Ganoderma lucidum inhibits proliferation and induces apoptosis in human prostate cancer cells PC-3

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Abstract. *Ganoderma lucidum* (Reishi), an oriental medical mushroom, has been widely used in Asian countries for centuries to prevent or treat different diseases, including cancer. However, the mechanism(s) responsible for the effects of *Ganoderma lucidum* on cancer cells remain to be elucidated. We have previously demonstrated that *Ganoderma lucidum* down-regulated the expression of NF-κB-regulated urokinase plasminogen activator (uPA) and uPA receptor (uPAR), which resulted in suppression of cell migration of highly invasive human breast and prostate cancer cells. In this study, we investigated the effects of *Ganoderma lucidum* on cell proliferation, cell cycle, and apoptosis in human prostate cancer cells PC-3. Our data demonstrate that *Ganoderma lucidum* inhibits cell proliferation in a dose- and time-dependent manner by the down-regulation of expression of cyclin B and Cdc2 and by the up-regulation of p21 expression. The inhibition of cell growth was also demonstrated by cell cycle arrest at G2/M phase. Furthermore, *Ganoderma lucidum* induced apoptosis of PC-3 cells with a slight decrease in the expression of NF-κB-regulated Bcl-2 and Bcl-xl. However, the expression of pro-apoptotic Bax protein was markedly up-regulated, resulting in the enhancement of the ratio of Bax/Bcl-2 and Bax/Bcl-xl. Thus, *Ganoderma lucidum* exerts its effect on cancer cells by multiple mechanisms and may have potential therapeutic use for the prevention and treatment of cancer.

Introduction

Prostate cancer is the most common malignancy in men in the United States, accounting for about 33% of all cancers diagnosed in males (1). Prostate cancer cells initially respond to androgen ablation therapy, but long-term antiandrogen

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treatment of prostate cancer patients results in loss of responsiveness. Prostate cancers finally progress from androgen-dependent to androgen-independent with highly metastatic behavior. The transcription factor, nuclear factor-κB (NF-κB), is overexpressed in highly invasive prostate cancers (2-4), and its activity has been linked to cancer chemo-resistance (5). NF-κB is also associated with tumor cell proliferation, invasion, and angiogenesis (6,7), and further activation of NF-κB has been demonstrated by various carcinogens and tumor promoters, such as benzopyrene, UV radiation, and phorbol esters (8-10). Therefore, activation of NF-κB promotes cell proliferation and survival, while suppression of NF-κB decreases cell proliferation and sensitizes the cells to apoptosis induced by cytokines and chemotherapeutic agents (11-13). Furthermore, constitutively active NF-κB contributes to the resistance of tumors to conventional therapy by mechanisms employing the up-regulation of antiapoptotic genes such as Bcl-2, Bcl-xl, and the cell cycle regulator cyclin D1, all of which are overexpressed in highly invasive prostate cancer cells (14-17). We have recently demonstrated that constitutively active NF-κB controls cell adhesion and migration in highly invasive prostate cancer cells (18), suggesting that the inhibition of NF-κB may be especially important for the treatment of highly invasive and androgen-independent prostate cancer.

Ganoderma lucidum (Reishi), a basidiomycetous fungus, is widely used in China and other Asian countries to treat various human diseases, such as hepatitis, hepatopathy, hypertension, nephritis, and cancers (19-21). In addition, many bioactive components isolated from *Ganoderma lucidum* have been demonstrated to possess antioxidative, antihypertensive, and anticancer effects (22-25). For example, polysaccharides from *Ganoderma lucidum* exert anticancer effects against HL-60 and U937 leukemic cell lines (26). Some of the triterpenes isolated from *Ganoderma lucidum* also exhibit cytotoxic activity against mouse sarcoma and mouse lung carcinoma cells *in vitro* (27). Furthermore, we have recently reported that *Ganoderma lucidum* suppresses cell migration of highly invasive human breast and prostate cancer cells by inhibiting constitutively active NF-κB, which resulted in the down-regulation of expression of urokinase-type plasminogen activator (uPA) and its receptor uPAR (28). Therefore, the NF-κB-regulated genes may be suitable targets of *Ganoderma lucidum* for treatment of prostate cancer.

