



Triterpene-enriched extracts from *Ganoderma lucidum* inhibit growth of hepatoma cells via suppressing protein kinase C, activating mitogen-activated protein kinases and G2-phase cell cycle arrest

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Abstract

The medicinal mushroom *Ganoderma lucidum* (*G. lucidum*) has been used in the Orient for the prevention and treatment of various diseases including cancer. Except for the immune enhancing properties of its polysaccharide constituent, very little is known about the anticancer activity of another major constituent, triterpenes. In this report, we studied the anticancer mechanism of triterpene-enriched extracts from *G. lucidum*. The triterpene-enriched fraction, WEES-G6, was prepared from mycelia of *G. lucidum* by sequential hot water extraction, removal of ethanol-insoluble polysaccharides and then gel-filtration chromatography. We found that WEES-G6 inhibited growth of human hepatoma Huh-7 cells, but not Chang liver cells, a normal human liver cell line. Treatment with WEES-G6 caused a rapid decrease in the activity of cell growth regulative protein, PKC, and the activation of JNK and p38 MAP kinases. The changes in these molecules resulted in a prolonged G2 cell cycle phase and strong growth inhibition. None of these effects were seen in the normal liver cells. Our findings suggest that the triterpenes contained in *G. lucidum* are potential anticancer agents.

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Keywords: *G. lucidum*; Triterpenes; Tumoricidal; PKC; MAP kinases

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Introduction

Medicinal mushrooms have a long history of use in folk medicine. *Ganoderma lucidum* (*G. lucidum*) a basidiomycetes mushroom, is one of the most popular chemopreventive mushrooms in oriental countries. Many bioactive components have been identified from its fruit bodies, mycelia, spores, and culture media. Polysaccharides and triterpenes are two major categories of the bioactive ingredients. It has been found previously that polysaccharides from *G. lucidum* exert their in vitro and in vivo anticancer effect via an immune-modulatory mechanism [7,17,31]. Some researches have reported that the triterpenes possess the bioactivities of antioxidation [34], hepatoprotection [13], cholesterol stasis [15], anti-hypertension [14,16] and inhibiting platelet aggregation [29] due to the inhibition of enzymes such as β -galactosidase, cholesterol synthase, angiotension converting enzyme, etc. Recently, triterpenes isolated from *Ganoderma spp.* were reported to exhibit cytotoxic activity against tumor cells [8,22,24,33]. A triterpene from *Ganoderma tsugae* was found to induce cell apoptosis and cell cycle arrest in human hepatoma Hep3B cells, but the molecular mechanism was not investigated [8]. In this study, we report that a triterpene-enriched fraction from mycelia of *G. lucidum* inhibits growth of human hepatoma Huh-7 cells while exerts little effect on a normal human liver cell line. Mechanistic study showed that the extract can suppress the activity of protein kinase C (PKC), activate the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and p38 mitogen-activated protein (MAP) kinases, as well as prolong the G2 cell cycle phase in Huh-7 cells. Our findings present the first evidence on the signaling molecules involved in the anticancer activity of ganoderma triterpenes.

Materials and methods

Preparation of ganoderma extracts

Hot water extracts (WE) and triterpene-enriched ethanol soluble fractions (WEES) from mycelia of *G. lucidum* were prepared as described previously [17,31]. WEES was obtained by adding a one-fourth volume of ethanol to WE and filtering off the insoluble polysaccharides. The WEES was evaporated to dryness, dissolved in deionized water, and separated further by gel-filtration chromatography using a 2.5 × 40 cm column packed with Sephadex G-25 (Pharmacia, Uppsala, Sweden). Ten fractions (WEES-G1 to G10) were collected; each of them was evaporated to dryness, carefully weighed and dissolved in deionized water for the cell experiments. The presence of triterpenes was characterized by silica gel thin layer chromatography and visualized by UV light shadowing. Ten micrograms of sample was spotted onto silica gel 60 plates (20 × 20 cm, MERCK KGaA, Darmstadt, Germany) and developed by ethyl acetate/n-hexane (4:1). Triterpenes were visualized as fluorescent spots under long wavelength UV light.

Cell cultures

Dulbecco's modified Eagle medium (DMEM), fetal calf serum (FCS), penicillin, and streptomycin were purchased from Life Technologies, Inc. (Gaithersburg, MD). Human Huh-7 hepatoma cells and non malignant Chang liver Cells were cultured in DMEM supplemented with 10% FCS, 100 units/ml penicillin and 100 mg/ml streptomycin at 37 °C under 5% CO₂. Chang liver cell line (purchased from

American Type Culture Collection, ATCC-CCL13) was established by R.S. Chang at Department of Microbiology, Harvard School of Public Health (Boston, MA) from nonmalignant human tissue [3].

