Chapter 12
An Evidence-based Perspective of *Ganoderma Lucidum* (Lucid Ganoderma) for Cancer Patients

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Abstract *Ganoderma lucidum* (lucid ganoderma), a traditional Chinese herb, has been used extensively in East Asian for thousand years, and the biological activities and pharmacological functions of lucid ganoderma have been successively studied. Nowadays, some researches use scientific methods and techniques to study its anticancer effect on the cancer patients in clinical trial. For example, PC-SPES extracted from a mixture of lucid ganoderma and seven herbs decreased the prostate-specific antigen in patients with chemotherapy-induced hormone-independent prostate cancer. Lucid ganoderma extracts also improved the immune-stimulating response, such as the increase of plasma interleukin (IL)-2, IL-6, and interferon-γ concentrations, the enhance of natural killer cell activity, the decrease of plasma IL-1 and tumor necrosis factor-α concentrations, etc, as well as had low adverse effects in the cancer patients. The anticancer effects of lucid ganoderma in cell and animal models could be the strong references for the clinical trials. The components with anticancer potential in lucid ganoderma include triterpenoids, steroids, polysaccharides, fatty acids, and novel proteins such as LZ-8. On the basis of *in vitro* and *in vivo* study, the anticancer mechanism of lucid ganoderma treatment against the growth of cancer cells in clinical trial might be mediated by cell cycle arrest, apoptosis, anti-invasion, anti-migration, immunomodulation, anti-angiogenesis, etc. Additionally, the combination from lucid ganoderma and other herbs or foods as an alternative treatment might exhibit synergistic anticancer efficacy. However, more studies regarding the safety and application in clinical trial need to be processed in the future for providing more evidences.

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12.1 Introduction

Ganoderma lucidum (lucid ganoderma) is a kind of saprophytic fungus and usually grows well in a wet and ventilated environment with high temperature. Lucid ganoderma is classified as *Mycota, Basidiomycotina, Hymenomycetes, Holobasidiomycetidae, Aphyllophorales, Polyporaceae, Ganodermoideae, Ganoderma* according to the modern categorized system in Western (Shiao 1992). Lucid ganoderma is one of the Chinese precious medical fungi in the medicine since ancient times. Different cultivation methods of lucid ganoderma lead to the complicated and changeable chemical composition (Han et al. 2005; Liu and Zhang 2007).

The major constitutes contains carbohydrates such as polysaccharides (Zhu and Lin 2005), proteins such as enzymes and glycoproteins (Miura et al. 2002; Wang et al. 2002), and other bioactive compounds such as triterpenoids, steroids, and fatty acids (Akihisa et al. 2007; Fukuzawa et al. 2008). Because novel triterpenoids in numerous chemical compositions especially exists in lucid ganoderma, they have already been used to be indicators for taxonomy and quality in pharmacology (Chen et al. 1999). Many physiological functions of lucid ganoderma have been investigated, including anticancer activity (Nonaka et al. 2008), live-protective effect (Yang et al. 2006), antioxidation (Yuen and Gohel 2008), anti-blood platelet agglutination (Su et al. 2000), immune adjustment (Zhuang et al. 2009), cholesterol reduction (Hajjaj et al. 2005), anti-viral activity (Li and Wang 2006), and hypoglycemic effect (Zhang and Lin 2004).

In this chapter, we have reviewed recent researches regarding the effect of lucid ganoderma treatment on the subjects with cancer. The active components and possible mechanisms of anticancer activity *in vitro* and *in vivo* of lucid ganoderma will be discussed to expound how lucid ganoderma improved the constitutions of human being, especially cancer patients. In addition, the safety of lucid ganoderma for normal human subjects will also be described. These evidences may support the application of lucid ganoderma for the treatment of cancer patients.

12.2 Clinical Trials of Cancer Patients

According to the clinical results of de la Taille et al. (2000), it has been reported that two case reports regarding effect of PC-SPES extract, an herbal mixture contained lucid ganoderma, chrysanthemuism, isatis, licorice, ginseng, *Rabdosia rubescens*, saw palmetto, and scutellaria on hormone-refractory prostate cancer patients. A 73-year-old man with Gleason 9 (4+5) prostate cancer underwent surgery and treatment with bicalutamide for androgen blockade, but his high prostate-specific antigen (PSA) level suggested that the prostate cancer is not controlled well. After taking 3 tablets of PC-SPES per day for 6 months, his PSA level was decreased from 100 to 24 ng/ml (76% reduction) without any side effect. In the other case, an 80-year-old man with Gleason 8 T2 N0 M0 prostate cancer received radiation

therapy and successively took the medicine such as leuprolide, bicalutamide, ketoconazole, and hormonal steroids¸ but his PSA levels did not always be lowered and maintained. However, the PSA level was decreased from 386 to 114 ng/ml (72% reduction) and remained stably after treated with PC-SPES (6 tablets/day) for 4 months, and no side effects were observed. These two cases suggested that PC-SPES might have some potential activity against hormone-independent prostate cancers. Because PC-SPES has a strong estrogenic activity *in vitro* and *in vivo*, it suggested that PC-SPES might be an alternative drug to treat hormone-independent prostate cancers (de la Taille et al. 2000; Hsieh and Wu 2002).

