Ganoderma lucidum – mecanismos de suas atividades antitumoral e imunoregulatória

Anti-tumor and immunoregulatory activities of *Ganoderma lucidum* and its possible mechanisms

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ABSTRACT

Ganoderma lucidum (*G lucidum*) is a medicinal fungus with a variety of biological activities. It has long been used as a folk remedy for promotion of health and longevity in China and other oriental countries. The most attractive character of this kind of medicinal fungus is its immunomodulatory and anti-tumor activities. Large numbers of studies have shown that *G lucidum* modulate many components of the immune system such as the antigen-presenting cells, NK cells, T and B lymphocytes. The water extract and the polysaccharides fraction of G *lucidum* exhibited significant anti-tumor effect in several tumor-bearing animals mainly through its immunoenhancing activity. Recent studies also showed that the alcohol extract or the triterpene fraction of G *lucidum* possessed anti-tumor effect, which seemed to be related to the cytotoxic activity against tumor cells directly. Preliminary study indicated that antiangiogenic effect may be involved antitumor activity of *G lucidum.*

INTRODUCTION

The *fungi Ganoderma lucidum* (Leyss ex fr) Karst (Lingzhi) has been used for long time in China to prevent and treat various human diseases such as bron-chitis, hepatitis, hypertension, tumorigenic diseases, and immunological disorders[1]. Ancient Chinese medical scholars suggested that *G lucidum* could strengthen body resistance and consolidate the constitution of patients, ie, "Fuzheng Guben", which is one of the major principles in the therapeutics of traditional Chinese medicine[1]. Modern pharmacological and clinical investigations demonstrated that *G lucidum* had anti-tumor and immunomodulatory activities. Its anti-tumor and immunomodulatory properties, along with low cytotoxi-city, raise the possibility that it could be effective in the cancer patients receiving conventional chemotherapy and/or radiation treatment, to build up

immune resistance and decrease toxicity. The potential clinical value and wide acceptability of *G lucidum* have attracted intense interest in the search for its pharmacological component. Previous data have reported that *G lucidum* extract, *Ganoderma* polysaccharides and *Ganoderma* triterpenoids possessed anti-tumor and/or immuno-modulatory effects. A number of reports have demonstrated that *G lucidum* polysaccharides stimulated immune function both *in vivo* and *in vitro*. And the anti-tumor effect of *G lucidum* was supposed to be the results of its immune-related mechanism or possible direct cytotoxic activity mechanisms[2].

IMMUNOMODULATORY ACTIVITY AND MECHANISMS OF *G LUCIDUM*

There is a general consensus that the immuno-modulating effects of *G lucidum* were extensive, including promoting the function of antigen-presenting cells, mononuclear phygocyte system, humoral immunity and cellular immunity, and the action site of *G lucidum* was speculated to be located in the course of proliferation and differention of immune precursor cells to effector cells.

Ganoderma lucidum (Leyss ex Fr) Karst: inmatured (A); matured (B).