Quantitation of viable cells by ACP assay

The cells (3×10^3 cells/200 μ l DMEM) were seeded in 96-well plates and cultivated overnight before WEES-G6 was added. After 72 h, cell viability in each well was measured by determining cellular acid phosphatase activity as described previously [18,19]. Briefly, the media were aspirated, and the cells were washed with phosphate-buffered saline (PBS) and incubated with 10 mM *p*-nitrophenyl phosphate containing 0.1 M sodium acetate and 0.1% Triton X-100. The reaction was terminated by adding NaOH solution, and the absorbance at 410 nm was measured by an ELISA reader. The data presented were the averages derived from a minimum of three independent experiments, each performed in triplicate.

Assay of PKC activity

The cells (5×10^5 cells/6 ml DMEM) were seeded in 10-cm petri dishes and cultivated overnight before WEES-G6 was added. After 8 h, the cells were rinsed with ice-cold PBS three times and subsequently lysed at 4 °C in lysis buffer (20 mM Tris-HCl, 5 mM EDTA, 2 mM EGTA, 1 mM NaF, 150 mM NaCl, 1 mM PMSF, 10 μ g/ml leupeptin, 20 μ g/ml aprotinin, 1 mM Na₃VO₄, 0.1% Triton X-100). The protein content of the lysate was determined by BCA protein assay reagent (Pierce, Rockford, IL). PKC activity in the cell lysate was determined using the Peptag nonradioactive PKC assay kit (Promega, Madison, WI). According to the procedure provided by the manufacturer, phosphorylation by PKC of its specific substrate alters the peptide's net charge from +1 to -1. This change in the net charge of the substrate allows the phosphorylated and nonphosphorylated versions of the substrate to be separated on a 1.5% agarose gel at neutral pH. The negatively charged bands were quantified densitometrically with Gel-Pro Analyzer software (Media Cybernetics, Silver Spring, MD).

Western-blot analysis

Polyclonal antibodies for detecting unphosphorelated ERK, JNK, and p38 were purchased from Santa Cruz Biotech (Santa Cruz, CA.). Monoclonal antibodies to phosphorylated ERK, JNK and p38 were purchased from Cell Signaling (Beverly, MA). Monoclonal antibodies to cyclins A, B and D were obtained from Upstate Biotechnology Inc. (Lake Placid, NY). The horseradish peroxidase-labeled goat anti-rabbit and anti-mouse secondary antibodies were purchased from Zymed Laboratories Inc. (South San Francisco, CA). For the analysis of cellular protein by Western blotting method, the cells were seeded in 10-cm petri dishes and incubated overnight before WEES-G6 was added. After 4, 8, 12, 24 and 48 h, the cells were harvested and lysed in lysis buffer as described above. Cell lysates containing 40 μ g of protein were loaded onto a 10% SDS-polyacrylamide gel, electrophoresed and were then transferred to a nitrocellulose membrane. The membrane was immuno-blotted by primary antibody, then by HRP conjugated secondary antibody. The immuno-reactive bands were revealed by chemiluminescence-detecting reagent (New England Nuclear Life Science Products, Boston, MA) and exposure of X-ray films. Band intensity on X-ray films was quantitated using Gel-Pro Analyzer software.

Cell cycle analysis by flow cytometry

The cells (5×10^4 cells/3 ml DMEM) were seeded in 6-cm petri dishes and cultivated overnight before being treated with WEES-G6. After 24- and 48-h cultivation, the cells were harvested by trypsinization, washed with PBS, resuspended in 0.5 ml of PBS, and fixed in 0.5 ml of ice-cold absolute ethanol at $-20\text{ }^\circ\text{C}$ for 30 min. The cells were collected by centrifugation, resuspended in 0.5 ml of PBS containing 100 $\mu\text{g/ml}$ RNase, and incubated at $37\text{ }^\circ\text{C}$ for a further 30 min. In the next step, 0.5 ml of propidium iodide solution (10 $\mu\text{g/ml}$) was added, and the mixture was allowed to stand on ice for 30 min. The cells were analyzed with FACScan flow cytometer (Becton Dickinson, San Jose, CA). Cell cycle phase distribution was analyzed using ModFit LT software as described previously [19].