The present study was undertaken to further characterize the effect of *Ganoderma lucidum* on prostate cancer cells and to determine its effect on NF-κB-regulated genes, which are involved in cell proliferation and apoptosis. Here we demonstrate that *Ganoderma lucidum* inhibits cell proliferation through cell cycle arrest at G2/M phase and induces apoptosis in highly invasive prostate cancer cells PC-3. Growth inhibition is linked to the down-regulation of expression of cyclin B and Cdc2 and to the up-regulation of p21 expression. *Ganoderma lucidum* also induced apoptosis with moderate down-regulation of expression of antiapoptotic Bcl-2 and Bcl-xl. Furthermore, the expression of proapoptotic Bax protein was increased. Thus, *Ganoderma lucidum* exerts its effect on cancer cells by multiple mechanisms and may have potential therapeutic use for the prevention and treatment of cancer.

Materials and methods

Materials. *Ganoderma lucidum* (Reishimax) was purchased from Pharmanex (Provo, UT). According to the manufacturer, this sample contains 13.5% polysaccharides and 6% triterpenes. *Ganoderma lucidum* was dissolved in boiled water, stored at 4˚C, and reheated to 70˚C for 10 min before every experiment.

Cell culture. The human prostate cancer cell line PC-3 was obtained from ATCC (Manassas, VA). 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 proliferation assay. Cell proliferation was determined by the tetrazolium salt method, according to the manufacturer's instructions (Promega, Madison, WI). Briefly, PC-3 cells ($5x10^3$/well) were cultured in a 96-well plate and treated at indicated times with *Ganoderma lucidum* (0-0.5 mg/ml). At the end of the incubation period, the cells were harvested and absorption was determined with an ELISA plate reader at 570 nm. Data points represent mean ± SD in one experiment repeated at least twice.

Cell cycle analysis. PC-3 cells ($1x10^6$) were seeded and after 24 h treated with *Ganoderma lucidum* (0.5 mg/ml) for the indicated period of time (0-48 h). After incubation, the cells were harvested by trypsinization, washed with Dulbecco's phosphate-buffered saline (DPBS) containing 2% FBS, and resuspended in propidium iodine (50 μg/ml). Cell cycle analysis was performed on a FACStar[PLUS] flow cytometer (Becton-Dickinson, San Jose, CA), as previously described (29). Data are the mean ± SD from six independent experiments.

Nuclear fragmentation assay. PC-3 cells ($1x10^4$/well) were grown in multichamber slides (Nalgene Nunc Inc., Naperville, IL) and treated with *Ganoderma lucidum* (0-1.0 mg/ml) for 48 h. After incubation, the cells were quickly washed in ice-cold DPBS, fixed in methanol at -20˚C for 15 min, and dried and stained with DNA-specific fluorochrome DAPI (2 μg/ml) for 5 min. Stained cells were washed twice with DPBS, and

the changes in nuclei were observed with a Leica fluorescence microscope through UV filter.

DNA laddering. PC-3 cells ($1x10^6$/well) were cultured in 100-mm dish and treated with *Ganoderma lucidum* (1.0 mg/ml) for different times (0-72 h). After treatment, adherent and non-adherent cells were collected, centrifuged at 5,000 g for 5 min, and lysed with cold lysis buffer [5 mM Tris (pH 8.0), 20 mM EDTA, 0.5% Triton X-100] on ice for 45 min. DNA was extracted with phenol:chloroform:isoamyl alcohol (25:24:1), again extracted with chloroform, and precipitated with ethanol at -20˚C. The DNA pellet was resuspended in TE buffer (pH 8.0) with 100 μg/ml RNase A and incubated at 37˚C for 1 h. DNA laddering was detected by electrophoresis on 1.5% agarose gels containing ethidium bromide and visualized by ultraviolet light.

Annexin V staining. PC-3 cells ($2.5x10^5$) were treated with *Ganoderma lucidum* (0-1.0 mg/ml) for 48 h. After incubation, the cells were harvested and labeled with annexin V conjugated to fluoroscein (Roche Diagnostics, Indianapolis, IN). The labeled apoptotic cells were analyzed by flow cytometry, as previously described, on a FACStar[PLUS] flow cytometer (29).