In the clinical trial of 47 patients (27 men and 20 women, average 48.4 ± 7.0 years old) with advanced colorectal cancer, 35 and 45 subjects received surgical resection and previous chemotherapy/radiotherapy, respectively. These patients were treated with 5.4 g/day Ganopoly polysaccharide product, a polysaccharide-enriched fraction from lucid ganoderma fruiting body, for 12 weeks, and a small increase of CD3, CD4, CD8, and CD56 lymphocytes counts, plasma interleukin (IL)-2, IL-6, interferon (IFN)-γ concentrations, and natural killer (NK) cell activity, as well as a small decrease of plasma IL-1 and tumor necrosis factor (TNF)-α concentrations in 41 assessable cancer patients compared to the baseline data. The lucid ganoderma fraction consists of 98.8% polysaccharides with glucose (61.2%), xylose (15.5%), fructose (14.4%), galactose (4.8%), and rhamnose (4.1%) linked together by β-glycosidic linkages, and the clinical results for cancer patients might be correlated with the potential immune-modulating effect of lucid ganoderma (Chen et al. 2006).

Gao et al. (2003) also reported the effect of lucid ganoderma extract containing 25% (w/w) crude polysaccharides on the immune functions of 34 patients (31–77 years old; 20 men and 14 women) with different cancer origins including lung (7 subjects), colon (6 subjects), breast (5 subjects), liver (5 subjects), prostate (4 subjects), bladder (2 subjects), brain (2 subjects), and unknown (3 subjects). Except for 2 patients without previous treatment, the patients were treated with surgery (23 subjects), chemotherapy (6 subjects), radiotherapy (10 subjects), immunotherapy (12 subjects), endocrine treatment (5 subjects), traditional Chinese medicine (19 subjects), and more than two of above treatments excluding surgery (18 subjects). The statistic results of whole subjects compared to the baseline levels showed that oral administration of lucid ganoderma extract (1,800 mg, equal to 90 g of fruiting body, three times daily before meals) for 12 weeks significantly increased the mean concentrations of plasma IL-2, IL-6, and IFN-γ ($P < 0.05$) and significantly decreased the levels of IL-1 and TNF-α ($P < 0.05$). In terms of the mean absolute number of lymphocyte subset, a significant increase of $CD56^+$ (NK cells) ($P < 0.05$) and a small increase of $CD3^+$ (T lymphocyte), $CD4^+$ (T helper cells), and $CD8^+$ (T suppressor cells) with unchanged CD4:CD8 T cell ratios were induced by lucid ganoderma extract as compared to the baseline levels. Lucid ganoderma extract also significantly enhanced the proliferation of phytohemagglutinin-stimulated lymphocyte compared to the pretreatment baselines and increased the mean NK cells activity compared to the baselines ($P < 0.05$). It indicated that lucid ganoderma extract stimulated the immune responses in patients with advanced-stage cancer in clinical trial (Gao et al. 2003).

A total of 105 cancer patients (33–84 years old) receiving chemotherapy and/or radiotherapy were subjected in the study of Zhuang et al. (2009), including 60 breast cancer patients, 24 colorectal cancer patients, 14 nasopharyngeal cancer patients, and 7 lung cancer patients with disease stages I-IV except for 5 patients with unknown stage. Chinese medicinal herb complex (CCMH) is a mixture of citronellol powder (273.6 mg) and extracts of lucid ganoderma (3 mg), *Codonopsis pilosula* (27.1 mg), and *Angelicae sinensis* (64.5 mg) in one capsule. Either 9 capsules of CCMH ($n=55$) or placebo (control group) ($n=50$) were supplied by the cancer patients every day for 6 weeks, and the mean percentages of leukocytes and neutrophils in CCMH group were significantly higher than those in control group ($P<0.05$). Supplement of CCMH also maintained the mean percentages of CD4 lymphocytes and NK cells compared to control group. Therefore, treatment with CCMH improved the immune function in the cancer patients undergoing chemotherapy and/or radiotherapy (Zhuang et al. 2009).

In an open-label study of Yoshimura et al. (2010), 17 patients (60–80 years old: 15 patients; <60 years old: 2 patients) with biochemical failure after radical treatment for non-metastasized prostate cancer with different clinical stages including B0 (3 subjects), B1 (4 subjects), B2 (7 subjects), and C1 (3 subjects). After supplement of Rokkaku Reishi, a lucid ganoderma product in Japan, for 6 months, although the results indicated that Rokkaku Reishi did not exhibit significant anticancer effects, but no patients had serious adverse effects due to Rokkaku Reishi, including blood/bone marrow, dermatology/skin, and gastrointestinal events (Yoshimura et al. 2010).

In order to understand the anticancer effect of lucid ganoderma, we prefer to discuss what active compounds are contributed to and what mechanisms are mediated. However, the evidences, such as survival prolongation, adverse effects reduction, etc, of lucid ganoderma treatment against cancer in the patients are not enough to clarify the efficacy, thus the inhibitive effect of lucid ganoderma on the cancer cells *in vitro* and *in vivo* must be explicated as well.