Results

Triterpene-enriched extracts from *G. lucidum* inhibit growth of Huh-7 cells

The hot water extracts (WE) from mycelia of *G. lucidum* and its ethanol-insoluble (polysaccharides) fraction exhibit no growth inhibitory activity on either Huh-7 cells or Chang liver cells at concentrations of 1 ~ 100 mg/ml. However, WEES (the ethanol-soluble fraction of WE) effectively inhibits growth of Huh-7 cells. The concentration of WEES required to inhibit 50% of cell growth (IC_{50}) is about 450 $\mu\text{g/}$

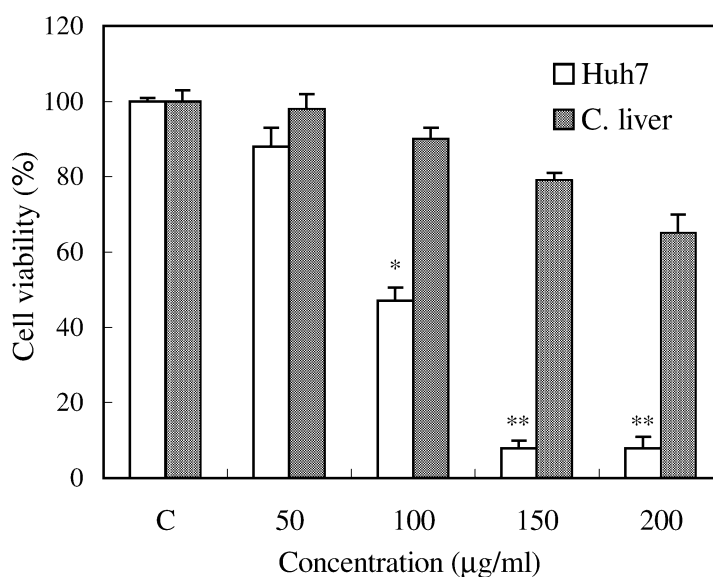


Fig. 1. Growth inhibitory effect of WEES-G6 on Huh-7 and Chang liver cells. The cells were cultivated alone as a control group (C) or treated with indicated concentrations of WEES-G6 for 72 h. At the end, cumulative viable cells were determined by acid phosphatase (ACP) assay. The data presented are averages derived from at least triplicate experiments. One asterisk and two asterisks indicate $P < 0.05$ and $P < 0.01$, respectively, as compared with the control group (Student's t -test).

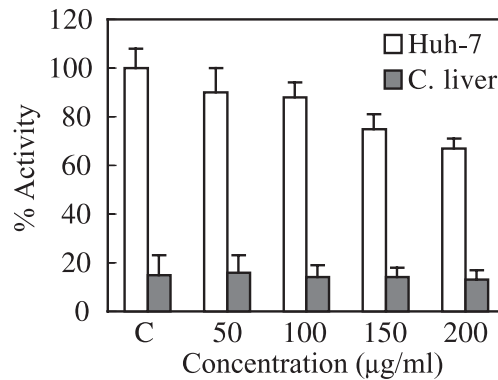


Fig. 2. Effect of WEES-G6 on PKC activity in Huh-7 cells and Chang liver cells. The cells were cultivated alone as a control group (C) or incubated with WEES-G6 at concentrations of 50, 100, 150 or 200 µg/ml. After 8 h, cell lysates were prepared; 40 µg of total protein from each sample was subjected to PKC activity analysis using Peptag nonradioactive PKC assay kit. The results were quantitation of phosphorylated product bands by densitometry as described in Materials and Methods. The PKC activity in drug-treated groups was presented as a percentage of the control group.

ml for Huh-7 cells, as measured 72 h after drug addition. After further separation of WEES by gel-filtration chromatography into ten fractions, each of them was screened for the growth inhibitory effect on Huh-7 cells and Chang liver cells. The sixth fraction (WEES-G6) was found to possess the highest amount of growth inhibitory activity. It effectively inhibited growth of Huh-7 cells and had little effect on the viability of Chang liver cells (Fig. 1). After 72-h treatment, IC_{50} is about 100 µg/ml for Huh-7