DNA transfection and chloramphenicol acetyltransferase (CAT) assay. PC-3 cells were transfected with NF-κB-CAT reporter constructs and ß-galactosidase expression vector pCH110, as previously described (28). Twenty-four hours after transfection, cells were treated with *Ganoderma lucidum* for an additional 24 h at 37˚C, as indicated in the text. Cell lysates were prepared and CAT assays performed, as described (28). Data points represent the mean ± SD of three independent transfection experiments.

Western blot analysis. PC-3 cells ($1x10^7$) were treated with *Ganoderma lucidum* (1.0 mg/ml) for 24, 48, 72, and 96 h. After incubation, cells were washed twice with ice-cold DPBS, lysed with 1 ml of ice-cold lysis buffer [50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 1 mM EGTA, 1 mM EDTA, and protease inhibitor cocktail Complete™ (Boehringer Mannheim, Indianapolis, IN)]. Cells were lysed at 4˚C for 30 min with occasional vortexing. The lysates were collected and cleared of nuclei by centrifugation for 10 min at 14,000 rpm. The protein concentration was determined according to the manufacturer's protocol (Bio-Rad Laboratories, Hercules, CA). For Western blot analysis, equal amounts of proteins (20 μg/lane) were separated on 15% SDS-PAGE and transferred to a PVDF membrane (Millipore, Bedford, MA). The membrane was incubated with the corresponding primary antibodies diluted 1:1,000 in blocking solution, as follows: a mouse anti-Bax monoclonal antibody (Biomol Research Laboratories Inc., Plymouth Meeting, PA); a mouse anti-Bcl-2 monoclonal antibody (Zymed Laboratories Inc., South San Francisco, CA); a rabbit anti-Bcl-xl polyclonal antibody (Transduction Laboratories, Lexington, KY); and a mouse anti-cyclin D1 monoclonal antibody, a rabbit anti-Cdk4 polyclonal antibody, a mouse anti-cyclin B monoclonal antibody, a mouse anti-Cdc2 monoclonal antibody, a rabbit anti-p21 polyclonal antibody, and a mouse anti-actin monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA).
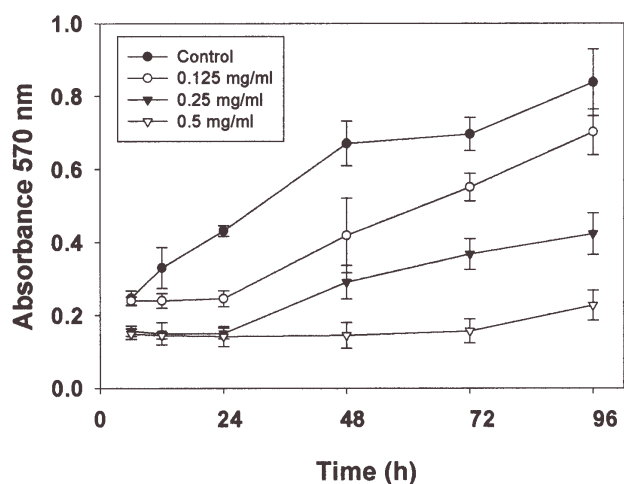
Figure 1. *Ganoderma lucidum* inhibits proliferation of PC-3 cells. PC-3 cells were treated with 0, 0.125, 0.25, and 0.5 mg/ml of *Ganoderma lucidum*. Proliferation was assessed after 5, 12, 24, 48, 72 and 96 h, as described in Materials and methods. Each bar represents the mean ± SD of three experiments.

Table I. Effect of *Ganoderma lucidum* on cell cycle distribution.

Time (h)	G0/G1	S	G2/M
0	21.3±6.8	55.4±4.4	23.3±5.5
24	20.3±11.6	41.7±6.0[a]	36.0±6.0[a]
48	19.4±5.1	37.2±8.7[a]	43.4±5.2[a]

[a]$p<0.01$

Anti-mouse or anti-rabbit secondary antibody horseradish peroxidase conjugate (1:5,000) (Amersham Biosciences, Buckinghamshire, UK) was used to detect and visualize by the ECL Western Blotting Detection system (Amersham Biosciences, Buckinghamshire, UK).

