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Ganoderma lucidum Polysaccharides: Immunomodulation and Potential Anti-Tumor Activities

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Abstract: *Ganoderma lucidum* (*G. lucidum*), a basidiomycete white rot fungus, has long been prescribed to prevent and treat various human diseases, particularly in China, Japan, and Korea. Several classes of bioactive substances have been isolated and identified from *G. lucidum*, such as triterpenoids, polysaccharides, nucleosides, sterols, and alkaloids, among others. This paper examines the potential role of *G. lucidum* polysaccharide (GLPS) in tumor therapy and the possible mechanisms involved. Both *in vitro* and *in vivo* studies suggested that the anti-tumor activities of GLPS are mediated by its immunomodulatory, anti-angiogenic, and cytotoxic effects. GLPS affects immune cells and immune-related cells including B lymphocytes, T lymphocytes, dendritic cells, macrophages, and natural killer cells. In addition, recent data also suggest that GLPS suppresses tumorigenesis or inhibits tumor growth through direct cytotoxic effect and anti-angiogenic actions. However, many questions still need to be answered before both *G. lucidum* and GLPS can be widely accepted and used as anti-tumor agents.

Keywords: *Ganoderma lucidum*; Polysaccharides; Immunomodulation; Anti-Angiogenesis; Anti-Proliferation.

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Introduction

The fruiting body of *Ganoderma lucidum* (*G. lucidum*, Reishi, or Ling-Zhi) was once considered a panacea in ancient China. In some old legends and fairy tales, the fungus was frequently described as a magical drug that could bring the dead back to life. In medicine, it has been widely used for centuries as a famous and precious traditional Chinese herb in China, Japan, Korea, and other oriental countries. Recently, *G. lucidum* has been used to prevent and treat various human diseases such as bronchitis, allergies, hepatitis, hypertension, immunological disorders, and cancer (Chen *et al.*, 1980; Yuen *et al.*, 2005; Boh *et al.*, 2007; Wicks *et al.*, 2007). Accumulated studies show that *G. lucidum* extracts possess anti-proliferative effects on many tumor cell lines *in vitro* such as the colorectal prostate cancer cell line (Berovic *et al.*, 2003), lung cancer cell line (Jiang *et al.*, 2005), acute myelogenous leukemia cancer cell line (Cheng *et al.*, 2007), breast cancer cell line (Thyagarajan *et al.*, 2006), colorectal cancer cell line (Xie *et al.*, 2006), and bladder cancer cell line (Paterson, 2006). The chemical components of *G. lucidum* are complicated: they involve polysaccharides, flavonoids and alkaloids, amino acids, steroids, oligosaccharides, proteins, mannitol, etc. (Zjawiony, 2004). Among these components, *G. lucidum* polysaccharide (GLPS) has been identified as one of its major bioactive components, showing multiple pharmacological effects, such as immunomodulation, anti-oxidation, hepatoprotection, anti-proliferation, and anti-angiogenesis. Recently, many researchers have focused on the anti-tumor effects of GLPS and have obtained some promising data both *in vivo* and *in vitro*. In this review, the beneficial potential of GLPS as an anti-tumor agent is summarized and the underlying mechanisms involved are explored.

The Immunomodulatory Effects

It has been established that the immune system plays an important role in tumorigenesis, and immunotherapy of cancer is one of the strategies for oncotherapy (Baxevanis *et al.*, 2009; Loose *et al.*, 2009). Many types of cancer are antigenic and could be recognized by the immune system (Boon *et al.*, 1996). The immune system tumor interaction is represented by the concept of cancer immunoediting, which emphasizes that immunity may subserve either classical cancer immunosurveillance functions or promote the eventual outgrowth of immunoevasive cancer cells. Immune surveillance of the immune system is crucial in the detection and prevention of tumors. However, the immune system establishes selective pressures that shape and may even generate new variants that display an increase in tumorigenicity (Goyos *et al.*, 2009). Both *in vivo* and *in vitro* studies have demonstrated that B lymphocytes, T lymphocytes, dendritic cells (DCs), natural killer cells (NKs), and mononuclear phagocyte cells are responsible for generating anti-tumor immune responses.

