concentrations of mushroom extract and also a pair-fed control group that was gavaged with water. From this *in vivo* study, we demonstrated that our mushroom extract decreased tumor size in a dose-dependent manner. Therefore, our findings on white button mushrooms indicate that the intake of mushrooms could reduce the incidence of breast cancer and prostate cancer. An effective chemopreventive agent should not significantly alter quality of life and is ideally inexpensive, safe, and well tolerated. This prevention method should be readily available and affordable to the general population, including underserved populations.

While it is exciting to find that mushroom extract contains anti-aromatase and anti-5 α -reductase chemicals, it is reasonable to think that mushroom chemicals also affect other cellular pathways. As the first step, we have performed gene expression microarray analysis on MCF-7aro tumors from the mushroom-fed animals and those from the control animals. It was our goal to identify additional gene targets whose expression could also be modulated by

the mushroom diet. We have performed the analysis using Affymetrix Human Genome U133A among three biological replicates—i.e., RNAs were isolated from three individual animals and analyzed separately. The intensities of the probe signals on GeneChips were statistically normalized following a background noise subtraction and were analyzed using algorithms in R/Bioconductor. We compared more than 22,000 gene expressions using two-sample *t* tests and applied the Benjamini–Yekutieli *p*-value correction to control the false discovery rate. Using a minimum fold change criteria of 1.2 and an adjusted *p*-value cutoff of 0.05, we identified 515 genes that were upregulated and 1805 genes that were downregulated in tumors from mushroom-fed mice versus those from control mice. The results indicate that a higher percentage of signal transduction genes are upregulated, and a higher percentage of genes involved in DNA processing and transcription/translation are downregulated in tumors from mushroom-fed mice versus those from control mice. A careful evaluation of the results from microarray analysis will yield novel insights into the mechanisms as well as important signal-transduction pathways regulated by the phytochemicals in white button mushrooms.

Recently, we extended our studies on other medicinal mushrooms. *Ganoderma lucidum* (W.Curt.: Fr.)Lloyd and several strains of *Fomitopsis officinalis* (Vill.) Bond. et Singer have been found to contain chemicals that can suppress aromatase and steroid 5 α -reductase.