12.3 Anticancer Activity of Lucid Ganoderma and Its Crude Extracts

In an anticancer animal model, the growth of MM 46 mammary carcinoma inoculated in C3H/HeN mice were inhibited after taking AIN-93 M feed containing 2.5% lucid ganoderma fruiting body for 28 days (Nonaka et al. 2008). Lucid ganoderma (2.5%) containing diet also suppressed tumor growth elongated the life span of ddY mice inoculated with Sarcoma 180 cells after 100 days treatment (Nonaka et al. 2006). Hot water extracts from lucid ganoderma fruiting body (100 mg/kg bw) and spores (1,000 mg/kg bw) also possessed inhibitory activities on Sarcoma 180 cell growth in the implanted mice (Yue et al. 2008a).

In terms of cancer cell experiments, the water extract from lucid ganoderma fruiting body exhibited anti-proliferative effects on myeloid leukemia HL-60, U937,

and K562 cells, lymphoblastic leukemia Blin-1 and Nalm-6 cells, and multiple myeloma RPMI8226 cells, and their ED_{50} (the effective dose which inhibited 50% growth) were 26–40 μg/ml (Muller et al. 2006). It also reported that the growth of human small cell lung cancer drug-sensitive (H69) and multi-drug resistant (VPA) cells were inhibited by the water extract from lucid ganoderma fruiting body, and the IC_{50} (the concentration of the sample to inhibit cell growth by 50%) were 60 and 80 μg/ml, respectively (Sadava et al. 2009). Against human breast cancer MCF-7 and MDA-MB-231 cell lines, the aqueous extracts of different parts of lucid ganoderma, including whole fruiting body, pileus, and stipe, have different potential inhibitive activity (Yue et al. 2006).

In addition to aqueous extract, crude methanolic extract of lucid ganoderma fruiting body caused cell death of murine cancer L1210 and 3LL cells (Tomasi et al. 2004), and the column-chromatography semipurified fraction from lucid ganoderma methanolic extract reduced cell viability of human acute promyelocytic leukemia NB4 cells and mouse IL-3 dependent lymphoma DA-1 cells (Calvino et al. 2010a, b). It has also been demonstrated that the ethanolic extract of lucid ganoderma fruiting body induced apoptosis in MCF-7 as well as human colonic carcinoma HT-29 and gastric carcinoma AGS cell lines (Hu et al. 2002; Hong et al.

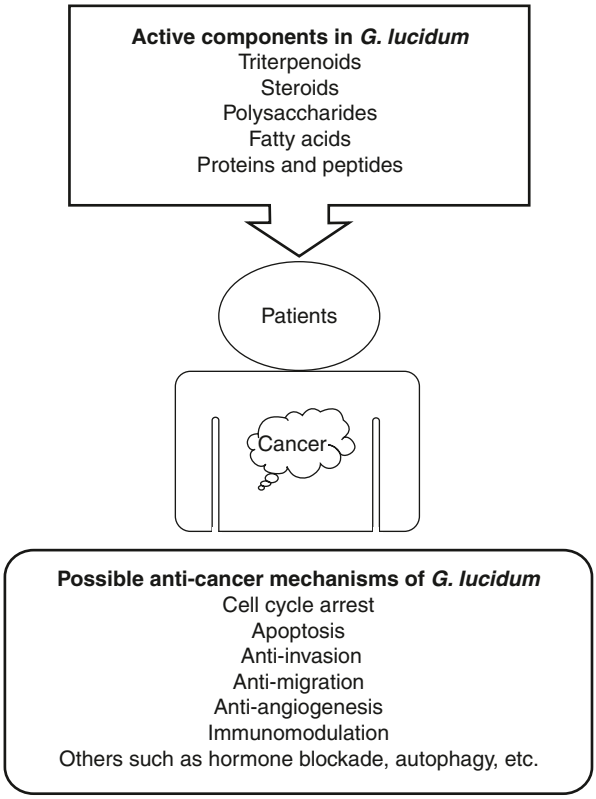

Fig. 12.1 The active components in *Ganoderma lucidum* (lucid ganoderma) and their possible anticancer mechanisms on the cancer patients

2004; Jang et al. 2010). The crude water or ethanolic extracts of lucid ganoderma spores also exhibited anticancer activity against MDA-MB-231, prostate cancer PC-3, and cervix uteri tumor HeLa cells (Zhu et al. 2000; Sliva et al. 2002, 2003; Lu et al. 2004).

Therefore, in order to support more evidences for cancer patients treated with lucid ganoderma, the active compounds with anticancer activity in lucid ganoderma and possible anticancer mechanisms of lucid ganoderma will be further discussed as follows (Fig. 12.1).