The immunomodulatory effects of *G. lucidum* have been comprehensively summarized in previous reviews (Lin *et al.*, 2004; 2005a). Notably, *G. lucidum* also demonstrates immunocompetence in horses (Lai *et al.*, 2004). However, the material basis for these immunomodulatory effects has not been fully elucidated. Experimental data suggest that GLPS, as a class of the main bioactive components of *G. lucidum,* contributes to, at least in

part, the immunomodulatory effects of *G. lucidum*. However, such effects have not been fully examined due to the complicated structure and composition of GLPS.

Effect of GLPS on B Lymphocytes

B lymphocytes play a key role in humoral immune response by producing antibodies against antigens. They also act as antigen presenting cells, and they eventually develop into memory B cells after activation by antigen interaction.

Bao *et al.* isolated three polysaccharides, two heteroglycans (PL-1 and PL-4), and one glucan (PL-3) from the fruit bodies of *G. lucidum* by anion-exchange and gel-filtration chromatography. Immunological investigation suggests that all of these polysaccharides enhance the proliferation of T- and B-lymphocytes to some extent *in vitro*. PL-1 was found to be more effective than PL-3 and PL-4, which might be due to their structural difference. Further investigation in mice revealed that PL-1 stimulates the proliferation of T- and B-lymphocytes and the production of antibodies (Bao *et al.*, 2002). However, it had little effect on serum IgG and complement (C_3) levels (Bao *et al.*, 2002). Using successive chromatographic steps, Zhang *et al.* isolated a kind of GLPS, a proteoglycan with a carbohydrate:protein ratio of 11.5:1. It was found to stimulate the proliferation and activation of mouse spleen B lymphocytes by enlarging the B cells, increasing CD71 and CD25 expression on the cell surface, and enhancing the secretion of immunoglobulin. It also enhanced the expression of protein kinase C_α and protein kinase C_γ in B cells and slightly increased the production of IL-2 without affecting IL-4 secretion and intracellular Ca^{2+} concentration (Zhang *et al.*, 2002). Zhu *et al.* reported a GLPS with a molecular weight of 584,900 and a 93.61–6.49% ratio of polysaccharides to peptides. In cyclophosphamide-induced immunosuppressed mice, intraperitoneal injection of a low-dose GLPS (2.5 mg/Kg) once daily accelerated the recovery of bone marrow cells, red blood cells, white blood cells, splenic NKs, and natural killer T cells. T and B cell proliferation responses, cytotoxic T lymphocyte activity, and NK cell and lymphokine-activated killer (LAK) cell activity were also enhanced without any side effects (Zhu *et al.*, 2007).

The mechanisms underlying the activation of B cells by GLPS were unknown to a large extent. Shao *et al.* discovered that cell membrane Ig (mIg) and toll-like receptor 4 (TLR4) are required for GLPS-mediated B cell activation and that the latter is also involved in GLPS-mediated macrophage activation. They also identified a unique 31 kDa serum protein and two intracellular proteins (ribosomal protein S7 and a transcriptional co-activator) that can bind with GLPS in co-precipitation experiments (Shao *et al.*, 2004). Further studies found that the GLPS fraction of Reishi (Reishi-F3) causes mouse splenic B cell activation and differentiation to IgM-secreting plasma cells, and the process depends on the Reishi-F3-mediated induction of Blimp-1. In human peripheral B lymphocytes, Reishi-F3 fails to induce B cell activation. However, it can enhance antibody secretion, which is associated with Blimp-1 mRNA induction. This function of Reishi-F3 is TLR4/TLR2 dependent, and the interaction of Reishi-F3 with TLR4/TLR2 followed by signaling through p38 mitogen-activated protein kinase (p38 MAPK) is involved in the induction of Blimp-1 mRNA. Meanwhile, signaling through ERK, p38 MAPK, JNK, and IKK complex

is involved in Reishi-F3-mediated Ig secretion. Therefore, the different mechanisms of Reishi-F3 in mouse and human B cell activation are probably due to the presence of the Blimp-1 regulatory site in human CD86 promoter (Lin *et al.*, 2006a).

Effect of GLPS on T Lymphocytes

T cells belong to a group of white blood cells known as lymphocytes, and play multiple roles in cell-mediated immunity. These specialized cells respond to stimulation by DCs plus a peptide antigen. They can be distinguished from other lymphocyte types such as B cells and NK cells by the presence of a special receptor on their cell surface called T cell receptors. In horses, the pure mycelia of *G. lucidum* could increase the percentage of $CD5^+$, $CD4^+$, and $CD8^+$ T lymphocytes in peripheral blood lymphocytes (Lai *et al.*, 2004).

