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Biologic Activity of Spores and Dried Powder from *Ganoderma lucidum* for the Inhibition of Highly Invasive Human Breast and Prostate Cancer Cells

DANIEL SLIVA, Ph.D.,[1,2] MIROSLAV SEDLAK, Ph.D.,[3] VERONIKA SLIVOVA, B.S.,[1] TATIANA VALACHOVICOVA, B.S.,[1] FRANK P. LLOYD, Jr., M.D.,[1,2] and NANCY W.Y. HO, Ph.D.[3]

ABSTRACT

Objective: *Ganoderma lucidum* has been used in East Asia as a home remedy to prevent or cure cancer. Furthermore, *Ganoderma lucidum* is one of the herbs in the herbal mixture PC-SPES that has become an alternative herbal therapy for prostate cancer. Because the dried powder of ganoderma is commercially available as a dietary supplement itself, the purpose of this study was to evaluate the biologic activity of samples of *Ganoderma lucidum* from different sources.

Methods: Samples of *Ganoderma lucidum* were characterized morphologically and evaluated for their ability to inhibit cell migration of highly invasive breast cancer MDA-MB-231 cells and prostate cancer PC-3 cells. Because the inhibition of cell motility is directly linked to the inhibition of the signaling pathway for constitutively active NF-κB in breast and prostate cancer cells, we determined how different samples of *Ganoderma lucidum* inhibit constitutively active NF-κB in a reporter gene assay.

Results: Some of the samples of *Ganoderma lucidum* demonstrated strong inhibition of cancer cell migration comparable to the inhibition of constitutively active NF-κB, whereas other samples showed less or no activity in highly invasive estrogen receptor-negative breast cancer cells or androgen receptor-negative prostate cancer cells, respectively. Interestingly, we did not find any correlation between the purity and composition (spores versus powder) of *Ganoderma lucidum* and biologic activity.

Conclusions: *Ganoderma lucidum* has demonstrated strong activity against breast and prostate cancer cells. Nevertheless, the composition of samples did not correlate with their ability to inhibit cell migration and activation of NF-κB *in vitro*.

INTRODUCTION

Herbal therapies have been used for centuries in traditional Asian medicine, and their use in the form of dietary supplements for the treatment of cancer has been increasing recently in the United States (Eisenberg et al., 1998). One of the popular herbal mixtures used by patients with prostate cancer is PC-SPES, which was successfully tested in clinical trials (de la Taille, 1999, 2000a, 2000b; DiPaola et al., 1998; Pfeifer et al., 2000; Small et al., 2000). Be-

[1]Cancer Research Laboratory, Methodist Research Institute, Clarian Health Partners Inc., Indianapolis, IN.
[2]Department of Medicine, School of Medicine, Indiana University, Indianapolis, IN.
[3]Laboratory of Renewable Resources Engineering, Purdue University, West Lafayette, IN.

cause the synthetic drugs diethylstilbestrol, indomethacin, and warfarin were identified in the herbal mixture PC-SPES, the Food and Drug Administration (FDA) published a medical alert, the clinical trial with PC-SPES was stopped, and all grants using PC-SPES funded by the National Center for Complementary and Alternative Medicine of the National Institute of Health were placed on hold (Sovak et al., 2002; White, 2002).

One of the eight herbs originally used in the herbal mixture PC-SPES is basidiomycetous fungi *Ganoderma lucidum*, Karst. A dried powder from ganoderma has been used for centuries to cure various diseases such as hepatitis, hepatopathy, hypertension, nephritis, bronchitis, and cancer. We have recently demonstrated that the potential of *Ganoderma lucidum* to suppress the motility of highly invasive breast and prostate cancer cells is caused by the inhibition of transcription factors NF-κB and AP-1, with subsequent inhibition of urokinase-type plasminogen activator (uPA) and its receptor uPAR (Sliva et al., 2002a). Previous studies suggested that NF-κB, AP-1, and uPAR are suitable targets for cancer therapy, and the inhibition of NF-κB by proteosome inhibitor PS-341 was recently evaluated in preclinical and clinical studies (Adams, 2002; Ludes-Meyers et al., 2001; Mazar, 2001; Wang et al., 1999). Therefore, *Ganoderma lucidum* can be used in therapy to inhibit constitutively active NF-κB as an alternative herbal intervention for cancer treatment. In the present study, we compared the activity of different dietary supplements containing *Ganoderma lucidum* for their effectiveness in inhibiting cell migration and suppression constitutively active NF-κB in highly invasive breast and prostate cancer cells.