12.4 Active Components with Anticancer Activity in Lucid Ganoderma

12.4.1 Triterpenoids and Steroids

Most of the researches reported that triterpenoids have direct inhibitory activity against cancer cells *in vitro*. The triterpenoids extracts isolated from lucid ganoderma fruiting body have been demonstrated to inhibit proliferation of human cancer cells, including HT-29, LNCaP (prostate cancer), MCF-7, MDA-MB-231, HeLa, HL-60, and HepG2 (liver cancer) cells, and their IC_{50} values were among 71.6 and 219.5 μg/ml (Yue et al. 2008c; Liu et al. 2009a, b; Thyagarajan et al. 2010). After treated with triterpenoids-rich extract from lucid ganoderma fruiting body, there were 14 proteins were regulated in HeLa cells according to two-dimensional gel electrophoresis-based comparative proteomics, including proliferation, cell cycle, apoptosis, and oxidative stress-related proteins (Yue et al. 2008c). Ganoderiol F, ganoderol B, ganodermadiol, ganodermnonol, ganodermaondiol, genoderic acids (A, AM1, D, E, F, H, K, and T), lucialdehydes (A, B, and C), and lucidenic acids (A, B, C, and N) were identified from lucid ganoderma fruiting body and exhibited novel inhibitive effects on various cancer cell lines, and their IC_{50} values were around 2.8–27.9 μg/ml (Wu et al. 2001; Gao et al. 2002; Tang et al. 2006; Weng et al. 2007, 2008; Liu et al. 2007, 2009a, 2010; Hsu et al. 2008a; Jiang et al. 2008; Yue et al. 2008b, c, 2010). Ganoderic acids from the fermentation products of lucid ganoderma inhibited the growth of human hepatoma BEL7402 cells, but not a normal human liver L02 cells (Yang 2005). Ganoderic acid Me existed in fermentation mycelia and showed anti-metastatic activity against human highly metastatic lung tumor 95-D cells (Chen et al. 2008).

In animal model, the methanol extract containing total terpenoids or purified methanol extract containing mainly acidic terpenoids from lucid ganoderma fruiting body (100 mg/kg bw/day) effectively inhibited the growth of implanted murine melanoma B16 cells in C57BL/6 mice after 24 days treatment (Harhaji Trajkovic et al. 2009). HT-29 cells were implanted in male nude mice, and lucid ganoderma triterpene extract containing a mixture of lanostanoid triterpenes (6 mg/kg bw/day for 23 days) inhibited the tumor growth (Thyagarajan et al. 2010). After tak-

ing 200 mg/kg bw/day lucidenic acid-rich lucid ganoderma extract for 68 days, the growth of HepG2 cells implanted into ICR mice were suppressed (Weng et al. 2009). Ganoderic acid T has been demonstrated to suppress tumor growth in the model of male BALB/c implanted with 95-D cells and celiac injected with ganoderic acid T (12.5 mg/kg bw/day) for 12 days (Tang et al. 2006). Ganoderic acid T at 7 mg/kg bw/day (celiac injection, 10 days) also decreased tumor growth and metastasis of murine Lewis lung carcinoma (LLC) cells implanted into male C57B/6 mice (Chen et al. 2010). From lucid ganoderma fermentation mycelia, 28 mg/kg bw/day of ganoderic acid Me (16 days) were intraperitoneally administrated to inhibit both lung tumor growth and lung metastasis of Lewis lung carcinoma in C57BL/6 mice (Wang et al. 2007).

In addition to triterpenoids, ethanolic extract of lucid ganoderma fruiting body contained sterols and exhibited anti-proliferative activities against MCF-7, MDA-MB-231, HepG2, and HL-60 cell lines (Liu et al. 2009b). Two ergosterol derivatives, ergosterol peroxide, and 9,11-dehydroergosterol peroxide, form ethanolic extract of fermentation mycelia were also isolated and had cytotoxicity against human hepatocellular carcinoma Hep3B cells (Chen et al. 2009).

12.4.2 Polysaccharides

A Ganopoly polysaccharides product showed cytotoxicity against Hep3B, HepG2, SiHa (cervical carcinoma), CaSki (cervical carcinoma), HT-29, HCT116 (colorectal adenocarcinoma), and MCF-7 cells (Gao et al. 2005). Furthermore, oral administration of Ganopoly polysaccharides product (20 mg/kg bw/day) also reduced the tumor weight in C57BL/6 J mice bearing Sarcoma 180 after 21 days treatment (Gao et al. 2005). It also reported that oral supplement of lucid ganoderma polysaccharides from fruiting body at 50 mg/kg bw/day for 10 days exhibited tumor inhibitory against Sarcoma 180 in BALB/c mice (Li et al. 2008). It has been demonstrated that daily intraperitoneal injection of a glucan extracted from lucid ganoderma spores (50 mg/kg bw for 2 weeks) had tumor-suppress activity against Lewis lung cancer bearing in C57BL/6 mice (Guo et al. 2009). Additionally, sulfated and carboxymethylated lucid ganoderma polysaccharides were synthesized for stronger inhibition of the growth of sarcoma 180 tumor cells *in vitro* and *in vivo* (Wang et al. 2009).