Cao *et al.* isolated a kind of polysaccharide peptide with a molecular weight of 584,900 from a boiling water extract of wood-cultured *G. lucidum*, followed by ethanol precipitation, dialysis, and protein depletion. The GLPS can increase mRNA expression of IFN-γ and protein expression of granzyme B. It can also promote the cytotoxicity of specific cytotoxic T-lymphocytes induced by DCs, which were pulsed with P815 tumor antigen during the stage of antigen presentation. The mechanism of cytotoxicity is assumed to be mediated through the IFN-γ and granzyme B pathways (Cao and Lin, 2003). Long-term (for 14 days) treatment of mice with an antler-shaped fruiting body of *G. lucidum* (Rokkaku-Reishi), which contains more than 40% β-D-glucan, can activate both T cells and splenic macrophages. β-D-glucan is the major ingredient of GLPS. Further, IFN-γ production by splenocytes in response to both lipopolysaccharide (LPS) and concanavalin A *in vivo* is also significantly increased (Kohguchi *et al.*, 2004). GLPS promotes the release of TNF-α and IFN-γ from T lymphocytes in a dose-dependent manner (Wang *et al.*, 1997).

Meanwhile, some studies showed that GLPS does not promote the proliferation of T cells. Zhang *et al.* (2002) demonstrated that GLPS is a new B cell-stimulating factor with a three to fourfold increase in the percentage of B cells and in the activation of mouse spleen B lymphocytes. In contrast, the secretion of IL-4 is not affected, suggesting that GLPS shows a slightly increased proliferation of T cells. Cao and Lin (2004) also found that a kind of GLPS peptide (GLPP) cannot promote T lymphocyte proliferation and neutral red phagocytosis of the peritoneal macrophages of nude mice.

Effect of GLPS on DCs

DCs are white blood cells with the function of collecting antigen from pathogens and host cells in tissues, and presenting multiple antigen samples to naive T-cells in the lymph node.

Cao *et al.* (2002) reported a GLPS comprised of D-rhamnose, D-xylose, D-fructose, D-galactose, D-mannose, and D-glucose with molar ratios of 0.793:0.964: 2.944: 0.167: 0.389: 7.94, which are linked together by b-glycosidic linkages. It can increase the co-expression of CD11c and I-A/I-E molecules on the DCs surface, promote IL-12p40 mRNA expression in DCs, and enhance the protein production of IL-12p40 in culture

supernatants. The lymphocyte proliferation of a mixed lymphocyte culture induced by mature DCs is also enhanced by this GLPS. It can promote not only the maturation of cultured murine bone marrow-derived DCs *in vitro* but also the immune response initiation induced by DC. Lin *et al.* (2005b) showed that treatment of DCs with GLPS results in enhanced cell-surface expression of CD80, CD86, CD83, CD40, CD54, and human leukocyte antigen-DR. Production of IL-12p70, p40, and IL-10 and IL-12p35, p40, and IL-10 mRNA expression are also enhanced, and the capacity for endocytosis is suppressed. This GLPS induced activation and maturation of human monocyte-derived DCs are mediated by the NF-κB and p38 MAPK pathways.

GLPS can also stimulate the maturation of monocyte-derived DCs. GLPS and GM-CSF/IL-4 simultaneous treatment induces the transformation of THP-1 cells into typical DC morphology, whereas GLPS alone induces only proliferative response in THP-1 and U937 cells. Furthermore, the transformation of THP-1 DCs results in a significant increase in the expression of HLA-DR, CD40, CD80, and CD86 and in similar antigen-uptake capability. However, it has less potency in inducing allogeneic T cell proliferation (Chan *et al.*, 2008).

Gene chip microarray analysis of human monocyte-derived DCs demonstrated that purified GLPS decreases the genes associated with phagocytosis (CD36, CD206, and CD209) and increases those associated with pro-inflammatory chemokines (CCL20, CCL5, and CCL19), cytokines (IL-27, IL-23A, IL-12A, and IL-12B), and co-stimulatory molecules (CD40, CD54, CD80, and CD86). This was confirmed by examining the effect of PS-G on antigen-specific antibodies and cytokine production in BALB/c mice. Therefore, GLPS can effectively promote the activation and maturation of immature DCs, preferring a Th1 response (Lin *et al.*, 2006b).