Effect of *G lucidum* on the function of mononuclear phagocyte system Lin *et al* found that the water extracts of the fruiting bodies of *G lucidum* and *G lucidum* polysaccharides D6 administrated ig significantly enhanced the phagocytosis of chicken red blood cells by the peritoneal macrophages in mice[3]. Gu *et al* reported that an injection prepared from mycelia of *G capense*, when cultured in the concentration of 5-20 mg/L with mouse peritoneal macrophages over 24 h could enhance the phagocytosis of neutral red and increased the content of lysozyme in the macrophages. In addition, water extract of *G capense* synergistically promoted the lipopolysaccharide-stimulated interleukin-1 (IL-1) release from macrophages[4]. Treatment of mice with water extract from *G lucidum* spores by sc injection resulted in a considerable increase in the activities of lysozyme, acidic phosphatase, and β-glucoronidase and promoted the formation of H_2O_2, indicating that the water extract from *G lucidum* spores is able to activate macrophages[5]. Gao and Yang provided evidence that *G applanatum* stimulated IL-1 like substance secretion from macrophages *in vitro*[6]. Li demonstrated that IL-1a and tumor necrosis factor (TNF-α) production was significantly increased by mouse peritoneal macrophages treated with *Ganoderma* polysacchaides[7]. Berovic *et al* also reported that one polysaccharide isolated from *G lucidum* which were mainly composed of beta-*D*-glucanes could induce TNF-α synthesis in primary cultures of human peripheral blood mononuclear cells (PBMC)[8]. Further studies also showed that the addition of *G lucidum* polysaccharides (25-400 g/L) to the *in vitro* macrophages culture media, resulted in an significantly increased TNF-α mRNA expression in a concentration-dependent manner[9]. Following the administration of crude *G lucidum* extract (GLE) at 5, 10, and 20 g/kg by forced stomach tube feeding, TNF-α mRNA expression in the peritoneal macrophages was increased markedly[10]. These results indicate that the water extract and the polysaccharides fraction of *G lucidum* could induce TNF-α expression *in vivo* and *in vitro*. *G lucidum* could decrease the production

of free radicals and increase the intracellular level of free calcium in the peritoneal macrophages[11,12]. *Ganoderma* polysaccharides also increased the production of cAMP in a concentration- and time-dependent manner in murine peritoneal macrophages[13]. A recent study revealed that exposure of human neutrophils to *G lucidum* polysaccharides time-dependently caused increases in protein kinase C (PKC), p38 mitogen-activated protein kinase (MAPK), hematopoietic cell kinase (HCK) and another tyrosine kinase Lyn activities, these maybe the action that corresponded to an enhanced unspecific immune function[14]. Hsu *et al* recently reported that *G lucidum* was able to enhance phagocytic activity and migration of human primary neutrophils, and inhibit spontaneous and Fas-induced neutrophil apoptosis *in vitro* primarily relied on activation of Akt-regulated signaling pathways[15].

Effect of *G lucidum* on maturation and function of dendritic cells and NK cells Dendritic cells (DC), a kind of professional antigen-presenting cells, are pivotal for initiation of primary immune response. Recently, Cao and Lin have shown that *G lucidum* polysaccharides (*Gl*-PS) at the concentration of 0.8, 3.2, and 12.8 mg/L could increase the co-expression of CD11c and I-A/I-E molecules on DC surface, promote mRNA expression of cytokine IL-12 p40 in DC, and augment protein production of IL-12 p40 in culture supernatants. The lymphocyte proliferation of mixed lymphocyte culture (MLC) induced by mature DC was also enhanced by *Gl-PS*. These data demonstrated that *G lucidum* polysaccharides was shown to promote not only the maturation of cultured murine bone marrow derived DC *in vitro*, but also the immune response initiation induced by DC[16]. Further data showed that *Gl*-PS was able to promote the cytotoxicity of specific cytotoxic T lymphocytes (CTL) induced by DC during the stage of antigen presentation mainly through IFN-γ and granzyme B pathways[17].

Chien *et al* reported that treatment with the water-soluble extract of *G lucidum* (F3) could increase the presence of the natural killer cells (CD56(+) marker) significantly from 1.1 % to 3.2 % in UCB mononuclear cells, indicating that F3 quantitatively influenced NK cells activities[18].

Effect of *G lucidum* on the T lymphocytes The cell-mediated immune function was also enhanced by *G lucidum*, as suggested by the observations that *G lucidum* promoted the mixed lymphocyte reaction (MLC)[19,20]. It also exerted an increasing effect on the induction of delayed hypersensitivity to protein antigen. BN3A, BN3B, and BN3C, three kinds of *G lucidum* polysaccharides, significantly increased the lymphocyte proliferation induced by ConA and the IL-2 production in the normal mice, as well as in the aged mice *in vitro*. BN3A and BN3C also could antagonize the suppressive effect of hydrocortisone on the proliferation of mouse spleen cells[21]. Further study showed that *G lucidum* polysaccharides increased the DNA synthesis of spleen cells in MLC through the enhancement of DNA polymerase induction in the young and aged mice[20]. It was found that *G lucidum* polysaccharides not only increased the contents of nuclear DNA and RNA but also remarkably changed the cell ultrastructure in the murine splenocytes[22]. Moreover, *G lucidum* increased the production of IFN-γ and significantly increase IFN-γ mRNA expression in the T-lymphocytes[9]. *G lucidum* also was effective in repairing the damage of subset T-cells in the spleen of gamma-irradiated mice[23].