Results

Ganoderma lucidum inhibits proliferation of human prostate cancer cells. We have recently shown that *Ganoderma lucidum* inhibits cell migration of highly invasive human prostate cancer cells PC-3 (28). To examine the effect of *Ganoderma lucidum* on cell proliferation, PC-3 cells were treated with increasing concentrations of *Ganoderma lucidum* (0-0.5 mg/ml), and cell growth was determined. As seen in Fig. 1, *Ganoderma lucidum* markedly inhibited proliferation of PC-3 cells in a time-dependent manner. Growth inhibition was also dose-dependent because 96 h of treatment with 0.125, 0.25, and 0.5 mg/ml of *Ganoderma lucidum* inhibited proliferation of PC-3 cells by 7.6%, 53.8%, and 79.6%, respectively (Fig. 1). Therefore, our current data clearly demonstrate the anti-proliferative effect of *Ganoderma lucidum* on highly invasive human prostate cancer cells.

Ganoderma lucidum arrests PC-3 cells at G2/M phase of the cell cycle. Since a recent report demonstrated that alcohol extract of *Ganoderma lucidum* inhibited cell proliferation of breast cancer cells through cell cycle arrest at G1 phase (30), we examined whether the same mechanism is also employed in prostate cancer cells. PC-3 cells were treated with 0.5 mg/ml of *Ganoderma lucidum* and cell cycle distribution was determined by flow cytometry after 24 and 48 h. As shown in Table I, *Ganoderma lucidum* induced cell cycle arrest at G2/M phase in PC-3 cells. Thus, the treatment of PC-3 cells did not significantly change the amount of cells at G0/G1 phase of the cell cycle, but resulted in an increase of 57.1% and 86.3% of the cell population in G2/M phase after 24 and
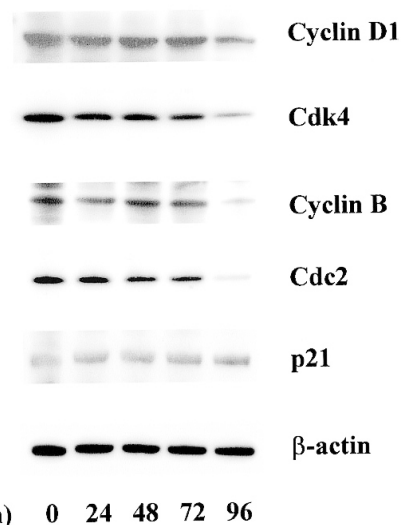

Figure 2. Effect of *Ganoderma lucidum* on cell cycle regulatory proteins. PC-3 cells were treated with *Ganoderma lucidum* (1.0 mg/ml) for indicated times. Whole cell extracts were subjected to Western blot analysis with anti-cyclin D1, anti-Cdk4, anti-cyclin B, anti-Cdc2, and anti-p21 antibodies. The equivalent amount of protein was verified by reprobing the blot with anti-ß-actin antibody. The results are representative of three separate experiments.

48 h, respectively. These data suggest that *Ganoderma lucidum* inhibits the growth of prostate cancer cells by cell cycle arrest at G2/M phase.