12.4.3 Other Active Compounds

Except for triterpenoids, steroids, and polysaccharides, long chain saturated (C19:0, C18:0, C17:0, and C16:0) and unsaturated (C19:1, C18:1, C17:1, and C16:1) fatty acids in the ethanolic extract from lucid ganoderma spores inhibited the proliferation, and especially ethanolic extract and 19-carbon fatty acids induced apoptosis

in HL-60 cells (Fukuzawa et al. 2008). It also reported that polysaccharides peptide (polysaccharides:peptides is 94.8:5.2) from boiling water extract of lucid ganoderma fruiting body at 50 mg/kg bw/day effectively suppressed either sarcoma 180 (10 days treatment) or human lung carcinoma PG cells (33 days treatment) implanted into BALB/c mice (Cao and Lin 2004). However, their cancer-inhibitory effects *in vivo* need to be further studied to support more adequate references for treating cancer in clinical trial.

12.5 Anticancer Mechanisms for Lucid Ganoderma

12.5.1 Cell Cycle Arrest

It has been reported that water extract of lucid ganoderma fruiting bodies induced cell cycle arrest at G2/M phase in human immune system-related cancer cell lines such as myeloid leukemia HL-60, U937, and K562 cells, lymphoblastic leukemia Blin-1 and Nalm-6 cells, and multiple myeloma RPMI8226 cells (Muller et al. 2006) and at S phase in H69 cells (Sadava et al. 2009). The ethanolic extract from lucid ganoderma fruiting body up-regulated p21/Waf1 and down-regulated cyclin D1, cdk4 (cyclin-dependent protein kinase 4) and transcription factor E2F to arrest cell cycle at G0/G1 phase in MCF-7 cells (Hu et al. 2002). A powdered mixture (containing 6% triterpenoids and 13.5% polysaccharides) from fruiting body extract and spores also induced G0/G1 phase arrest which might be correlated with the down-regulation of Akt/NF-κB signaling pathway, cyclin D1, and cdk4 in MDA-MB-231 cells (Jiang et al. 2004).

Regarding anti-proliferation potential of triterpenoids, it reported that a crude ganoderic acid-rich extract from submerged culture of lucid ganoderma blocked the cell cycle at the transition from G1 to S phase in BEL7402 cells (Yang 2005). A triterpenoids-enriched extract arrested the cell cycle of human hepatoma Huh-7 cells at G2 phase mediated by decreasing the PKC activity and JNK and p38 MAP kinases activation (Lin et al. 2003). Lucidenic acids A, C, and N from fruiting body induced cell cycle arrest at G1 phase in HL-60 cells (Hsu et al. 2008a). Both ganoderic acids A and H inhibited the activities of transcription factors AP-1 (activator protein-1) and NF-κB resulting in the decrease of Cdk4 expression and the suppression of uPA (urokinase-type plasminogen activator) secretion in MDA-MB-231 cells (Jiang et al. 2008). It has been demonstrated that ganoderic acid D induced G2/M phase arrest in HeLa cells mediated by binding six isoforms of 14-3-3 protein family, annexin A5, and aminopeptidase B (Yue et al. 2008b).

Some researches modified the structures of polysaccharides from lucid ganoderma by the method of chemical synthesis for giving better anticancer activity. For example, it also reported that chemical modified lucid ganoderma polysaccharides such as sulfated and carboxymethylated polysaccharides induced cell-cycle arrest in the G2/M phase to inhibit the proliferation of sarcoma 180 tumor cells

(Wang et al. 2009). But the safety and bioactivity of these new compounds have to be further investigated using animal models.

12.5.2 Apoptosis

Cell death of four hematopoietic cell lines such as HL-60, U937, Blin-1, and RPMI8226 cells were caused by water extract from lucid ganoderma fruiting bodies *via* apoptosis (Muller et al. 2006). A water extract from fruiting bodies increased DNA fragmentation, TUNEL staining for DNA breaks, and specific activities of caspase-3 and -9, but not caspase-8 by colorimetric assays (Sadava et al. 2009). Aqueous extract of lucid ganoderma fruiting bodies induced apoptosis accompanied with the increasing the expressions of Bax, p53, mdm2, and cleavaged caspase-3 in DA-1 cells (Calvino et al. 2010b).

A column-chromatography semipurified fraction from lucid ganoderma methanolic extract caused reduction of the Bcl2/Bax ratio, both unphosphorylated and phosphorylated Akt (protein kinase B) levels, and Erk1/2 (extracellular signal-regulated kinase) levels in NB4 cells (Calvino et al. 2010a). It has been reported that methanolic extract containing total terpenoids form fruiting body induced caspase-dependent apoptotic cell death which might be mediated by producing reactive oxygen species, up-regulated p53, and inhibited Bcl-2 expression in B16, mouse fibrosarcoma L929, and rat astrocytoma C6 cells (Harhaji Trajkovic et al. 2009). Ethanolic extract of lucid ganoderma also has pro-apoptotic function. For example, ethanolic extract of fruiting body increased the activity of caspase-3 in HT-29 cells (Hong et al. 2004). AGS cell line was treated with ethanolic extract of fruiting body, and then the phenomena of two apoptotic pathways were observed as follows: (1) mitochondria-mediated intrinsic pathway, including the activation of caspase-3 and -9, the cleavage of Bid, and the degradation of poly(ADP-ribose) polymerase (PARP); (2) death receptor-mediated extrinsic pathway, including the increase of death receptor-related proteins such as death receptor 5 and tumor necrosis factor-related apoptosis-inducing ligand, the activation of caspase-8, and the down-regulation of IAP family proteins such as XIAP and survivin (Jang et al. 2010).