MATERIALS AND METHODS

Ganoderma lucidum

Samples of *Ganoderma lucidum* were purchased from different sources: Zihang, Zihang, Beijing, China (sample A); Zhongke, Nanjing, Zhongke, China (sample B); Jilan, DaSahn, Lyong-Linh-Chi City, Jilan Province, China (sample C); Two Urn, Shen Yang City, Liaoning Province, China (sample D); Double Crane, Yung-Chien Company, Tainan, Taiwan (sample E); and ReishiMax, Pharmanex, Provo, UT (sample F). Nancy W.Y. Ho, Ph.D., Purdue University, provided the authentication and morphological characterization (with a Nikon 200 TE inverted microscope) of samples. The samples were dissolved in boiled water at a concentration of 50 mg/mL, stored at 4°C, and reheated to 70°C for 10 minutes before every experiment.

Cell culture

The human breast cancer cell line MDA-MB-231 and human prostate cancer cell line PC-3 were obtained from ATCC (Manassas, VA). MDA-MB-231 cells were maintained in Dulbecco's modified Eagle medium (DMEM) and PC-3 cells were maintained in F-12 medium containing 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).

Cell migration assay

MDA-MB-231 and PC-3 cells were harvested and incubated for 4 hours and 24 hours, respectively, with ganoderma samples, as indicated in the text. Chemokinesis was assessed in Transwell chambers, as previously described (Sliva et al., 2002a). Data points represent the average ± standard deviation (SD) of individual filters within one representative experiment repeated at least twice.

DNA transfection and chloramphenicol acetyltransferase assay

Both MDA-MB-231 and PC-3 cells were transfected with both NK-κB-CAT and β-galactosidase expression vector pCH110. Twenty-four hours after transfection, cells were treated with ganoderma samples for an additional 24 hours at 37°C, and chloramphenicol acetyltransferase (CAT) assays were performed as described (Sliva et al., 2002a). Data points represent the average ± SD of 3 to 6 independent transfection experiments.

Statistical analysis

Data are presented as means ± SD. Statistical comparisons between groups of data were carried out using the Student's *t* test.

RESULTS

We have recently demonstrated that constitutively active NF-κB is responsible for the increased motility of highly invasive breast cancer cells and that *Ganoderma lucidum* suppresses the motility of breast and prostate cancers by inhibiting NF-κB (Sliva et al., 2002a, 2002b). Because *Ganoderma lucidum* is available as a dietary supplement in various forms (mushroom powder, purified whole spores, broken spores, or mixtures), we compared their biologic activity on cell migration and inhibition of NF-κB in highly invasive breast and prostate cancer cells. First, the composition of samples purchased from commercial sources from China (samples A, B, C, D), Taiwan (sample E), and the United States (sample F) was morphologically characterized by microscopy. As seen in Table 1, Ganoderma samples were in the form of whole spores (samples A, C), broken spores (sample B), or ground body particles/mushroom powder (samples D, E). Only sample F (ReischiMax), which claimed to contain 6% triterpenes and 13.5% polysaccharides, was characterized chemically. To assess the power of *Ganoderma lucidum* to inhibit cell motility of cancer cells, we preincubated increased concentrations of samples (0, 0.5, 1.2, 2.5 mg/mL) with breast MDA-MB-231 and prostate PC-3 cancer cells, and determined cell migration under standard cell migration assay conditions (Sliva et al., 2002a). As seen in Figure 1A (only the 2.5 mg/mL concentration is shown), dietary supplements containing *Ganoderma lucidum* demonstrated various activities in the inhibition of migration of breast cancer cells MDA-MB-231. The samples containing mushroom powder inhibited cell migration by 86.5% (sample E) and 54.3% (sample D). The broken spores inhibited cell migration by 71.9% (sample B), whereas the whole spores inhibited migration by 89.3% (sample A) or showed virtually no effect (9.5%, sample C). Interestingly, the sample containing powdered extract with spores (sample F) was the most potent in inhibiting migration (99%). We observed the same pattern on the inhibition of migration of PC-3 cells (Fig. 1B), where ganoderma powder inhibited cell motility by 93.6% (sample E) and 39.3% (sample D). The broken spores suppressed motility of PC-3 cells by 95.9% (sample B). The whole spores suppressed activity by 83.8% (sample A) or did not significantly inhibit cell migration (16.5%, sample C). The powdered extracts with spores (sample F) inhibited cell motility by 89.0%. Therefore, the samples showed comparable activity in inhibiting migration of breast and prostate cancer cells.