Effects of Ganoderma lucidum on cell cycle regulatory proteins. Since the dysregulation of cell cycle control is a hallmark of cancer (31), and since, as shown above, *Ganoderma lucidum* induced cell cycle arrest of PC-3 cells at G2/M phase, we investigated how *Ganoderma lucidum* modulates expression of cell cycle regulatory proteins. Therefore, we examined the expression of cyclin D1 and Cdk4, which control G1/S transition, and the expression of cyclin B and Cdc2, which are involved in G2/M transition (31). PC-3 cells were treated with *Ganoderma lucidum* (0-96 h) and whole cell extracts were prepared and subjected to Western blot analysis with specific antibodies against cell cycle regulatory proteins. As seen in Fig. 2, *Ganoderma lucidum* moderately down-regulated the expression of the G0/G1 regulatory proteins, cyclin D1 and Cdk4. However, the same treatment markedly decreased expression of cyclin B and Cdc2, which control
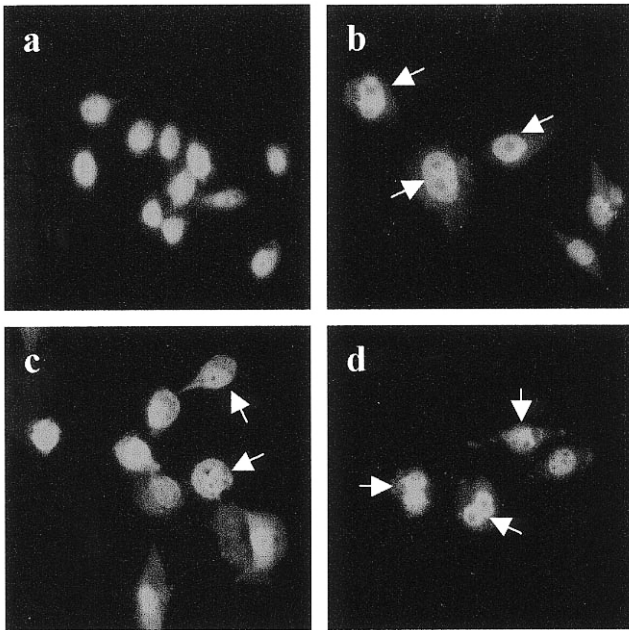
Figure 3. DAPI staining after treatment with *Ganoderma lucidum*. PC-3 cells were incubated with treated *Ganoderma lucidum* for 24 h. Nuclear staining with DAPI was examined by fluorescence microscopy, as described in Materials and methods. (a), Control; (b), 0.25 mg/ml; (c), 0.5 mg/ml; (d), 1.0 mg/ml of *Ganoderma lucidum*. The arrows indicate apoptotic cells. The results are representative of three independent experiments.
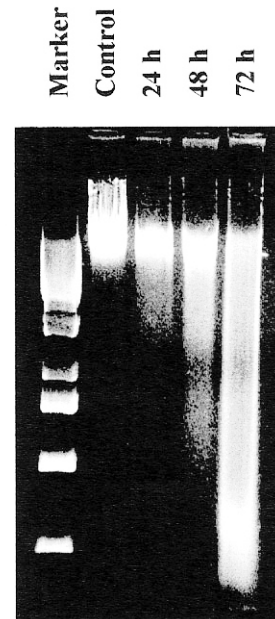

Figure 4. DNA laddering in PC-3 cells. PC-3 cells were treated with *Ganoderma lucidum* as follows: 1.0 mg/ml for 24, 48, and 72 h. Isolated DNA was separated by gel electrophoresis and stained with ethidium bromide, as described in Materials and methods. The results are representative of three separate experiments.

G2/M transition (Fig. 2). Furthermore, *Ganoderma lucidum* increased the expression of p21 in PC-3 cells, confirming cell cycle arrest at G2/M phase. Growth arrest at G2/M phase through an increase of p21 was previously reported for breast, prostate, non-small lung carcinoma, and head and neck squamous cancer cells (32). Therefore, our data suggest that G2/M phase cell growth arrest in PC-3 cells by *Ganoderma lucidum* is mediated through the down-regulation of expression of cyclin B and Cdc2 and the up-regulation of expression of p21 proteins.

Ganoderma lucidum induced apoptosis in human prostate cancer cells. As we demonstrated above, the inhibition of cell proliferation is linked to cell cycle arrest. Another reason for the growth inhibition of PC-3 cells by *Ganoderma lucidum* could be the induction of programmed cell death, apoptosis. Furthermore, alcohol extracts of *Ganoderma lucidum* induced apoptosis of breast cancer cells (30). To examine whether *Ganoderma lucidum* also induces apoptosis in prostate cancer cells, we incubated PC-3 cells with *Ganoderma lucidum* and determined cell death. As seen in Fig. 3a, DAPI staining in the absence of *Ganoderma lucidum* showed nuclei with homogeneous chromatin distribution, whereas the treatment of PC-3 cells with concentrations of *Ganoderma lucidum* (0.25, 0.5, and 1.0 mg/ml) induced nuclear shrinkage, chromatin condensation, and nuclear fragmentation (Fig. 3b-d). Apoptosis was also assessed by analyzing DNA fragmentation, where treatment with *Ganoderma lucidum* induced the formation of DNA laddering (Fig. 4). Finally, in accordance with the nuclear DNA fragmentation data, flow cytometric analysis of annexin V binding confirmed that *Ganoderma lucidum* significantly induced the percentage of apoptotic cells in a dose-response

