Effects of Ganoderma Lucidum sporoderm-broken spore extracts on proliferation and apoptosis in prostate cancer PC-3 cells

Xingya Wang1, Susan M. Fischer2, Steven K. Clinton1, Russell D. Klein1
1Dept. of Human Nutrition and Comprehensive Cancer Center, Cancer Chemoprevention Program, The Ohio State University, Columbus, OH 43210.
2The University of Texas M.D. Anderson Cancer Center, Science Park Research Division, P.O.Box 389, Park Rd. 1c, Smithville, TX 78957

ABSTRACT

Ganoderma Lucidum (LiuZhi or Reishi), the most popular mushroom used in Traditional Chinese Medicine (TCM), has been used in Asian countries over thousands of years for the treatment and prevention of many diseases, including cancer. However, the molecular mechanisms responsible for the anti-cancer effects of G. Lucidum remain to be elucidated. The objective of the current study was to determine the effects of the sporoderm-broken spore water extracts (SBSWE) of G. Lucidum on cell proliferation, apoptosis and the molecular targets in prostate cancer PC-3 cells. Our data demonstrated that the SBSWE (1.25 mg/ml - 10 mg/ml) significantly inhibited the proliferation of PC-3 cells in both a time- and dose-dependent manner. SBSWE treatment at 10 mg/ml for 72 h produced maximal inhibitory effects on the PC-3 cell growth by 85% compared to the control. Western blot revealed that the growth inhibitory effects were associated with activation of caspase-3, caspase-6, and the cleavage of the poly (ADP-ribose) polymerase (PARP) in PC-3 cells. In addition, the phosphorylation of the extracellular signal-regulated kinase (ERK) was significantly inhibited by SBSWE in both a time- and dose-dependent manner. Phosphorylated Akt was also downregulated by SBSWE treatment in these cells. However, the activity of p38 was significantly upregulated by SBSWE treatment in these cells. We also determined that SBSWE were able to inhibit the growth of the normal human prostate epithelial isolate PrEC and the prostate non-malignant RWPE-1 cells in a dose-dependent manner. Taken together, these observations suggest that the SBSWE of G.Lucidum contain very potent phytochemicals which effectively inhibited the growth of prostate PC-3 cells by targeting multiple signaling pathways.

INTRODUCTION

Effect on Prostate Cell Growth

An aqueous extract of sporoderm-broken spores of G. Lucidum will have anti-proliferative and pro-apoptotic effects on human prostate cancer cells.

OBJECTIVE

To determine the effects of sporoderm-broken spore water extracts on cell proliferation in prostate cancer and non-cancer cells.

To examine the mechanisms by which the sporoderm-broken spore water extracts regulate the growth of human prostate cells.

MATERIALS

The sporoderm-broken spore product of G. Lucidum was provided as a kind gift from Chinese Academy of Science. The antibodies were purchased from Cell Signaling (Beverly, MA). The 3-(4,5-dimethylthiazol-2-yl)-5-diphenyltetrazolium bromide (MTT) were obtained from Sigma (St. Louis, MO).

Water extraction. The sporoderm-broken spores of G. Lucidum (100g) were extracted with 2 L of water at 70º C in a water bath with agitation for 12 h. The aqueous fraction obtained from extraction was lyophilized with a lyph-Lock 4.5 Freeze Dry System (Corona, CA) and stored at -20º C. The freeze-dried sporoderm-broken spore water extracts (SBSWE) were then dissolved in appropriate medium at indicated concentrations for subsequent experiments.

Cell Proliferation Assay. Cells were plated in 96-well microtiter plates at an initial density of 2 x 104 cells per well. All experiments were repeated three times. Data are represented as Mean ± SD. A p-value of < 0.05 is considered as significant.

RESULTS

Effect on Prostate Cell Growth

The sporoderm-broken spore product of G. Lucidum inhibited the growth of human prostate cancer PC-3 cells in a time- and dose-dependent manner.

Effect on MAPKs and Akt Signaling

The sporoderm-broken spore product of G. Lucidum inhibited growth promoting and survival pathways involving MAPKs, Akt, caspases, and PARP.

CONCLUSIONS

- Water extracts of the sporoderm-broken spores of G. Lucidum inhibit the growth of human prostate cancer PC-3 cells in a time- and dose-dependent manner.
- Water extracts of the sporoderm-broken spores of G. Lucidum inhibit growth promoting and survival pathways involving MAPKs, Akt, caspases, and PARP.
- To further define the effects of SBSWE on cellular and molecular pathways involved in growth inhibition and activation of apoptotic pathways in prostate cancer cells.
- To determine the effects of SBSWE on prostate tumorigenesis and carcinogenesis in vivo.
- To isolate and quantitate the bioactive components in SBSWE of G. Lucidum.

FUTURE DIRECTIONS

- To further define the effects of SBSWE on cellular and molecular pathways involved in growth inhibition and activation of apoptotic pathways in prostate cancer cells.
- To determine the effects of SBSWE on prostate tumorigenesis and carcinogenesis in vivo.
- To isolate and quantitate the bioactive components in SBSWE of G. Lucidum.

ACKNOWLEDGEMENTS

We thank Dr. Steven Schwartz for providing extraction facilities, Rachel Kopeck for technique support, all the members from Dr. Klein and Dr. Clinton’s laboratory for suggestions and help. This study was supported by a Vision grant from the department of human nutrition at OSU.

In memoriam (1962-2006): Russell D. Klein, Ph.D passed away on December 1, 2006 after a year long battle with leukemia at the James Cancer Hospital. He will be greatly missed by his colleagues and students as a superb scientist, mentor, and gentleman.