Effect of GLPS on Macrophages

Macrophages help to destroy bacteria, protozoa, and tumor cells. They also release substances that stimulate other cells of the immune system and cells involved in antigen presentation by carrying the antigen on their surface and presenting it to T cells.

A kind of GLPS peptide with an average molecular weight of 513,000, which contains 16 kinds of amino acids, protects mice peritoneal macrophages injury induced by reactive oxygen species (You *et al.*, 2002). Stimulation of mouse macrophages with alkali-extracted GLPS from the spore of *G. lucidum* can increase macrophage volume and its capability to phagocytize latex beads (Tang *et al.*, 2004). A purified proteoglycan fraction from *G. lucidum* significantly enhanced the proliferation of bone marrow macrophages in a dose-dependent manner. RAW264.7 derived macrophages were enlarged and formed a pseudopodia after GLPS exposure. The NO production, cellular respiratory burst activity, and gene expressions of IL-1β, IL-12p35, and IL-12p40 were also significantly increased (Ji *et al.*, 2007).

Recently, Wei *et al.* (2007) found that GLPS not only enhances the J774A.1 macrophage surface expression of TLR4 and CD14 as well as LPS binding and phagocytosis internalization, but it also reduces the adhesion time constant and increases the force

constant of the binding interaction. Treatment with F3, an active ingredient of GLPS, increased the population of $CD14^+ CD26^+$ monocyte/macrophage in human umbilical cord blood mononuclear cells (Chien *et al.*, 2004). Dynamic gene expression profiles showed that F3-treated THP-1 cells exhibit enhanced macrophage differentiation as demonstrated by changes in cell adherence, cell cycle arrest, nitroblue tetrazolium reduction, and expression of differentiation markers including CD11b, CD14, CD68, MMP-9, and myeloperoxidase, which might be mediated by caspase cleavage and p53 activation (Hsu *et al.*, 2009).

In trans-retinoic acid-treated HL-60 cells, GLPS can enhance the phagocytic activities of human primary neutrophils and differentiate neutrophilic-phenotype cells. Furthermore, GLPS efficiently inhibits spontaneously and Fas-enhanced neutrophil apoptosis and increases neutrophil migration. Pharmacological inhibitors assay demonstrated that the capabilities of GLPS to enhance neutrophil function in phagocytosis and chemotaxis are mediated by PI3 K, p38 MAPK, Src tyrosine kinases, and PKC (Hsu *et al.*, 2003).

Effect of GLPS on NKs

NKs are small lymphocytes that originate in the bone marrow and develop fully in the absence of the thymus. NKs look for a "banner" flown by normal cells. If the NK cell recognizes the "banner," it spares that cell. If the "banner" is absent, the NK cells attach to the target cells and release a burst of chemicals that penetrate the wall of the target cell, causing it to break up. NKs can kill a wide range of cancer cells and so are promising tools for cell therapy in cancer. NK cell cytotoxicity is regulated by a balance between stimulatory and inhibitory signals (Cho *et al.*, 2009).

Chien *et al.* (2004) demonstrated that a fucose-containing glycoprotein fraction (F3) isolated from the water-soluble extracts of *G. lucidum* stimulates the activity of $CD56^+$ NKs in cord blood. After F3 treatment, NKs mediated cytotoxicity was found to be significantly enhanced. The polysaccharide component with a branched $(1 \rightarrow 6)$-β-D-glucan moiety of *G. lucidum* (PS-G) has also been reported to exert anti-tumor activity and to activate NKs (Lin *et al.*, 2005b).

Recently, it was reported that GLPS could significantly decrease the amount of cytokine in LAKs and cytokine-induced killer cell culture. However, it showed no significant effect on the proliferation, cytotoxicity, and phenotype of LAKs or of cytokine-induced killer cells induced by cytokines at higher doses alone. This activity of GLPS can mostly be blocked by anti-CR3 (Zhu *et al.*, 2007).