manner (data not shown). Therefore, these results suggest that the inhibition of PC-3 cell proliferation in cells treated with *Ganoderma lucidum* might also be caused by the induction of apoptosis.

Effects of Ganoderma lucidum on the expression of apoptotic proteins. Based on our recent data that *Ganoderma lucidum* inhibits constitutively active NF-κB in prostate cancer cells (28), we speculated that the induction of apoptosis could be caused by the down-regulation of proapoptotic proteins Bcl-2 and Bcl-xl, the expression of which is controlled by NF-κB (8). PC-3 cells were treated with *Ganoderma lucidum* (0-96 h), and the whole cell extract was prepared and subjected to Western blot analysis. As seen in Fig. 4, *Ganoderma lucidum* slightly decreased the expression of Bcl-2 and Bcl-xl. To further elucidate the mechanisms by which *Ganoderma lucidum* induces apoptosis in PC-3 cells, we analyzed the expression of proapoptotic Bax protein (14). Although the expression of Bax is not regulated by NF-κB, *Ganoderma lucidum* markedly up-regulated the expression of Bax in a time-dependent manner (Fig. 5). Therefore, the significant shift in the ratio of Bax/Bcl-2 and Bax/Bcl-xl corresponds with the induction of the apoptotic process.

Ganoderma lucidum inhibits NF-κB in human prostate cancer cells. We have previously demonstrated that *Ganoderma lucidum* decreased DNA binding of NF-κB and constitutively active NF-κB in the reporter gene assay (28). Therefore, the inhibition of NF-κB at the transactivation level is a sufficient marker for its biological effect. In our original report, we showed the effect of *Ganoderma lucidum* with spores and fruiting body, which were not chemically characterized (28); however, in the present study, we used *Ganoderma lucidum*,
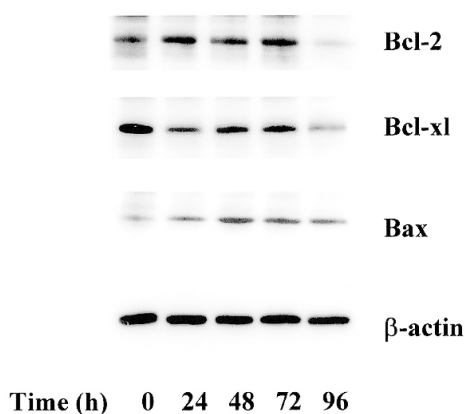
Figure 5. Effect of *Ganoderma lucidum* on the expression of Bcl-2, Bcl-xl, and Bax in PC-3 cells. PC-3 cells were treated with *Ganoderma lucidum* (1.0 mg/ml) for indicated times. Whole cell extracts were subjected to Western blot analysis with anti-Bcl-2, anti-Bcl-xl, and anti-Bax antibodies. The equivalent amount of protein was verified by reprobing the blot with anti-ß-actin antibody. The results are representative of three separate experiments.
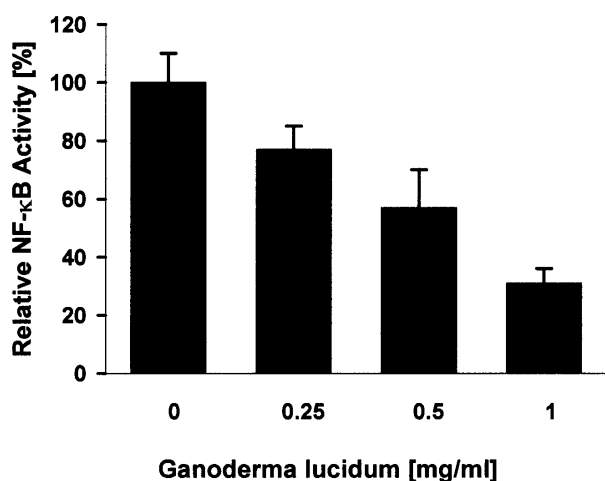

