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Ganoderma and Health

Pharmacology and Clinical Application

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Ganoderma and Health

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Chapter 1
Immunomodulating Effect of *Ganoderma* (Lingzhi) and Possible Mechanism

Xin Wang and Zhibin Lin

Abstract *Ganoderma* (Lingzhi) has been used for a long time in China to prevent and treat various diseases. Accumulated studies have demonstrated that the *Ganoderma* modulates immune function both in vivo and in vitro. The immunomodulating effects of *Ganoderma* were extensive, including promoting the innate immune function, humoral immunity, and cellular immunity. In particular, *G. lucidum* polysaccharides may affect immune cells and immune-related cells including B and T lymphocytes, dendritic cells, macrophages, and natural killer cells, with the promotion of immune organ growth, cytokine release, and other immune regulatory functions. Furthermore, cellular and molecular immunomodulatory mechanisms, possible receptors involved, and triggered signaling pathways have also been summarized. However, whole animal experiments are still needed to further establish the mechanism of the immunomodulating effects by *Ganoderma*. Importantly, evidence-based clinical trials are also needed.

Keywords *Ganoderma* · Polysaccharides · Immunomodulatory effects · Macrophages · Cytokines

It has been deeply investigated and well recognized that the variety of pharmacological activities of *Ganoderma* (Lingzhi) and its active components including polysaccharides were mainly through its extensive immunomodulatory effects. A number of studies have demonstrated the immunomodulating effects both in vivo and in vitro, including promoting the proliferation, differentiation, and function of antigen-presenting cells (APC) such as dendritic cells, enhancing the phagocytic function of mononuclear macrophages and natural killer (NK) cells, and enhancing humoral and cellular immunity, such as promoting immunoglobulin production, promoting T and B lymphocyte proliferative responses, and promoting cytokine production. *Ganoderma* can restore immune dysfunction induced by various causes

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[1]. The immunomodulatory effect of Lingzhi is one of the potential mechanisms of "Fuzheng Guben (supporting the healthy energy, strengthening and consolidating body resistance)," which is one of the major principles in the therapeutics of traditional Chinese medicine [2–4]. This review provides a comprehensive summary of the cellular and molecular mechanisms of immunomodulation by *Ganoderma* on the basis of our research and highlights recent advances delineating immunobiological mechanisms that have been made toward integrating immunomodulatory therapies in the clinic.

1.1 *Ganoderma* Enhances Innate Immune Function

Innate immunity, also known as natural immunity or non-specific immunity, is a series of defense mechanisms that not only responds quickly to various invading pathogenic microorganisms but also plays an important role in the initiation and effect processes of specific immunity. Studies have found that a variety of innate immune cells such as natural killer (NK) cells, dendritic cells (DCs), and macrophages can regulate the innate immune response. Enhancing the body's non-specific immunity is of great significance to improve the overall immune function of the body.

1.1.1 Ganoderma Promote Maturation and Function of Dendritic Cells

Dendritic cells (DCs) are the most powerful professional antigen-presenting cells (APCs) in the body. DCs are the initiator of immune response and play a unique role in the induction of immune response. Mature DCs can activate the initial T cells effectively.

Cao and Lin (2002) firstly established the culture of murine bone marrow-derived DC in vitro and further explored whether *G. lucidum* polysaccharides (Gl-PS) have regulatory effects on maturation and function of DC. The results showed that Gl-PS at the concentration of 0.8, 3.2, and 12.8 μg/mL upregulated the co-expression of I-A/I-E and CDl1c molecules on DC surface and promoted mRNA expression and protein secretion of IL-12 p40 unit, which indicated that Gl-PS could promote the maturation of DC in the presence of 1 mg/mL lipopolysaccharide (LPS). On the other hand, the upregulation of co-expression of I-A/I-E and CD11c on the DC surface also indicated the mechanism by which Gl-PS promotes the maturation of DC may be related to its effect on I-A/I-E expression. Further results confirmed that Gl-PS could promote the proliferation of one-way MLC induced by DC, indicating the modulating effects of Gl-PS on innate immune response primed by mature DC. These data demonstrate that Gl-PS promotes not only the maturation of cultured

murine bone marrow-derived DC in vitro but also the immune response initiation induced by DC [5]. Further data show that Gl-PS are able to promote the cytotoxicity of specific cytotoxic T lymphocyte (CTL) induced by Gl-PS-treated DC pulsed with P815 tumor cell lysates during the stage of antigen presentation, with the mechanism mainly through interferon (IFN)-γ and granzyme B pathways [6].

Lin et al. (2005, 2006) investigated the effects of the polysaccharide component with a branched (1 → 6)-β-D-glucan moiety of *G. lucidum* (PS-G) on human monocyte-derived DC. Treatment of DC with PS-G (10 μg/mL) resulted in the enhanced cell surface expression of CD80, CD86, CD83, CD40, CD54, and human leukocyte antigen (HLA)-DR, as well as the enhanced mRNA expression and production of IL-12 p70, IL-12 p40, and IL-10, while the capacity for endocytosis was suppressed in DC. In addition, treatment of DC with PS-G resulted in enhanced T-cell stimulatory capacity and increased T-cell secretion of IFN-γ and IL-10 [7, 8].