In terms of pure triterpenoid compounds, lucidenic acid B induced apoptosis through mitochondria pathway in HL-60 cells, including the loss of mitochondria membrane potential, the decrease of the ratio of pro- and anti-apoptotic Bcl-2 family expressions, the release of mitochondria cytochrome c, and the activation of caspase-3 and -9, and the cleavage of PARP (Hsu et al. 2008a). Ganoderic acid T had similar pro-apoptotic mechanism against 95-D cells (Tang et al. 2006).

12.5.3 Anti-invasion and Anti-migration

Treatment of lucid ganoderma (2.5%)-containing AIN-93M (14 days) showed the anti-metastatic activity against the implanted LLC cells to the lung in C57BL/6

mice (Nonaka et al. 2008). The water extract from lucid ganoderma either spores or dried fruiting body inhibited invasion and migration of both PC-3 and MDA-MB-231 cells mediated by inhibiting the activities of transcription factors AP-1 and NF-κB, decreasing the expressions of uPA and uPAR (uPA receptor), and suppressing the secretion of uPA (Sliva et al. 2002, 2003). The lucid ganoderma fruiting body extract containing 13.5% polysaccharides and 6% triterpenoids inhibited oxidative stress-induced migration of MCF-7 cells through down-regulation of MAPK signaling pathway such as the suppression of oxidative stress stimulated Erk1/2 phosphorylation, the decrease of c-Fos expression, the inhibition of AP-1 and NF-κB activities, and the suppression of oxidative stress-mediated IL-8 secretion from MCF-7 cells (Thyagarajan et al. 2006).

In phorbol-12-myristate-13-acetate-induced invasion of HepG2 cells, lucidenic acid-rich lucid ganoderma fruiting body extract reduced the expression of MMP-9 (matrix metalloproteinase-9) as well as inhibited the phosphorylations of ERK1/2 and Akt in the cytosol and the expressions of AP-1, NF-κB, c-Jun, and c-Fos in the nucleus (Weng et al. 2008, 2009). The possible active compounds with anti-invasion in lucid ganoderma fruiting body might be lucidenic acids A, B, C, and N (Weng et al. 2007). In animal model, supplement of the lucidenic acid-rich extract (200 mg/kg bw/day for 68 days) decreased the number of tumor foci and the activities of serum MMP-2 and MMP-9 in the ICR-nu/nu mice implanted with HepG2 cells (Weng et al. 2009). Ganoderic acid Me was isolated from lucid ganoderma fermentation mycelia and showed the inhibitory effect on the cell adherence to extracellular matrix of highly metastatic 95-D cells mediated by suppressing mRNA and protein expressions of MMP-2 and MMP-9 (Chen et al. 2008). Additionally, ganoderic acid T inhibited the invasion of HCT116 cells, and the results suggested that ganoderic acid T mediated the inhibition of nuclear translocation of NF-κB and the degradation of IκB-α inhibitor resulting in the decrease of expressions of MMP-9, iNOS (inducible nitric oxide synthase), and uPA (Chen et al. 2010). In LLC cells implanted male C57B/6 mice, ganoderic acid T (7 mg/kg bw/day) *via* celiac injection for 10 days) suppressed LLC metastasis and decreased the expressions of *MMP-2* and *MMP-9* mRNA levels (Chen et al. 2010).

12.5.4 Anti-angiogenesis

Lucid ganoderma inhibits the early event in angiogenesis (Stanley et al. 2005). It reported that a mixture from fruiting body extract and spores suppressed the phosphorylation of Erk1/2 and Akt kinases, and then inhibited the secretion of vascular endothelial growth factor and TGF-β1 in highly invasive PC-3 cells (Stanley et al. 2005). The polysaccharides and peptides from lucid ganoderma fruiting body showed anti-angiogenic potential because it inhibited the proliferation of human umbilical cord vascular endothelial cells and decreased vascular endothelial growth factor in PG cells (Cao and Lin 2004, 2006).

12.5.5 Immunomodulation

AIN-93 M feed containing lucid ganoderma (2.5% for 100 days) inhibited tumor growth and elongated the life span in the C3H/He mice implanted with MM 46 mammary carcinoma mediated by decreasing splenic CD8 cell number and IFN-γ production in regional lymph nodes (Nonaka et al. 2006). A hot water extract from sporoderm-broken spores stimulated the production of cytokines such IFN-γ, IL-4, and IL-6 of spleen lymphocytes resulting in its tumor inhibitory activity in sarcoma 180 bearing mice (Yue et al. 2008a). It reported that crude triterpenoids extract suppressed the production of TNF-α and IL-6 in LPS-induced endotoxemic mice (Dudhgaonkar et al. 2009). Ganoderic acid Me enhanced the activity of NK cells and increased the expressions of IL-2 and IFN-γ in LLC cells implanted C57BL/6 mice (Wang et al. 2007).