Effect of *G lucidum* on the B lymphocytes The plaque forming cells (PFC) response is a specific method to examine the effect of medicine on the animal's humoral immune function. *Ganoderma* polysaccharides (BN_3C) ip injection promoted PFC response to the sheep red blood cells (SRBC) not only in the normal mice but also in the aged mice[21]. *In vitro*, *G lucidum* polysaccharides also significantly increased the lymphocyte proliferation induced by LPS[24,25]. A bioactive fraction(GLIS), isolated from the fruiting body of *G lucidum* could stimulate the activation, proliferation, differentiation of B lymphocyte. The B lymphocytes were enlarged, expressed CD71 and CD25 on the cell surface, and showed an increase in the secretion of immuno-globulin. Furthermore, the activation of B lymphocytes by GLIS did not depend on the activation of T lymphocytes. It was associated with stimulation of the expression of protein kinase C alpha and protein kinase C gamma in B lymphocytes by GLIS directly. However GLIS did not influence the intracellular Ca^{2+} concentration of lymphocytes. According to these results, it showed that GLIS was a new B cell-stimulating factor [26]. It has been indicated that *G lucidum* polysaccharides (in particular active β-*D*-glucans) could bind to lymphocyte surfaces through specific receptors or serum-specific proteins, leading to alteration of the activities of macrophages, T-helper, NK cells, and other effector cells. These maybe gave some explanation on the phenomenon why the immuno-modulating effects of *G lucidum* were so extensive[27]. A preliminary investigation indicates that the effect of ingestion of *G lucidum* mycelium on gut humoral immunity was investigated using mice as an animal model. The oral immunization protocol used in this study elicited an anti-cholera toxin (CT)-antibody response. The consistent outcome of low specific anti-CT IgA level in luminal contents of small intestine, fecal pellets and serum suggest that *G lucidum* mycelium depressed mucosal IgA responses in orally immunized young adult mice. However, this study did not provide information on the component(s) in *G lucidum* mycelium that were active in depressing the specific IgA antibody response in mice[28].

Other immunomodulatory effect of *G lucidum* Most of the studies demonstrated that *G lucidum* possessed the immune-enhancing action, while some other studies showed that *G lucidum* also could down-regulate the excessive immune function. It appears that the cytokines-modulating effect of *G lucidum* polysaccharides would be tissue-specific. *G lucidum* polysaccharides had potent healing effect on indomethacin-induced gastric lesions in the rat due partly to the suppression of gene expression of TNF-α[29]. Application of *G lucidum* polysaccharides also significantly mitigated hepatic tumefaction, decreased ALT enzyme release, and NO production in serum or supernatant, improved the pathological changes of chronic and acute inflammation in the BCG-induced immune liver injury in mice. Moreover, the immunohistochemical result showed that *G lucidum* polysaccharides inhibited iNOS protein expression in BCG-immune hepatic damage model[30]. The triterpenoids isolated from *G lucidum* also showed significant protective effects against immunological liver damage induced by BCG plus LPS in mice both *in vivo* and *in vitro*[31]. Recently the study from our lab demonstrated that *G lucidum* polysaccharides ip injection could decrease the serum glucose level and the prevalence of diabetes in the multiple low dose streptozotocin-induced autoimmune diabetes[32]. Kino *et al* reported that LZ-8, an immunomodulatory lectin isolated from *G lucidum*, had immunosuppressive activity *in vivo*. Intraperitoneal administration of LZ-8, twice weekly into the mice (8 and 12 mg/kg) greatly prevented the production of antibody to HBs Ag with the inhibition rate of 83.3 % and 96.8 % respectively in C57BL/10 and C57BL/10BR mice[33]. Similarly, a polysaccharide with a molecular weight of 1.26×10^5, obtained from the sporoderm-broken spores of *G*