In summary, present studies demonstrated that GLPS is a potent immunomodulator that exerts a significant and comprehensive impact on immune cells including B lymphocytes, T lymphocytes, NKs, macrophages, and DCs. These immunodulatory effects are likely to have been mediated by its complex multiple components and can be one of the underlying anti-tumor mechanisms of *G. lucidum* to some extent. The potential effects of GLPS on immune cells are summarized in Fig. 1.
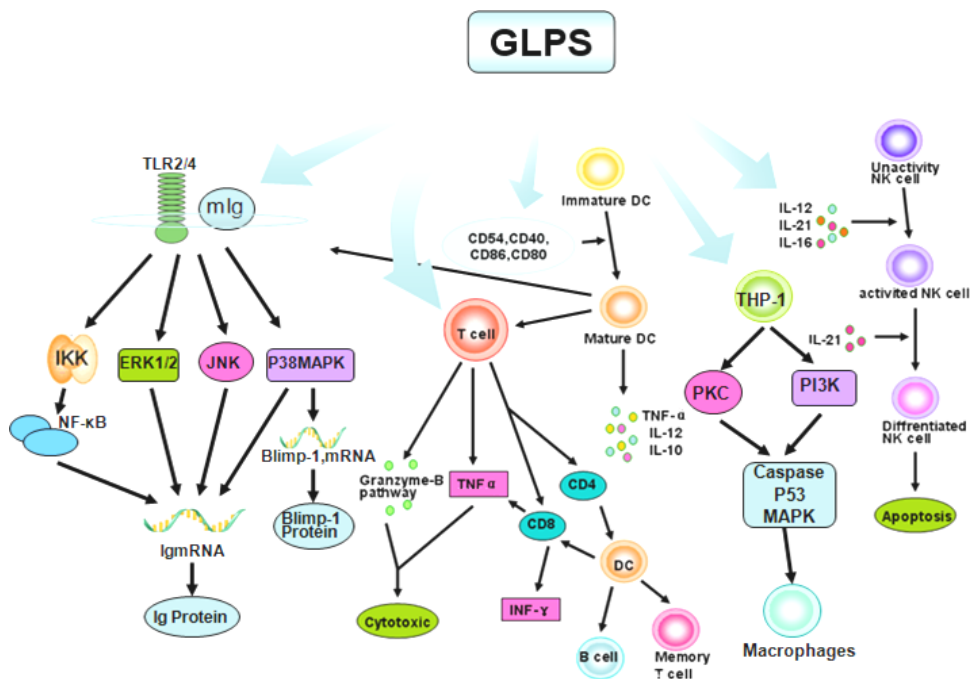
Figure 1. The immunomodulatory effects of GLPS on immune cells. GLPS exerts an obvious impact on immune cells. It stimulates B cell proliferation and activation, promotes T cell release of TNF-α and interferon-γ, enhances activation and maturation of immature DC, promotes macrophage differentiation and maturation, and sensitized NK cell-mediated cytotoxicity. These effects are mediated through different signaling pathways, which might be due to the multiple components of GLPS. GLPS, *G. lucidum* polysaccharide; TLR2/4, Toll-like receptor 2/4; DCs, dendritic cells.

The Anti-Angiogenetic Effects

A solid tumor forms an organ-like entity comprised of neoplastic cells and non-transformed host stromal cells embedded in an extracellular matrix. Similar to normal tissues, blood vessels nourish the cells residing in tumors. However, unlike normal blood vessels, tumor vasculature has an abnormal organization, structure, and function. The angiogenesis has become an innovative and identified target in cancer therapy. A GLPS peptide was isolated from the fruiting body of *G. lucidum* (*Leyss ex Fr*) Karst (*Gl*). It directly inhibited human umbilical vein endothelial cells (HUVECs) proliferation *in vitro*, but it did not affect PG cell (a human lung carcinoma cell line) proliferation when it was added directly to the cultured medium. Notably, the serum collected from tumor-bearing mice after GLPS peptide treatment for 33 days showed inhibition of PG cell proliferation clearly. Chick chorioallantoic membrane (CAM) assay revealed that GLPS peptide and GLPS peptide treated serum showed anti-angiogenic effect. Furthermore, GLPS peptide dramatically reduced the xenograft (human lung carcinoma cell PG) in BALB/c nude mice *in vivo*, suggesting that

GLPS has anti-angiogenic effects (Cao *et al.*, 2004). The same group also found that the anti-proliferative effect of the GPLS peptide on HUVECs is due to its pro-apoptotic action, which might be mediated by the reduction of the anti-apoptotic protein Bcl-2 expression and by the increase of the pro-apoptotic protein Bax expression. Its downregulation of VEGF secretion might also contribute to this action (Cao *et al.*, 2006). In addition, culture soybean extracts with *G. lucidum* mycelia produced a cultivated product called genistein combined polysaccharide, which inhibited angiogenesis in CAM and the formation of new vessels induced by colon carcinoma cells *in vivo* (Miura *et al.*, 2002). Triterpenoid, another active ingredient of *G. lucidum*, was also found to possess anti-tumor and anti-angiogenic activities (Kimura *et al.*, 2002).