Figure 6. *Ganoderma lucidum* inhibits NF-κB in PC-3 cells. PC-3 cells were transfected with 1 μg NF-κB-CAT reporter construct and 3 μg ß-galactosidase expression vector pCH110. Twenty-four hours after transfection, the cells were treated with *Ganoderma lucidum*, and NF-κB was measured as described under Materials and methods. The results are expressed as the percentage of relative NF-κB activity. Each bar represents the mean ± SD of three experiments.

which contains 13.5% polysaccharides and 6% triterpenes. Furthermore, as demonstrated above, some of the biological effects of *Ganoderma lucidum* (such as inhibition of cell proliferation, the cell cycle arrest, and induction of apoptosis) are modulated by genes that are not controlled by NF-κB. Thus, to examine whether *Ganoderma lucidum* can inhibit transactivation of NF-κB, we transiently transfected PC-3 cells with NF-κB-CAT reporter gene plasmid and treated the cells with *Ganoderma lucidum* for 24 h. This treatment inhibited constitutive activation of NF-κB in a dose-dependent manner (0-1.0 mg/ml), confirming that *Ganoderma lucidum* is a potent inhibitor of NF-κB (Fig. 6).

Discussion

Ganoderma lucidum is a popular edible mushroom, the dried powder of which has been used in traditional Chinese medicine in many Asian countries. We have previously reported that spores or the fruiting body of *Ganoderma lucidum* down-regulated the expression of uPA and uPAR, which resulted in the inhibition of cell migration of highly invasive human breast and prostate cancer cells (28). In the present study, we examined the effects of *Ganoderma lucidum* on the proliferation of highly invasive prostate cancer cells. Here we show that *Ganoderma lucidum* inhibits the growth of prostate cancer cells, causes cell cycle arrest at G2/M phase, and induces apoptosis. Although *Ganoderma lucidum* inhibits NF-κB, some of its effects are independent of NF-κB-regulated genes, which are involved in the regulation of the cell cycle and apoptosis.

Different biologically active compounds with possible anticancer activities were recently isolated from *Ganoderma lucidum*. For example, polysaccharides from *Ganoderma lucidum* demonstrated activation of the immune response through the stimulation of production of interleukin-1ß (IL-1ß), IL-6, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) by macrophages and T-lymphocytes (26). Triterpenes were cytotoxic for hepatoma and lung carcinoma cells (27,33), phenols demonstrated antioxidant properties (34), and lipids inhibited the growth of hepatoma and sarcoma *in vivo* (35). In our study, we used commercially available dietary supplements of *Ganoderma lucidum*, which contain 13.5% polysaccharides and 6% triterpenes, and we demonstrated Ganoderma's biological 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 (27,33). In addition, there is some evidence that certain components in the natural 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 (36).

In the present study, we show that *Ganoderma lucidum* inhibits the growth of prostate cancer cells by the cell cycle arrest at G2/M phase. Since aberrantly active cell cycle regulatory proteins are responsible for the uncontrolled growth of cancer cells, these proteins are suitable therapeutic targets. Therefore, we were interested in whether *Ganoderma lucidum* affects the activity of cyclins and cyclin-dependent kinases (Cdks), which accelerate cell cycle progression. Cyclin D1 and Cdk4 are involved in the regulation of transition from G1 phase to S phase of the cell cycle, while Cdc2 and cyclin B are involved in progression from G2 phase to M phase of the cell cycle. Our data demonstrate that *Ganoderma lucidum* down-regulated the level of expression of Cdc2 and cyclin B, which confirms cell cycle arrest at the G2/M phase. Furthermore, we have also found up-regulation of the Cdk inhibitor p21. Our observation that *Ganoderma lucidum* induced cell cycle arrest at G2/M phase is in accordance with a recent report showing cell cycle arrest at the G2 phase of hepatoma cells by a triterpene-enriched fraction from *Ganoderma lucidum* (25). However, other studies have shown that alcohol