lucidum was found to have a strong suppressing effect on the antibody production and the Con A or LPS induced lymphocyte proliferation in mice[34]. In a pilot study, New Zealand Black/White (B/W) F1 lupus mice were fed with *Ganoderma tsugae* extract (the major components consisted of polysaccharide, nucleotide, tripenoids, and Ling-Zhi-8 identified by HPLA analysis) in an equivalent way to that used by patients for systemiclupus erythematosus. It was found that *Ganoderma tsugae* alone showed a therapeutic advantage compared with lupus control. *Ganoderma tsugae* improved the survival rate of lupus mice, increased body weight, and decreased the amount of proteinuria, decreased serum levels of anti-dsDNA autoantibody in B/W F1 mice. Pathology findings in lung, kidney, and liver tissues showed that *Ganoderma tsugae* decreased perivascular and parenchyma mononuclear cell infiltration[35].

ANTI-TUMOR ACTIVITY AND MECHANISMS OF *G LUCIDUM*

Anti-tumor activity of *G lucidum* in tumor-bearing mice In the past 30 years, the hot water extract or alcohol extract of *G lucidum* have been shown to inhibit the growth of Sarcoma 180 in mice, of fiblasarcoma in C3H mice and of azoxymethane- inducted colon cancers in male F344 rats[36-42]. Both the water extract of *G lucidum* and *G lucidum* polysaccharides inhibited the growth of S-180 in a dose-dependent manner[39,40,43]. Co-administration of *G lucidum* polysaccharides potentiated the anti-tumor activity of cyclophosphamide in mice. Inhibitory rate was significantly higher than those in the groups treated with polysaccharides or cyclophosphamide alone[10]. Hu and Lin found that the polysaccharides isolated from mycelia of *G lucidum* at 50 and 100 mg/kg inhibited the growth of S-180 in Balb/c mice and Kunming mice, with an inhibitory rates of 37.8 %-78.1 %[43]. *G lucidum* polysaccharides was also able to prolong the life-span of Lewis carcinoma-implanted C57BL/6 mice and promote anti-tumor activities of cytotoxic drugs and chemical immunomodulators[44]. The triterpenoid fraction (100 and 200 mg/kg) of the fruit bodies of *G lucidum* also inhibited not only the primary solid-tumor growth in the spleen and liver metastasis but also the secondary metastatic tumor growth in the liver in Lewis lung carcinoma (LLC)-implanted mice[45]. Recently, it was reported that both the lipids extracted from the germinating spores and the sporoderm-broken spores of *G lucidum* had remarkable anti-tumor effects in a dose-dependent manner, and could significantly inhibit mouse hepatoma, sarcoma S-180 with an inhibition of 80 %-90 %[46]. These results indicate that either *G lucidum* or its active component has anti-tumor activity in mice *in vivo*, and *Ganoderma* polysaccharides have synergic effect on the anti-tumor activity of cytotoxic drug such as cyclophosphamide.

Antitumor mechanisms of water extract and polysaccharides isolated from *G lucidum* Although the anti-tumor activity of *G lucidum* has been documented for a long time, the real mechanisms underlying this therapeutic effect still awaits to be elucidated. Does it elicit this effect through cytotoxic activity directly or through other pathways? First, the addition of either *G lucidum* water extract or *G lucidum* polysaccharides to the cultures of S-180 or HL-60 tumor cells directly had no inhibitory effect against the proliferation and apoptosis of tumor cells, even at the very high concentration such as 400 mg/L of *G lucidum* polysaccharides[39,40,43]. These results suggest that mechanisms other than direct cytotoxicity may be involved in the anti-tumor activity of *G lucidum*.