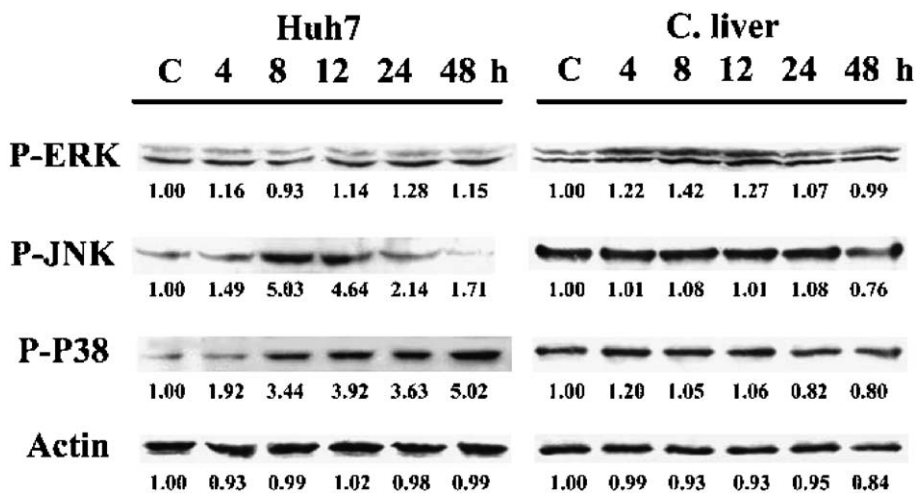


Fig. 3. Activation of MAP kinases in Huh-7 cells treated with WEES-G6. The cells were cultivated alone as a control group (C) or incubated with 250 µg/ml WEES-G6 for the indicated periods of time. Forty micrograms of total cellular proteins were analyzed by Western blot analysis for unphosphorylated and phosphorylated forms of ERK, JNK and p38. Actin was used as an internal control to ensure equal loading of each lane. The band intensity was quantified by densitometry and the intensity relative to control group was indicated under each band. The amounts of unphosphorylated ERK, JNK and p38 were not changed during the WEES-G6 treatment (not shown).

cells, while is > 1 mg/ml for Chang liver cells. Thin-layer chromatographic separation showed that WEES-G6 contained a major fluorescent spot ($R_f = 0.68$), and some fluorescent materials remained at the origin.

WEES-G6 affects the activity of PKC and MAP kinases in Huh-7 cells

By analyzing PKC activity of the cell lysates, we found that Huh-7 cells have higher PKC activity (5 ~ 6 folds) than Chang liver cells as shown in Fig. 2. The influence of WEES-G6 on PKC activity can