The Anti-Proliferation Effects

Uncontrolled proliferation is one of the characteristics of most tumors. In the last decade, research indicated that GLPS is not only a natural product that possesses potent functions in the immune system but also acted as an adjuvant drug suppressing the proliferation of tumor cells both *in vivo* and *in vitro* directly.

The GLPS peptide inhibits the proliferation of HUVECs in a dose-dependent fashion. Flow cytometry studies showed that it induces HUVECs apoptosis directly by decreasing anti-apoptotic protein Bcl-2 expression and increasing pro-apoptotic protein Bax expression (Cao *et al.*, 2006). In THP-1 cells, F3 mimics induces death receptor ligands such as TNF-α and TRAIL to initiate signaling *via* death receptor oligomerization, recruitment of specialized adaptor proteins, and activation of caspase cascade, followed by cell shrinkage and apoptosis (Cheng *et al.*, 2007). Recently, GLPS has been reported to inhibit the mouse melanoma cells of B16F10 proliferation *in vitro* (Man *et al.*, 2009).

Incubation of Ganopoly, an aqueous polysaccharide fraction extracted from *G. lucidum*, at 0.05–1.0 mg/ml for 48 hours showed a small or negligible cytotoxicity against human tumor CaSki, SiHa, Hep3B, HepG2, HCT116, HT29, and MCF7 cell lines *in vitro*. In contrast, 10 mg/ml of Ganopoly caused a significant cytotoxicity in all tumor cells tested, except for MCF7 cells, with marked apoptotic effects observed in CaSki, HepG2, and HCT116 cells (Gao *et al.*, 2005a).

However, some studies showed that GLPS cannot inhibit cell proliferation *in vitro*. GLPS with cytokine-containing mononuclear cell-conditioned media (PSG-MNC-CM) suppresses the proliferation and clonogenicity of both HL-60 and U937 leukemic cell lines. On the contrary, the GLPS peptide alone shows no such effects even at a higher dose of 400 mg/ml (Cao *et al.*, 2003). In a co-culture of normal R6 cells and R6/GFP-Ras cells (stable transformed R6 cells expressing green fluorescent protein-ras fusion protein), GLPS peptide shows no effect on the growth of individually cultured normal and transformed R6 cells, as well as the level or subcellular localization of the Ras protein (Hsiao *et al.*, 2004).

A GLPS peptide dose-dependently inhibited cell migration by inhibition of MMP9 expression and activity but showed no inhibitory effect on cell proliferation in human lung carcinoma cell (PG cell) (Qi-zhen *et al.*, 2007). Furthermore, GLPS also shows no cytotoxic effects on human prostate carcinoma PC-3M cells or on the proliferation of

HUVECs but significantly inhibited PC-3M adhesion to and migration through HUVECs (Ying-bo *et al.*, 2008).

The in vivo Anti-Tumor Effects

G. lucidum is widely prescribed by traditional Chinese physicians as the "Jun" herb (君药) in many medical formulas for tumor therapy. However, the real contribution of *G. lucidum* in the beneficial effects of these formulas is rather difficult to evaluate, and the underlying mechanisms involved are far from clear. The *in vivo* anti-tumor effects of GLPS provide some convincing evidence.