Results from the effects of Ganopoly (a *G lucidum* extract) on the immune functions in thirty four advanced-stage cancer patients revealed that treatment with 1800 mg

Ganopoly, three times daily orally for 12 weeks resulted in a significant increase in the mean plasma concentrations of some cytokines including IL-2, IL-6, and IFN-γ. PHA responses and natural killer activity after 12-week treatment with ganopoly were enhanced in most patients, when compared to pretreatment baselines[47]. Using serologic pharmacology method, after addition of *G lucidum* extract-treated serum to the *in vitro* S-180 culture media and the results showed that *G lucidum* extract-treated serum could inhibit proliferation of S-180 cells and induced their apoptosis *in vitro*[38]. Similarly, *G lucidum* polysaccharides β-treated serum also inhibited proliferation of HL-60 cells and induced apoptosis in these cells[39,44,48]. These results suggest that *G lucidum* extract or *G lucidum* polysaccharides β-treated serum may have the substances with anti-tumor activity.

What active substances are in the serums? TNF-α and IFN-γ are known to play important roles in suppressing tumor cell growth and inducing apoptosis of many different kinds of tumor cells. Many studies have shown that TNF-α and IFN-γ work together in inducing tumor cell apoptosis. They are also the endogenous active products by stimulating effect of *G lucidum* or *G lucidum* polysaccharides on immune system *in vivo*. Therefore, according to the results mentioned above, *G lucidum* extract or *G lucidum* polysaccharides β-treated serum may be associated with these two cytokines. To certify this speculation, the TNF-α activity and IFN-γ content in serum were detected. The results showed that the activity of TNF-α in serum treated with *G lucidum* extract 5, 10, and 20 (crude material) g/kg or *G lucidum* polysaccharides B 50, 100, and 200 mg/kg were increased by 18.3 %-40.1 % or 14.1 %-28.1 % respectively and the content of IFN-γ in serum treated with *Ganoderma* extract or *G lucidum* polysaccharides B were increased 3-7 or 4-8 folds respectively[39,40].

Next step is to study the effect of *G lucidum* polysaccharides on cytokines production by T lymphocytes and macrophages, and the effect of *G lucidum* polysaccharides β-conditioned medium with T lymphocytes or macrophages on proliferation and apoptosis of tumor cells. A pure population of macrophages or T lymphocytes was incubated with or without various concentrations of *G lucidum* polysaccharides B for 12-72 h, which were called macrophage culture medium with *G lucidum* polysaccharides B (GL-B-M-CM) and T lymphocyte culture medium with *G lucidum* polysaccharides B (GL-B-M-CM). At either concentration GL-B-M-CM and GL-B-M-CM significantly inhibited the HL-60 cells proliferation and induced apoptosis of HL-60 cells *in vitro*[39, 40]. Similar results were observed that conditioned medium with the polysaccharides isolated from mycelia of *G lucidum*-activated splenocytes or macrophages markedly induced HL-60 apoptosis[39,43,48].

The TNF-α level in the supernatant of 12.5-400 mg/L *G lucidum* polysaccharides B cultured with macrophages rised during 24 h as the dose increased. Similarly, the IFN-γ level in the supernatant of 12.5-200 mg/L *G lucidum* polysaccharides B cultured with T lymphocytes was increased during 24 h as the dose was increased to 400 mg/L. Moreover, it also indicated that there was a positive correlation between the level of TNF-α in GL-B-M-CM and IFN-γ in GL-B-M-CM and the anti-tumor effect of GL-B-M-CM and GL-B-M-CM[39]. The results also found that at the dose of 12.5, 50, and 200 mg/L, the macrophage culture medium with polysaccharides isolated from mycelia of *G lucidum* inhibited proliferation of HL-60 cells and induced its apoptosis significantly; with an increased TNF level in the cultured supernatant[48]. These results together with those from other laboratories suggest that all of these cytokines may be involved in the