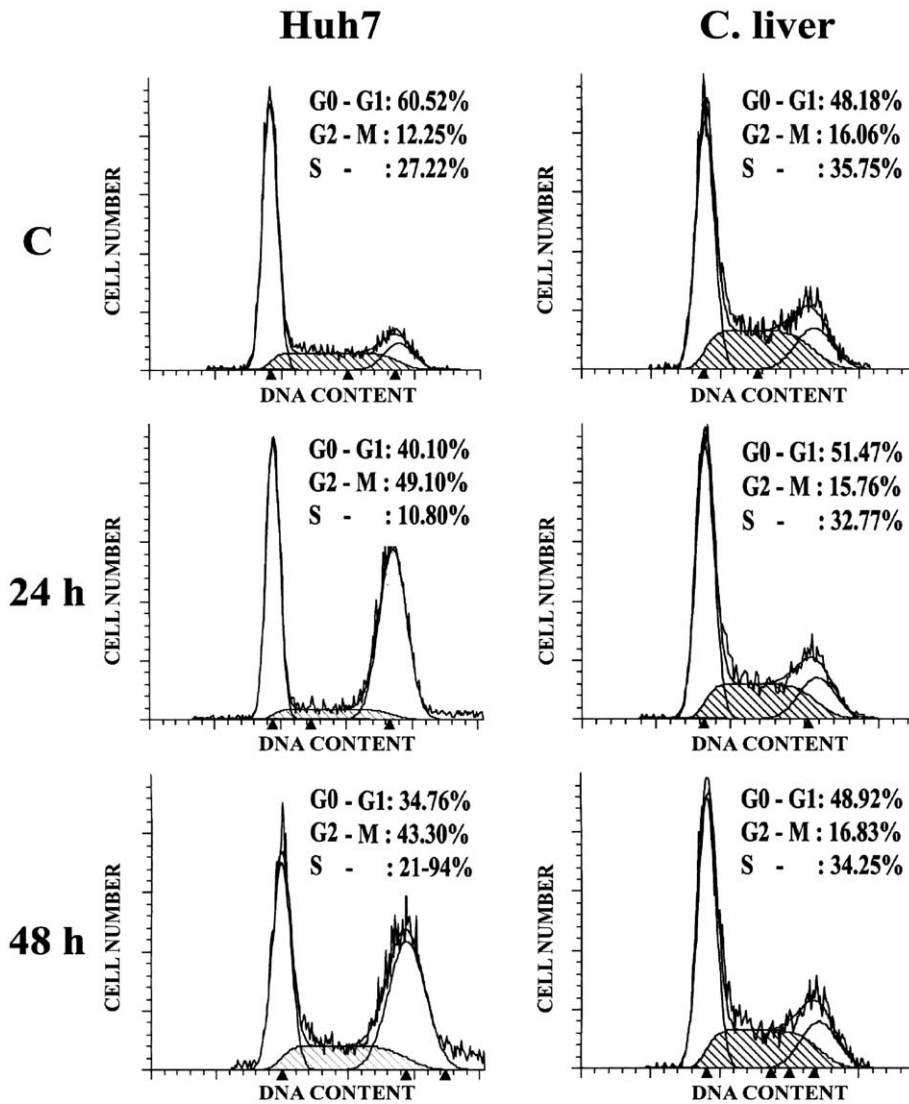


Fig. 4. WEES-G6 caused G2-phase cell cycle arrest in Huh-7 cells. The cells were cultivated alone as a control group (C) or cultured in the presence of 250 μ g/ml of WEES-G6 for 24- and 48 h. After that, the cells were harvested, stained with propidium iodide and analyzed by FACscan flow cytometer. The data were analyzed by ModFitLT software.

be observed 4 h after drug addition and is more pronounced at 8 h post drug-addition. As shown in Fig. 2, treatment of Huh-7 cells with WEES-G6 for 8 h caused decreased activity of PKC in a dose-dependent manner. On the other hand, the influence on Chang liver cells was minimal (Fig. 2). The influences of WEES-G6 on phosphorylated- ERK, JNK and p38 (P-ERK, P-JNK and P-p38, respectively) are shown in Fig. 3. In Huh-7 cells, activation of JNK and p38 (increases in the level of P-JNK and P-p38) were observed 8 h post drug-addition. The level of JNK activation reached five folds of the control group and lasted about 4 h (from 8 h to 12 h). The activation of p38 was seen from 8 h post drug-addition and became more pronounced as time went by. No significant change was observed in the level of P-ERK. In Chang liver cells, activation of the MAP kinases was not obvious. The levels of the unphosphorylated form of these MAP kinases were not changed in both cell lines (data not shown).

WEES-G6 causes G2-phase arrest in Huh-7 cells

WEES-G6 was found to affect cell cycle phase distribution in Huh-7 cells but not in Chang liver cells. Exponentially growing cultures were exposed to WEES-G6 for 24 h and 48 h, and the cell cycle distribution of the cells was analyzed by flow cytometry. As shown in Fig. 4, the treatment of Huh-7 cells with WEES-G6 caused an increased G2 phase population (49.1% and 43.3% for 24-h and 48-h treatment, respectively) that was about four folds of the control group (12.3%). Arrest of cells in the G2 phase could be due to deficiency in M phase promoting factors. We therefore analyzed the level of cyclins and found a gradual decrease in the level of cyclin B protein and no obvious changes in cyclins A and D (Fig. 5). Deficiency in cyclin B might account for the G2-phase cell cycle arrest. On the other hand, there was no cell cycle disturbance observed in the Chang liver cells.