Actually, there are more studies regarding immunomodulatiing functions of lucid ganoderma polysaccharides recently (Chen et al. 2004; Gao et al. 2005; Chan et al. 2007, 2008; Hsu et al. 2009; Lai et al. 2010). For example, the polysaccharides form fruiting body increased TNF-α and IFN-γ protein expressions and mRNA levels in splenocytes, the cytotoxicity of cytotoxic T lymphocytes, and the activity of NK cells in both healthy and sarcoma 180 bearing C57BL/6 J mice (Gao et al. 2005). A polysaccharide fraction prepared from lucid ganoderma fruiting body showed immune-stimulating activity in BALB/c mice, including increasing the number of dendritic cells and CD4, CD8, regulatory T, B, plasma, NK and NKT ($CD3^+$ NK-T/NK^+) cells in the spleen, elevating the levels of multiple cytokines and chemokines in the blood, and enhancing both Th1 and Th2 responses (Lai et al. 2010). In an *in vitro* study, it also found that the polysaccharide fraction induced the maturation of dendritic cells derived from human monocytes mediated by up-regulating CD40, CD54, CD80, CD83, CD86, and HLA-DR, enhancing mixed lymphocyte reaction, and stimulating the production of ten cytokines and six chemokines (Lai et al. 2010). A heteroglycan from lucid ganoderma fruiting body stimulated immune system to enhance the proliferation of T and B lymphocytes and the production of antibodies, but little effect on serum IgG and complement (C3) levels in inbred ICR female mice (Bao et al. 2002). The structure of the glycan was identified to be a backbone consisting of 1,4-linked α-D-glucopyranosyl residues and 1,6-linked β-D-galactopyranosyl residues with branches at *O*-6 of glucose residues and *O*-2 of galactose residues, composed of terminal glucose, 1,6-linked glucosyl residues, and terminal rhamnose (Bao et al. 2002). Additionally, supplement of lucid ganoderma extract (400 mg/kg bw/day) enhanced the recovery of immunocompetence such as the increase of the leukocytes, the relative weight of thymus, and the increase of CD4 and CD8 splenocytes in gamma-ray-irradiated ICR male mice after 28 days treatment (Chen et al. 1995a, b).

Except for polysaccharides, the protein fraction from lucid ganoderma mycelia and culture liquid also had immunomodulating activity (Jeurink et al. 2008). The immune-stimulating effect of recombinant LZ-8 (rLZ-8) expressed from the cloned lucid ganoderma *LZ-8* gene has been investigated recently, including the

rLZ-8-mediated signal-transduction pathways in the regulation of *IL-2* gene expression within human T cells (Hsu et al. 2008b), the rLZ-8-modulated the production of Th1 and Th2 cytokines by peripheral blood mononuclear cells and tumor NF-α by a macrophage cell line (Yeh et al. 2008), and the rLZ-8-induced activation and maturation of immature human monocyte-derived DCs (Lin et al. 2009). Although LZ-8 or rLZ-8 may be useful in cancer treatment, there is hardly any research to confirm the antitumor activity of LZ-8 or rLZ-8 in clinical trial for human being currently.

12.5.6 Others

The cell viability of estrogen-dependent MCF-7 cell line was decreased by the mixture of lucid ganoderma fruiting body extract and spores accompanied with the down-regulation of ERα (estrogen receptor-α) and *c-myc* expressions, the inhibition of constitutive transactivation activity of ER through estrogen response element, and the decrease of TNF-α-induced NF-κB activity (Jiang et al. 2006). It also reported that ganoderiols B and F and ganodermatriol isolated from lucid ganoderma fruiting body showed a binding activity to androgen receptor which might be related to the anti-proliferative effect of lucid ganoderma on LNCaP cells (Liu et al. 2007, 2009a, 2010). Therefore, it suggested that the progress of prostate cancer in the patients affected by PC-SPES containing lucid ganoderma might be correlated to this hormone-dependent pathway (de la Taille et al. 2000).

Moreover, it is worth to mention a new report that lucid ganoderma extract containing lanostanoid triterpenoids suppressed the phosphorylation of p38 MAPK and induced the expressions of Beclin-1 and LC-3 proteins resulting in induction of autophagy, programmed cell death Type II (Thyagarajan et al. 2010). This is a new pathway of cell death induced by lucid ganoderma.