anti-tumor effect of *Ganoderma* polysaccharides *in vivo*. The subsequent results showed that the addition of *G lucidum* polysaccharides (50-200 g/L) to the *in vitro* macrophages or T-lymphocytes culture media, resulted in an significantly increased TNF-α and IFN-γ mRNA expression in a concentration-dependent manner[10]. Following the administration of the water extract of *G lucidum* at 5, 10, and 20 g (crude material)/kg by forced stomach tube feeding, TNF-α and IFN-γ mRNA expression was increased markedly[40]. These results indicate that the water extract or the polysaccharides fraction of *G lucidum* could induce TNF-α and IFN-γ mRNA expression *in vitro* and *in vivo*.

Sliva *et al* reported that the spores or fruiting body of *G lucidum* inhibited cell migration of highly invasive breast cancer MDA-MB-231 cells and prostate cancer PC-3 cells. Because the inhibition of cell motility is directly linked to the inhibition of the signaling pathway, further results showed that *G lucidum* also inhibited constitutively active transcription factors AP-1 and NF-kB in MDA-MB-231 cells and PC-3 cells. It is of particular interest because recent studies suggested that AP-1 and NF-kB were potential targets for cancer treatment[48]. It has also been suggested that the urokinase-type plasminogen activator (uPA) and the uPA receptor (uPAR) played a crucial role in cancer metastasis. uPA can stimulate cell migration directly through its proteolytic activity by activating transforming growth factor-β (TGF-β) and fibroblast growth factor (FGF). The further evidences also showed that *Ganoderma* could inhibit the expression of uPA and uPAR, as well as the secretion of uPA, which resulted in the suppression of the migration of MDA-MB-231 and PC-3 cells[49].

Recently, we observe the ability of *G lucidum* polysaccharides peptide (GLPP) to inhibit in *vivo* angiogenesis using the chick chorioallantoic membrance (CAM) assay. There was potent inhibition of angiogenesis with GLPP (80 μg per disc) or GLPP 50 mg/kg-treated serum (10 μL per disc). Therefore, Anti-angiogenesis might represent an important mechanism underlying antitumor activity. To determine whether or not GLPP had effect on the endothelial cell proliferation, HUVEC proliferation assay had been done with MTT method and the result showed GLPP (1, 10, and 100 mg/L) directly inhibited HUVEC cell proliferation *in vitro*. And the mechanism may be connected with induced endothelial cell apoptosis by our primary result, the further experiment was still going on[50].

A number of studies indicate that polysaccharides isolated from *G lucidum* are main antitumor components *in vivo*. Antitumor action of polysaccharides differs greatly due to their chemical composition and configuration and physical properties. Antitumor activity is exhibited in a wide range of glycans extending from homopolymers to highly complex heteropolymers. Although it is difficult to correlate the structure and antitumor activity of complex polysaccharides, some possible relationships can be inferred. It has been reported that most of the antitumor polysaccharides show the same basic β-glucan structure with different types of glycosidic linkages. Therefore it is obvious that some structural features such as β-1,3-linkages in the main chain of the glucan and further β-1,6-branch points are needed for antitumor action. The β-glucans containing mainly 1,6-linkages have less activities. Glucans with high molecular weight appear to be more effective than those with low molecular weight. However, obvious variations of antitumor polysaccharides are also noted[36,52-55].

Anti-tumor mechanisms of *G lucidum* alcohol extract Although the pharmacology and clinical application of water extracts of *G lucidum* have been extensively documented, little is known regarding its alcohol extract.