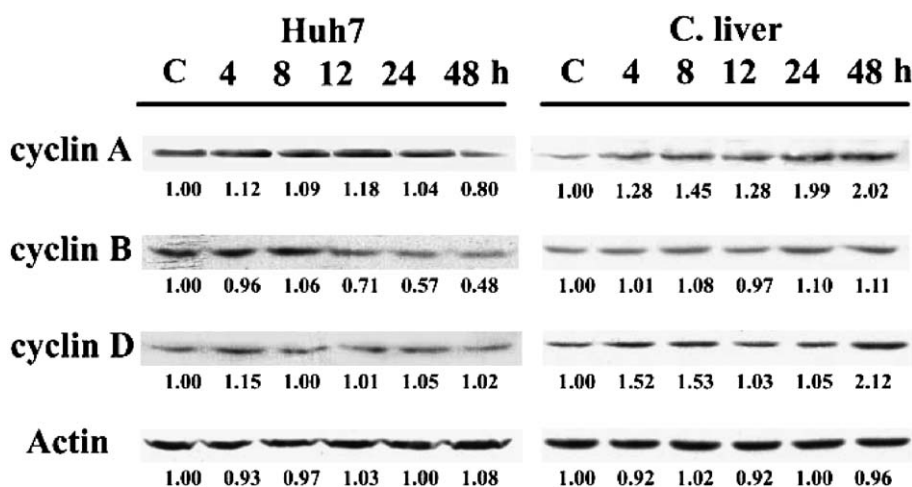


Fig. 5. Western blot analysis of cyclins in WEES-G6-treated cells. The cells were cultivated alone as a control group or incubated with 250 $\mu\text{g}/\text{ml}$ WEES-G6 for different periods of time. Forty micrograms of total cellular proteins were analyzed by Western blot analysis. Actin was used as an internal control to ensure equal loading of each lane. The band intensity was quantified by densitometry and the intensity relative to control group was indicated under each band.

Discussion

In the present study, we utilized one cancerous and one normal liver cell line to demonstrate the cancer-cell specific growth inhibitory activity of *G. lucidum*. The efficacy of *G. lucidum* as an anticancer and tonic agent has been recognized for thousands of years. These two cell lines may serve as a good bioassay system for screening anticancer drugs of this type. A parallel study of anticancer effects of WEES-G6 has found the involvement of oxidative stress induction in Huh-7 cells [20]. The anticancer components, as characterized by the UV-absorption and fluorescence properties, are likely triterpenes. Triterpene molecules are cyclic hydrophobic hydrocarbons. The WEES-G6 fraction, however, is soluble in both hot water and ethanol, implying that it may have hydrophilic, such as hydroxyl- or carboxyl-, functional groups. To elucidate structures of the active components, further purification and characterization are required. Recently, polysaccharides from *G. lucidum* were reported to reduce the cell proliferation rate and induce the neuron differentiation of PC-12 cells. In the report, ganoderma extract at 50 ~ 100 mg/ml was employed, and activation of MAP kinase (pERK 1/2) was found to mediate the bioactivity [4]. Our result that WEES-G6 executes its growth inhibitory effect and triggers intracellular signaling on Huh-7 cells at much lower concentrations (~ 100 µg/ml) could possibly attribute to its being a better signal transducer or containing purer constituents.

The mechanistic effects of WEES-G6 include down regulating PKC activity, activating JNK and p38 MAP kinases and G2-phase cell cycle arrest. PKC is a class of serine-threonine protein kinases related to cell proliferation and also involved in cell-cycle control [6]. A recent report indicated that the growth of Huh-7 cells was stimulated by PKC activators and inhibited by PKC inhibitors [12]. The decrease in PKC activity by WEES-G6, leading to G2-phase arrest, is similar to other known PKC inhibitors, such as ilmofosine and 12-O-tetradecanoyl phorbol-13-acetate (TPA) [1,10]. The G2-phase arrest of Huh-7 cells by WEES-G6 seems to be a result of delayed cell division caused by a lack of cyclin B. It has been reported that the ionization-induced division delay and resultant G2 arrest in HeLa cells was due to a lack of cyclin B [23]. The activity of WEES-G6 is also similar to anticancer drug Taxol, which activates MAP kinase signaling pathways and also caused G2/M phase arrest of the cell cycle in a variety of human cells [2,27,30,32]. MAP kinases are another group of serine/threonine specific kinases, which are activated in response to extracellular stimuli through dual phosphorylation at conserved threonine and tyrosine residues. MAP kinases consist of three main groups: the c-Jun N-terminal kinase (JNK), the extracellular signal regulated kinases (ERK), and the p38 kinase. Each of the three kinases has several sub-forms derived from different genes [9,28]. JNKs are first found responding to cellular stresses and are the archetypal stress-activated protein kinases (SAP kinase). Both the ERK and p38 kinase groups are also found responding to cellular stress later, so all three MAP kinase groups are SAP kinases under certain conditions [21,25]. It is possible that constituents in WEES-G6, by interacting with the cell membrane, cell surface receptors, or intracellular macromolecules, cause stress for Huh-7 cancer cells thus activate JNK and p38. Although the molecular events involved in WEES-G6 activity is similar to Taxol or other known PKC inhibitors, however, induction of cell apoptosis by WEES-G6 was not detected. It has been reported that apoptosis of cancer cells was induced by Taxol [2,30] or pharmacological inhibitors of PKC [5,11], or in liver cancer cells treated with isoenzyme-specific PKC antisense inhibitor [19,26].