As mentioned above, the suppression of cell motility is directly linked to the inhibition of constitutively active transcription factor NF-κB. To identify whether the samples of *Ganoderma lucidum* also inhibit constitutively active NF-κB in breast cancer cells, we transfected

Table 1. Source and Morphologic Characterization of *Ganoderma lucidum*

Sample	*Brand name*	*Origin*	*Morphology/composition*
A	Zihang[a]	China	Whole spores
B	Zhongke[b]	China	Broken spores
C	Jilin[c]	China	Whole spores
D	Two Urn[d]	China	Mushroom powder (fruiting body)
E	Double Crane[e]	Taiwan	Mushroom powder (fruiting body)
F	ReishiMax[f]	USA	Mushroom powdered extract with spores (20:1)

Microscopical analysis of *ganoderma lucidum* was performed with Nikon 200 TE inverted microscope (40× magnification).

[a]Zihang, Beijing, China.
[b]Zhongke, Nanjing, China.
[c]Da Sahn, Luong-Lin-Chi City, Jilan Province, China.
[d]Two Urn, Sheng Yang City, Liaoning Province, China.
[e]Yung-Chien Company, Tainan, Taiwan.
[f]Pharmanex, Provo, UT.

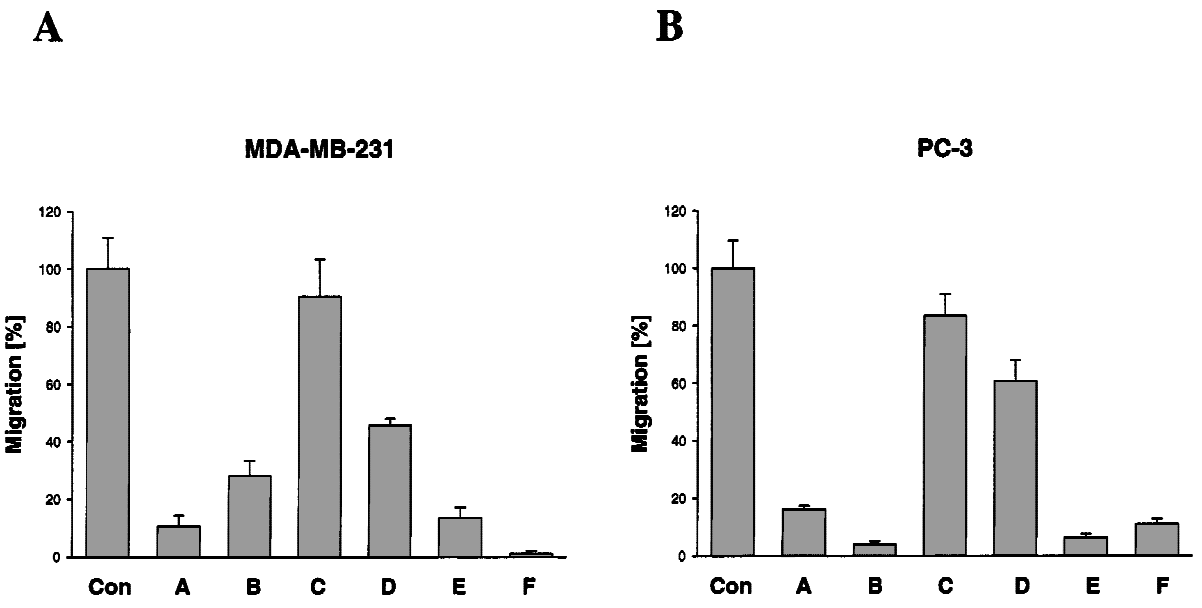

FIG. 1. Inhibition of migration of MDA-MB-231 and PC-3 cells by *Ganoderma lucidum*. **A**: MDA-MB-231 cells (1×10^6) were pretreated for 1 hour with Ganoderma samples (2.5 mg/mL), and cell motility was determined after 3 hours of incubation as described in the Materials and Methods section. Data are the mean ± standard deviation (SD) of triplicate determinations. Similar results were obtained in at least two additional experiments. **B**: PC-3 cells (1×10^6) were pretreated for 1 hour and, after an additional 23 hours of incubation, cell motility was determined as described in (**A**). Data are the mean ± SD of triplicate determinations. Similar results were obtained in at least two additional experiments. Con, control.