Obviously different from the mechanisms of water extract of *G lucidum*, the current studies showed that the alcohol extract of *G lucidum* elicited cytotoxicity directly on some kinds of tumor cells *in vitro*. Three new lanostante-type triterpene aldehydes, named lucialdehydes A-C, were isolated from the fruiting bodies of *G lucidum*. Lucialdehydes B, C showed cytotoxic effects on Lewis lung carcinoma (LLC), T-47D, Sarcoma 180, and Meth-A tumor cell lines. Lucialde-hyde C exhibited the most potent cytotoxicity against the tested cell lines with ED_{50} values of 10.7, 4.7, 7.1, and 3.8 mg/L, respectively[56]. Six new highly oxygenated lanostane-type triterpenes isolated from *Ganoderma* spores also showed direct cytotoxicity *in vitro* on the Meth-A and LLC tumor cell lines[57]. A triterpene from *G tsugae* was found to induce cell apoptosis and cell cycle arrest in human hepatoma Hep3B cells[58]. It has also been suggested that the triterpene-enriched fraction, WEES-G6, prepared from mycelia of *G lucidum* inhibited the growth of human hepatoma Huh-7 cells. 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, which resulted in a prolonged G2 cell cycle phase and strong growth inhibition of the hepatoma cells[59]. The alcohol extract of *G lucidum* also showed that it inhibited cell proliferation in a dose- and time-dependent manner, which might be mediated through up-regulation of p21/Waf1 and down-regulation of cyclin D1. Furthermore, it can directly induce apoptosis in MCF-7 cells, which might be mediated through up-regulation of a pro-apoptotic Bax protein and not by the immune system[60]. Zhu *et al* reported that two alcohol extracts (I and III) from *G lucidum* spores strongly inhibited the growth of HeLa cells. Moreover, extract III was shown to be capable of blocking the cell cycle at the transition from G1 to S phase and inducing a marked decrease of intracellular calcium level. These results imply that the effective extract might influence the cell cycle and cellular signal transduction by altering the calcium transport system[61].

Recent studies indicate that the anti-angiogenic activity of *G lucidum* might be linked with its plausible anti-tumor activity. Kimura *et al* found that the triterpenoid fraction of the fruit bodies of *G lucidum* at the concentration of 800 mg/L inhibited angiogenesis induced by Matrigel [a soluble basement membrane extract of Engelbreth-Holm-Swam(EHS) tumor] supplemented with vascular endothelial growth factor (VEGF) and heparin in an *in vivo* model[45]. *G lucidum* ethanol extract (GL) also showed strong anti-angiogenic activity in the CAM assay, which is very useful for detecting *in vivo* angiogenesis. When 1.25, 2.5, 5, or 10 mg GL per egg was applied, the inhibitory percentage of angiogenesis were found to be 47.1 %, 57.6 %, 64.7 %, or 67.1 %, respectively. At a dose of 10 mg per egg, its anti-angiogenic activity is comparable to that of retinoic acid (1 mg per egg) used as a control. Further investigation suggests that the GL reasonably inhibits LPS-induced NO production in macrophages, which corresponds with its anti-angiogenic activity[62].

CONCLUSIONS

All of the above studies clearly showed the *G lucidum* possessed immunomodulatory and anti-tumor potentials. The immunomodulatory activity of *G lucidum* includes enhancing the maturation and function of antigen-presenting cells such as dendritic

cells, promoting phagocytosis of mononuclear phagocytes and modulating humoral immunity and cellular immunity. The most attractive nature of this kind of fungus is its anti-tumor action, which was demonstrated to be mainly associated with its polysaccharides fraction. The water extract and the polysaccharides fraction of *G lucidum* exhibited significant anti-tumor effect in several tumor-bearing animals. However, they neither induced tumor cells apoptosis and nor inhibited their proliferation *in vitro* directly. Both of them could induce macrophage or T lymphocyte to secrete TNF-α and IFN-γ, which are known to play an important role in suppressing tumor cells growth and inducing apoptosis of tumor cells, suggesting that the anti-tumor activity of *G lucidum* water extract or the polysaccharides was mainly through its immunoenhancing activity in the tumor-bearing animals. A number of studies also showed that the alcohol extract or the triterpene fraction extracted from *G lucidum* also possessed anti-tumor effect which may be related to the cytotoxic activity on the tumor cells directly. Primary study also indicated that anti-angiogenesis is related to anti-tumor mechanism.

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