GLPS administration can inhibit tumor growth in S180 ascitic tumor-bearing mice. Intragastric administration of GLPS (100, 200, and 400 mg/kg) for nine consecutive days significantly decreases the tumor weight in a dose-dependent manner (Li *et al.*, 2008). Oral administration of Ganopoly for ten days with GLPS significantly reduces the tumor weight of sarcoma 180 in a dose-dependent manner, with inhibition rates of 32.3%, 48.2%, and 84.9% and with growth delays of 1.5, 3.5, and 13.1 days at 20, 50, and 100 mg/kg, respectively. The potential mechanism involved might include the increased serum IFN-γ levels (Li *et al.*, 2008), the enhanced TNF-α, IFN-γ expression, and cytotoxic T lymphocyte cytotoxicity and NK after GLPS treatment (Gao *et al.*, 2005a). The regulation of cell cycle and inhibition of DNA and RNA synthesis might also contribute to this effect (Jian-Jun *et al.*, 2007). A GLPS from *G. lucidum* aqueous extract demonstrated antitumor activities and a satisfactory tolerability after oral administration in different tumor strain lines (Ca755, s/c P388, s-180)-bearing mice. In addition, the aqueous extract of *Hericium erinaceus*, *Lentinus edodes*, and GLPS showed synergic effect in the inhibition of tumor growth (Bukhman *et al.*, 2007).

In addition, when applied in combination with chemotherapeutic drugs, GLPS exhibited positive effects. Treatment S180 ascitic tumor-bearing mice with beta-$(1 \rightarrow 6)$-branched beta-$(1 \rightarrow 3)$ glucohexaose, a hexaose isolated from GLPS, not only substantially increased the inhibition of S180 for chemotherapeutic agent cyclophospamide (CPA) but also decreased the toxicity caused by CPA (Ning *et al.*, 2003).

In some clinical studies, GLPS was reported as an adjuvant drug to treat patients with advanced cancer. However, the real effects and potential mechanisms need further study to evaluate and elucidate. In advanced-stage cancer patients, treatment with Ganopoly (1800 mg, three times daily orally before meals for 12 weeks) resulted in a significant increase in the mean plasma concentrations of IL-2, IL-6, and IFN-γ and a dramatic decrease in the plasma levels of IL-1 and TNF-α. The mean absolute numbers of CD56$^+$, CD3$^+$, CD4$^+$, and CD8$^+$ cells were significantly increased, whereas the CD4:CD8 T cell ratio was unchanged. Ganopoly treatment also enhanced phytohemagglutinin (PHA) responses and NK cell activities (Gao *et al.*, 2003). However, the same group also reported that the same treatment of similar patients with Ganopoly did not significantly alter the mean mitogenic reactivity to PHA; mean counts of CD3, CD4, CD8, and CD56; mean plasma concentrations of IL-2, IL-6, and IFN-γ; or NK activity. The results were significantly variable, but some cancer patients demonstrated markedly modulated immune functions. Changes in IL-1 were correlated with those in IL-6, IFN-γ, CD3, CD8, and NK

activity, and changes in IL-2 were correlated with those in IL-6, CD8, and NK activity. Therefore, some subgroups of cancer patients might be responsive to Ganopoly in combination with chemotherapy or radiotherapy. When used alone or in combination with chemotherapy or radiotherapy in lung cancer patients, the efficacy and safety of Ganopoly need to be examined further (Gao *et al.*, 2005b).

In conclusion, tumor therapy remains a serious challenge for oncologists worldwide. The therapeutic benefits of complementary and alternative medicines on tumor are now gradually being accepted by physicians. As a kind of dietary mushroom, *G. lucidum* is a popular food supplement in Asia, especially in China, Korea, and Japan. Recent pharmacological evaluation of *G. lucidum* provided strong evidence to support its nutritional value and beneficial effects on many kinds of diseases, especially cancer (Sliva, 2003; Yuen *et al.*, 2005; Mahajna *et al.*, 2009).

GLPS is one of the key bioactive ingredients of *G. lucidum*, which possesses extensive immunomodulatory activities including promotion of the function of B lymphocytes, T lymphocytes, DCs, and NKs. Both humoral immunity and cellular immunity were motivated by GLPS affecting the expression of cytokines such as IL, TNF-*a*, INF-γ, and so on. In the past few years, some fractions extracted from GLPS were also found to have anti-angiogenic and anti-proliferative activities, which contributed to its anti-tumor mechanisms. In traditional Chinese medicine, *G. lucidum* is one of the most widely prescribed herbs for the treatment of tumors or related diseases in combination with chemical drugs or other herbs. In view of the supporting evidence on the role of GLPS in cancer therapy, the antitumor potential of *G. lucidum* or its products should not be underestimated. However, more direct and scientific evidence needs to be obtained in further studies.

Acknowledgments

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