12.6 Anticancer Activity of Combination Treatment

Some researches proved the anticancer potential of lucid ganoderma combined with other herbs or foods using scientific methodology recently. For example, PC-SPES is a herbal mixture derived from eight different herbs including lucid ganoderma, *Dendranthema morifolium* Tzvel, *Panax pseudoginseng, Glycyrrhiza uralensis* Fisch, *Rabdosia rubescens* Hara, *Scutellaria baicalensis* Georgi, *Isatis indigotica* Fort, and *Serenoa repens*, and it effectively suppressed the growth of LNCaP cells (Hsieh and Wu 2002). Combined treatment with extracts of 150 μg/ml lucid ganoderma (triterpenoid-enriched fraction) and 100 μg/ml *Duchesnea chrysantha* (polysaccharide-enriched fraction) induced G1 phase arrest and apoptosis *via* mitochondria pathway in HL-60 cells, including the down-regulation of Bcl-2, the

translocation of Bax, the release of mitochondrial cytochrome c, and the activation of caspase-3 (Kim et al. 2007, 2008). Lucid ganoderma combined with crocodile egg extract and ginseng inhibited proliferation and colony formation of acute myelogenous leukemia KG1a cell line (Chui et al. 2006). A mixture composed of lucid ganoderma extract containing 13.5% polysaccharides and 6% triterpenes and green tea extract containing 97% polyphenols synergistically inhibited anchorage-dependent and -independent growth, adhesion, migration, and invasion of MDA-MB-231 cells with decreases of oncogene c-myc expression and uPA secretion (Thyagarajan et al. 2007). Traditional Botanical Supplement-101 containing lucid ganoderma, *Panax ginseng*, cranberry, green tea, grape skin, grape seed, and chamomile induced apoptosis in PC-3 cells (IC_{50}=1.4 μg/ml) *in vitro* and inhibited tumor growth and invasion in nude mice implanted PC-3 tumor cells with no toxicity *in vivo* (Evans et al. 2009).

In addition, the inhibitory effects of combinative treatments of lucid ganoderma and drugs on cancer were also studied. It reported that lucid ganoderma triterpenoids extract enhanced doxorubicin-increased reactive oxygen species production and doxorubicin-decreased Ku80 protein expression resulting in apoptosis in HeLa cells (Yue et al. 2008c). Oral administration of methanolic extract of lucid ganoderma (250 and 500 mg/kg body weight) prevented mice from cisplatin (an anticancer drug)-induced nephrotoxicity, and it suggested that lucid ganoderma has a potential therapeutic effect on cancer chemotherapy (Sheena et al. 2003).

According to the theory of Chinese medicine, some combination from the herbals, drugs, or foods with different attributes might exhibit better bioactivity in bodies. Therefore, lucid ganoderma combination treatment might be a good choice for cancer patients as well if their synergistic effect is obvious without any adverse effects.

12.7 Safety of Lucid Ganoderma in Clinical Trials

The safety and bioactivities of lucid ganoderma need to be carefully evaluated in normal human clinical trials before applying lucid ganoderma for cancer patients. For example, it has been reported that no adverse effects were observed in healthy subjects after intaking lucid ganoderma extract (2 g/day) for 10 days compared to placebo group (Wicks et al. 2007).

Clinical trials in another double-blinded, placebo-controlled, and cross-over intervention study, healthy volunteers took 0.72 g/day lucid ganoderma extract (equivalent to 6.6 g/day fresh mushroom) in the form of capsules for 10 days, and their plasma lipid standardized α-tocopherol concentration and urine antioxidant capacity were significantly increased ($P<0.05$) while plasma ascorbic acid and total alpha-tocopherol concentrations and erythrocyte superoxide dismutase and glutathione peroxidase activities were slightly increased (Wachtel-Galor et al. 2004a). Supplementation with lucid ganoderma capsules (1.44 g/day; equivalent to 13.2 g/day fresh mushroom) for 4 weeks also slightly lowered plasma total cholesterol,

total triglyceride, and low density lipoprotein levels while it slightly increased plasma lipid standardized α-tocopherol concentration and urine antioxidant capacity in healthy adults (Wachtel-Galor et al. 2004b).

In 40 male football players during a 28-day "living high-training low" training, lucid ganoderma capsules containing pure spores and fruiting body extract were administrated by subjects in the study of Zhang et al. (2008). The results suggested that lucid ganoderma (5 g/day) could ameliorate the variation of the $CD4^+/CD8^+$ ratio in "living high-training low" training, and the main active components contributing to immuno-modulating function might be lucid ganoderma polysaccharides (Zhang et al. 2008).

12.8 Conclusion

There are some evidences for supporting the anticancer potential of lucid ganoderma in the clinical trial of cancer patients recently. Lucid ganoderma contains active compounds contributing to inhibitory function against the growth of cancer cells in the patients, including triterpenoids, steroids, polysaccharides, fatty acids, and novel proteins. They showed direct (such as cell cycle arrest and apoptosis) and indirect (such as immunomodulation) inhibitory effects on cancer. They also exhibited anti-invasion, anti-migration, and anti-angiogenesis activities against cancer cells. Some combinations of lucid ganoderma and other herbs or foods might be applied to treat cancer patients as an alternative treatment on the basis of their synergistic efficacy. More investigations on the safety and treatment dosages for cancer patients, the immune-modulating responses for chemo-prevention in normal human being, and the different culture condition including fermentation process to produce lucid ganoderma with stronger anticancer activity are worth to be further studied in the future.

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