Our findings have demonstrated that mycelia of *G. lucidum* contain anticancer triterpene compounds, which affect important signaling molecules in hepatoma cells but not normal liver cells. Whether or not the effects on PKC and MAP kinases mediated by the same constituent requires advanced experiments

using dissected fractions of WEES-G6. However, a common feature of herbal medicine is the presence of multiple components that could act either independently or synergistically to elicit their pharmacological effects. Nevertheless, there are limitations to extrapolate in vitro findings to in vivo. Thus animal experiments demonstrating the anticancer efficacy of WEES-G6 is inevitable.

Acknowledgements

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References

- [1] Arita Y, Buffolino P, Coppock DL. Regulation of the cell cycle at the G2/M boundary in metastatic melanoma cells by 12-O-tetradecanoyl phorbol-13-acetate (TPA) by blocking p34cdc2 kinase activity. *Experimental Cell Research* 1998; 242:381–90.
- [2] Bacus SS, Gudkov AV, Lowe M, Lyass L, Yung Y, et al. Taxol-induced apoptosis depends on MAP kinase pathways (ERK and p38) and is independent of p53. *Oncogene* 2001;20:147–55.
- [3] Chang RSM. Continuous sub-cultivation of epithelial-like cells from normal human tissue. *Process in Social Experiment Biological Medicine* 1954;87:440–3.
- [4] Cheung WM, Hui WS, Chu PW, Chiu SW, Ip NY. Ganoderma extract activates MAP kinases and induces the neuronal differentiation of rat pheochromocytoma PC12 cells. *FEBS Letters* 2000;486:291–6.
- [5] Couldwell WT, Hinton DR, He S, Chen TC, Sebat I, et al. Protein kinase C inhibitors induce apoptosis in human malignant glioma cell lines. *FEBS Letters* 1994;345:43–6.
- [6] Fishman DD, Segal S, Livneh E. The role of protein kinase C in G1 and G2/M phases of the cell cycle. *International Journal of Oncology* 1998;12:181–6.
- [7] Furusawa E, Chou SC, Furusawa S, Hirazumi A, Dang Y. Antitumor activity of *Ganoderma lucidum*, an edible mushroom, on intraperitoneal implanted Lewis lung carcinoma in syngeneic mice. *Phytotherapy Research* 1992;6:300–4.
- [8] Gan KH, Fann YF, Hsu SH, Kuo KW, Lin CN. Mediation of the cytotoxicity of lanostanoids and steroids of *Ganoderma tsugae* through apoptosis and cell cycle. *Journal of Natural Products* 1998;61:485–7.
- [9] Garrington TP, Johnson GL. Organization and regulation of mitogen-activated protein kinase signaling pathways. *Current Opinion in Cell Biology* 1999;11:211–8.
- [10] Hofmann J, O'Connor PM, Jackman J, Schubert C, Ueberall F, et al. The protein kinase C inhibitor ilmofosine (BM 41 440) arrests cells in G2 phase and suppresses CDC2 kinase activation through a mechanism different from that of DNA damaging agents. *Biochemical and Biophysical Research Communication* 1994;199:937–43.
- [11] Jarvis WD, Turner AJ, Povirk LF, Traylor RS, Grant S. Induction of apoptotic DNA fragmentation and cell death in HL-60 human promyelocytic leukemia cells by pharmacological inhibitors of protein kinase C. *Cancer Research* 1994; 54:1707–14.
- [12] Kambe H, Kishima Y, Kuroda T, Enomoto H, Ogawa H, et al. Protein kinase C inhibitor, H-7 suppresses the growth activity of hepatoma-derived growth factor. *Hepatogastroenterology* 2000;47:1645–8.
- [13] Kim DH, Shim SB, Kim NJ, Jang IS. Beta-glucuronidase-inhibitory activity and hepatoprotective effect of *Ganoderma lucidum*. *Biological and Pharmaceutical Bulletin* 1999;22:162–4.
- [14] Kimura S, Tamura T. Dietary effect of *Ganoderma lucidum* mushroom on blood pressure and lipid levels in spontaneously hypertensive rats (SHR). *Journal of Nutritional Science and Vitaminology* 1988;34:433–8.
- [15] Komoda Y, Shimizu M, Sonoda Y, Sato Y. Ganoderic acid and its derivatives as cholesterol synthesis inhibitors. *Chemical and Pharmaceutical Bulletin* 1989;37:531–3.
- [16] Lee SY, Rhee HM. Cardiovascular effects of mycelium extract of *Ganoderma lucidum*: inhibition of sympathetic outflow as a mechanism of its hypotensive action. *Chemical and Pharmaceutical Bulletin* 1990;38:1359–64.
- [17] Lieu CW, Lee SS, Wang SY. The effect of *Ganoderma lucidum* on induction of differentiation in leukemic U937 cells. *Anticancer Research* 1992;12:1211–5.