extracts of *Ganoderma lucidum* can arrest the cells at G1/G0 phase in cervical and breast cancer cells (22,30). Therefore, it is possible that specific cancer cell lines respond differently to *Ganoderma lucidum*. Alternatively, specific compounds of *Ganoderma lucidum* can be responsible for its biological effect. Alcohol extracts arrested cells at G0/G1 phase (22,30), whereas triterpene-enriched ethanol-soluble water extracts caused arrest at G2 phase (25). In our experiments, *Ganoderma lucidum* containing polysaccharides and triterpenes arrested prostate cancer cells at G2/M phase of the cell cycle.

The inhibition of proliferation of prostate cancer cells by *Ganoderma lucidum* might also be caused by the induction of apoptosis. Apoptosis is a physiological process by which cells are removed when an agent damages their DNA (37), and the inhibition of apoptosis, rather than enhanced cell proliferation, is the critical factor that contributes to the development of cancer (14,38). Therefore, apoptosis can be considered an ideal way to remove cells (39,40). Our data clearly demonstrate that *Ganoderma lucidum* induced apoptosis, as confirmed by DAPI staining, DNA ladder detection, and annexin V staining by flow cytometry. These results agree with a recent study demonstrating induction of apoptosis by *Ganoderma lucidum* in human breast cancer cells (30). Since prostate cancer cells PC-3 are also characterized by the increased expression of the survival genes Bcl-2 and Bcl-xl, which protect cells from apoptosis (14,16,41,42), we speculated that *Ganoderma lucidum* would down-regulate their expression. However, the expression of Bcl-2 and Bcl-xl was only slightly decreased. Nevertheless, the expression of proapoptotic Bax (16,43) was up-regulated by treatment with *Ganoderma lucidum*, moving the balance in the ratio of proapoptotic and survival signaling proteins (Bax/Bcl-2 and Bax/Bcl-xl) toward cell death (44,45).

NF-κB controls the expression of various genes that are involved in cell proliferation, cell survival, cell migration, cell adhesion, cell invasion, and angiogenesis (5-8). NF-κB is constitutively active in highly aggressive and invasive cancers, and thus it has been proposed as a suitable target for cancer therapy (46). Based on our previous study (28), we speculated that *Ganoderma lucidum* inhibits cell growth and induces apoptosis of prostate cancer cells through the inhibition of NF-κB, which would result in the down-regulation of expression of NF-κB controlled genes. Although, as we demonstrate here, *Ganoderma lucidum* effectively inhibited both NF-κB and cell growth, the suppression of proliferation and cell cycle arrest seems to be only partially dependent on the inhibition of NF-κB. Originally, we expected significant down-regulation of NF-κB-regulated cyclin D1 and G0/G1 cell cycle arrest, as recently reported for breast cancer cells (30). However, *Ganoderma lucidum* markedly down-regulated the expression of cyclin B and Cdc2 and up-regulated the expression of p21. Although these genes are not directly controlled by NF-κB, the inhibition of NF-κB may result in the up-regulation of expression of GADD45, which subsequently inhibits cyclin B/Cdc2 complex and arrests cells at G2/M phase (47). As mentioned above, *Ganoderma lucidum* also induced apoptosis but only moderately inhibited expression of NF-κB-regulated Bcl-2 and Bcl-xl. In addition, *Ganoderma lucidum* up-regulated the proapoptotic Bax protein, suggesting that *Ganoderma lucidum* affects the expression of NF-κB-dependent as well as NF-κB-independent genes.

In conclusion, our data demonstrate that *Ganoderma lucidum* inhibited the growth of human prostate cancer cells by cell cycle arrest at G2/M phase and induced apoptosis. The biological effects of *Ganoderma lucidum* are mediated by the inhibition of multiple signaling pathways. Additional *in vivo* studies are necessary to establish *Ganoderma lucidum* as a potential agent for the prevention and/or treatment of prostate cancer.

Acknowledgements

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