MDA-MB-231 cells with reporter gene construct NF-κB-CAT and treated the cells with ganoderma samples (2.5 mg/mL) for an additional 24 hours. The cell extracts were prepared and the inhibitory effect on NF-κB was determined in CAT assay as described in the Materials and Methods section. As seen in Table 2, the whole spores (sample A), broken spores (sample B), mushroom powder (sample E), and mushroom powdered extract with spores (sample F) significantly inhibited NF-κB in MDA-MB-231 cells. In accordance with the migration assay, the whole spores (sample C) or mushroom powder (sample D) did not inhibit (sample C) or slightly inhibited (sample D) NF-κB activity. We found the same pattern of inhibition of NF-κB in PC-3 cells, where broken spores (sample B) and mushroom powdered extract with spores (sample F) demonstrated stronger inhibitory activity than in MDA-MB-231 cells (Table 2).

Altogether, our data demonstrate the correlation between potency to affect cell migration and the inhibitory effect on constitutively active NF-κB in breast MDA-MB-231 and prostate PC-3 cancer cells.

DISCUSSION

In this study we compared the biologic activity of an herbal dietary supplement, *Ganoderma lucidum*, against cell migration and con-

TABLE 2. INHIBITION OF NF-κB IN MDA-MB-231 AND PC-3 CELLS

	NF-κB activity (%)	
	MDA-MB-231	*PC-3*
Control	100 ± 5.7	100 ± 7.6
A	29 ± 4.6*	35 ± 14.5**
B	29 ± 0.8**	2 ± 0.2*
C	97 ± 3.9	83 ± 0.5*
D	68 ± 13.7	75 ± 0.9**
E	28 ± 9.2**	34 ± 3.0*
F	13 ± 1.9*	3 ± 0.8*

MDA-MB-231 or PC-3 cells were transfected with 1 μg of NF-κB-CAT reporter construct and 3 μg β-galactosidase expression vector pCH110. Twenty-four hours after transfection, the cells were treated with *Ganoderma lucidum* (2.5 mg/mL) for an additional 24 hours. Chloramphenicol acetyltransferase (CAT) activity was measured as described under Materials and Methods. Data are the mean ± standard deviation of 3 to 6 independent transfection experiments and were analyzed by Student's *t* test.

*$p < 0.05$.

**$p < 0.005$.

stitutively active transcription factor NF-κB in highly invasive breast and prostate cancer cells. Our purpose was to evaluate the activity of samples with different compositions and from different sources. We tested whole spores, broken spores, and mushroom powder from China; mushroom powder from Taiwan; and mushroom powdered extracts with spores from the United States. The chemical composition of the samples was unknown; only the sample from the United States declared 6% triterpenes and 13.5% polysaccharides among its contents. We found a correlation between the inhibitory activity of NF-κB and potency to inhibit cell migration in four samples. One sample did not inhibit cell migration or NF-κB, and one sample demonstrated a minimal effect on the cell motility and NF-κB activation. We did not find any correlation between the purity and composition of samples and their biologic activity. For example, some of the samples containing spores, broken spores, or mushroom powder (fruiting body) demonstrated high activity, whereas spores or mushroom powder from other sources were inactive in inhibiting cell migration and NF-κB. However, when we broke inactive spores (sample C) with glass beads we found the inhibition of cell migration comparable with active spores (sample A) and broken spores (sample B) (data not shown). Interestingly, we found the most potent effects against breast and prostate cancer cells in the sample containing mushroom powdered extract with spores. The difference in the potency to inhibit cancer cells of the different samples of *Ganoderma lucidum* can be the result of variation in the concentration of the active ingredients because it is not known in which stage of the sporulation the spores were collected, how the spores were collected, and how old the mushrooms used for the preparation of fruiting body were. Thus, the amount of active ingredients in different samples of *Ganoderma lucidum* can reflect nonstandardized procedures in collecting and preparing these dietary supplements.