- [18] Lin SB, Hsieh SH, Hsu HL, Lai MY, Kan LS, et al. Antisense oligodeoxynucleotides of IGF-II selectively inhibit growth of human hepatoma cells overproducing IGF-II. *Journal of Biochemistry* 1997;122:717–22.
- [19] Lin SB, Wu LC, Huang SL, Hsu HL, Hsieh SH, et al. In vitro and in vivo suppression of growth of rat liver epithelial tumor cells by antisense oligonucleotide against protein kinase C- α . *Journal of Hepatology* 2000;33:601–8.
- [20] Lin SB, Li CH, Chen YR, Kan LS, Lee SS. Triterpene extract from *Ganoderma lucidum* inhibits growth of hepatoma Huh7 cells: involvement of oxidative stress induction. In: Lin ZB, editor. *Ganoderma: Genetics, Chemistry, Pharmacology and Therapeutics*. Beijing: Beijing Medical University Press; 2002. p. 176–82.
- [21] Liu Y, Guyton KZ, Gorospe M, Xu Q, Lee JC, Holbrook NJ. Differential activation of ERK, JNK/SAPK and P38/CSBP/RK map kinase family members during the cellular response to arsenite. *Free Radical Biology and Medicine* 1996; 21:771–81.
- [22] Min BS, Gao JJ, Nakamura N, Hattori M. Triterpenes from the spores of *Ganoderma lucidum* and their cytotoxicity against meth-A and LLC tumor cells. *Chemical and Pharmaceutical Bulletin* 2000;48:1026–33.
- [23] Muschel RJ, Zhang HB, Iliakis G, McKenna WG. Cyclin B expression in HeLa cells during the G2 block induced by ionizing radiation. *Cancer Research* 1991;51:5113–7.
- [24] Noda Y, Kaiya T, Kohda K, Kawazoe Y. Enhanced cytotoxicity of some triterpenes toward leukemia L1210 cells cultured in low pH media: possibility of a new mode of cell killing. *Chemical and Pharmaceutical Bulletin* 1997;45:1665–70.
- [25] Price MA, Cruzalegui FH, Treisman R. The p38 and ERK MAP kinase pathways cooperate to activate Ternary Complex Factors and c-fos transcription in response to UV light. *EMBO Journal* 1996;15:6552–63.
- [26] Shen L, Dean NM, Glazer RI. Induction of p53-dependent, insulin-like growth factor-binding protein-3-mediated apoptosis in glioblastoma multiforme cells by a protein kinase C α antisense oligonucleotide. *Molecular Pharmacology* 1999;55:396–402.
- [27] Shtil AA, Mandlekar S, Yu R, Walter RJ, Hagen K, et al. Differential regulation of mitogen-activated protein kinases by microtubule-binding agents in human breast cancer cells. *Oncogene* 1999;18:377–84.
- [28] Su B, Karin M. Mitogen-activated protein kinase cascades and regulation of gene expression. *Current Opinion in Immunology* 1996;8:402–11.
- [29] Su CY, Shiao MS, Wang CT. Differential effects of ganodermic acid S on the thromboxane A2-signaling pathways in human platelets. *Biochemical Pharmacology* 1999;58:587–95.
- [30] Torres K, Horwitz SB. Mechanisms of taxol-induced cell death are concentration dependent. *Cancer Research* 1998;58:3620–6.
- [31] Wang SY, Hsu ML, Hsu HC, Tzeng CH, Lee SS, et al. The anti-tumor effect of *Ganoderma lucidum* is mediated by cytokines released from activated macrophages and T lymphocytes. *International Journal of Cancer* 1997;70:699–705.
- [32] Wang TH, Wang HS, Ichijo H, Giannakakou P, Foster JS, et al. Microtubule-interfering agents activate c-Jun N-terminal kinase/stress-activated protein kinase through both Ras and apoptosis signal-regulating kinase pathways. *Journal of Biological Chemistry* 1998;27:4928–36.
- [33] Wu TS, Shi LS, Kuo SC. Cytotoxicity of *Ganoderma lucidum* triterpenes. *Journal of Natural Products* 2001;64:1121–2.
- [34] Zhu M, Chang Q, Wong LK, Chong FS, Li RC. Triterpene antioxidants from *Ganoderma lucidum*. *Phytotherapy Research* 1999;13:529–31.