Previous studies using purified or semipurified fractions from *Ganoderma lucidum* demonstrated biologic activity through various mechanisms. Organic fractions containing triterpenes demonstrated cytotoxicity against mouse sarcoma and mouse lung carcinoma cells in vitro (Min et al., 2000). Polysaccharides isolated from Ganoderma suppressed the growth of sarcoma-180 solid tumors in mice (Miyazaki and Nishijima, 1981; Sone et al., 1985) and stimulated production of cytokines responsible for inhibiting proliferation of HL-60 and U937 leukemic cells (Wang et al., 1997). Bioactive proteoglycans containing a carbohydrate protein ratio of 11.5:1 also activated B mouse spleen lymphocytes (Zhang et al., 2002). Lipids extracted from the germinating spores of *Ganoderma lucidum* inhibited the growth of hepatoma and sarcoma tumors in mice (Liu et al., 2002). We have also observed the difference in potency against breast and prostate cancer cells. This difference is probably caused by the different characteristics of these cells. Although both breast cancer MDA-MB-231 and prostate cancer PC-3 cells are highly invasive and contain high levels of constitutively active transcription factor NF-κB, they also express different genes. For example, MDA-MB-231 cells express estrogen receptor β (ERβ), whereas PC-3 cells express ERα and ERβ (Tong et al., 2002; Lau et al., 2000). Because more than 100 terpenoids have been isolated from *Ganoderma lucidum* (Min et al., 2000) and some terpenoids have been shown to modulate estrogen signaling as agonists/antagonists for ERα and ERβ (Ikeda et al., 2002), it is possible that *Ganoderma lucidum* possesses phytoestrogenic activity and has the characteristics of a selective estrogen receptor modulator (SERM) (Kuiper et al., 1998). Therefore, different expressions of estrogen receptors in breast and prostate cancer cells can reflect the different levels of inhibition of MDA-MB-231 and PC-3 cells by *Ganoderma lucidum*. In addition, a recent study demonstrated that extracts from *Ganoderma lucidum* inhibited cell proliferation and downregulated the expression of estrogen receptor-α in human prostate cancer cells with the same potency as the herbal mixture PC-SPES (Hsieh and Wu, 2002).

In agreement with the practice of herbal medicine, we used commercially available dietary supplements of *Ganoderma lucidum* in the form of whole products to demonstrate the biologic activity on the cellular and molecular levels. Although the identification of biologically active components of *Ganoderma lucidum*

is important for the mechanistic characterization of their specific activity, some of these components demonstrated cytotoxicity (Min et al., 2000). In addition, there is some evidence that certain components in the herbal products can reduce the cytotoxicity of the whole product, and the interaction between different biologically active components is responsible for their in vivo effects (Wilasrumee et al., 2002). Although we did not characterize the chemical composition of tested samples, we expect that they contain different ratios of biologically active components responsible for their inhibitory activity against breast and prostate cancer cells. As mentioned above, originally inactive spores (sample C) increased their activity after mechanistic disruption, suggesting that they contain biologically active components, which can be released. However, the purpose of the present study was to determine the biologic activity in different forms of commercially available dietary supplements containing *Ganoderma lucidum*. Another possibility is that some of the samples contain other contaminants or vehicles that can alter the observed activity in different samples. Further studies to determine the exact chemical composition(s) and their ratios are currently in progress.

Our knowledge that *Ganoderma lucidum* can inhibit the signaling pathway responsible for the increased migratory potential of cancer cells (Sliva et al., 2002b) can be easily translated into clinical studies. Because cell migration, as well as cell adhesion and invasion, is one of the crucial processes responsible for cancer metastasis (Price et al., 1997), inhibiting the motility of cancer cells can prevent the metastatic spread of cancer into surrounding and distant tissues of human body. Therefore, the dietary supplement *Ganoderma lucidum* can be considered as an adjuvant therapy for the treatment of breast and prostate cancers.

The recent uncovering of the adulteration of the herbal mixture PC-SPES with synthetic drugs resulted in the demise of PC-SPES in alternative medicine (White, 2002). Therefore, the use of one biologically active herb available from different sources could probably decrease the possibility of potential product adulteration.

In summary, in our study we compared the biologic activity of *Ganoderma lucidum* in the form of spores and mushroom powder. Our data demonstrate that the purity and composition of tested samples do not directly correlate with potency to inhibit highly invasive breast and prostate cancer cells. Because *Ganoderma lucidum* in the form of a dietary supplement is available through various companies and distributors, it is advisable to test every new source for its biologic activity.

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Address reprint requests to:
Daniel Sliva Ph.D.
Cancer Research Laboratory
Methodist Research Institute
1800 North Capitol Avenue
E504
Indianapolis, IN 46202

E-mail: dsliva@clarian.org