Chan et al. (2007) demonstrated that extracts from different parts of *G. lucidum* can also stimulate the maturation of monocyte-derived DC cells. The crude/pure polysaccharide of *G. lucidum* mycelium (1, 10, 100 μg/mL) could induce proliferation of human peripheral blood mononuclear cells (PBMC) in a dose- and time-dependent manner; upregulate cell surface and costimulatory molecules HLA-DR, CD40, CD80, and CD86; promote the functional maturation of DC cells; and secrete IL-12, IL-12 p70, and IL-10. The results of homologous (allogenic) mixed lymphocyte experiment showed that DC treated with purified *G. lucidum* mycelium polysaccharide promoted T-cell proliferation. Contrarily, DC treated with purified *G. lucidum* spore polysaccharide inhibited T-cell proliferation. The contents of IL-10 and TGF-β in supernatants of DC/T mixed cells treated with *G. lucidum* spore polysaccharide did not change significantly [9].

Another study confirmed that combination of GLPS and GMCSF/IL-4 induces the transformation of THP-1 cells into typical DC cells, while GLPS alone can only induce the proliferation of THP-1 and U937 cells. In addition, when THP-1 was converted to DC, the expression of HLA-DR, CD40, CD80, and CD86 increased significantly, and the ability of antigen uptake also increased. However, its ability to induce allogeneic T-cell proliferation is weak [10].

Zhao et al. (2010) investigated the immunomodulation activities of *G. lucidum* polysaccharide (GLP) by way of regulating the enteric mucosal immune response. Mouse peripheral blood mononuclear cells (PBMCs), intestinal epithelial lymphocytes (IEL), and Peyer's patches lymphocytes (PPL), respectively, were co-incubated with different concentrations of GLP (250, 125, 62.5, 31.25 μg/mL) under stimulation of ConA (4 μg/mL). The MTT assay suggests that GLP can stimulate the proliferation of PBMC and enteric mucosal lymphocytes. ELISA and RT-PCR assay reveal that GLP obviously increased the production of IL-2 and IL-10, as well as TNF-α and IL-10 mRNA expression in PBMC, IEL, and PPL induced by ConA [11].

To investigate and analyze the effects of *G. lucidum* polysaccharides (GLPs) on cell phenotype and functional maturation of murine DCs, Meng et al. (2011) use conventional scanning electronic microscopy (SEM) and transmitted electron

microscopy (TEM) for the morphology of and intracellular lysosomes inside the DCs. Under SEM, the DC treated with 300 μg/mL GLP for 24 h shows more protrusions and rougher surface in morphology, which indicates matured DC to trigger T-cell response. Under TEM, the number of lysosomes inside the DC treated with GLP reduced significantly with gradual maturation compared with those in the untreated DC. Other assays included cellular immunohistochemistry for phagocytosis by the DCs, flow cytometry (FCM) for analyzing key surface molecule alteration, bio-assay for the activity of acid phosphatases (ACP), and ELISA for the production of pro-inflammatory cytokine IL-12. It was found that GLP induced phenotypic maturation, as evidenced by increased expression of key surface markers and receptors such as CD86, CD40, and MHC II. Functional experiments showed the downregulation of ACP inside the DCs, which occurs when phagocytosis of DCs decreased, and antigen presentation increased with maturation. GLP increased the production of IL-12, which would work as an intensified signal to activating $CD4^+$ T-cell response. These data reveal that GLPs exert positive modulation to DCs, markedly enhance DC maturation and function, as well as have a marked enhancement in the DC-$CD4^+$ T-cell pathway [12].

Lv et al. (2016) use high content imaging system to quantify the immunostimulation ability of one major fraction of *G. lucidum* polysaccharide, GLPII. GLPII-treated (40 μg/mL) Raw264.7 cells for 24 h significantly increased cell metabolic activity and changed the morphology of Raw264.7 cells toward dendritic-like cells (DC) (Fig. 1.1). Within 24 h, the sizes of cells became larger with extended dendritic pseudopods compared to untreated group. Cell irregularity increases quickly within 0–10 h and then gradually reached a plateau after 30 h. Raw264.7 cell differentiation by GLPII was also in a concentration-dependent manner; at the same time, the 30 h plateau increased with the concentration. It indicated that polysaccharides may induce a strong metabolic activity during cell differentiation instead of cell proliferation. Flow cytometry was used to examine phenotypic changes on Raw264.7 cells by polysaccharides. Typical phenotypic maturation surface markers involved MHCII, CD40, CD80, and CD86. After treatment with different concentrations of GLPII, all these cell surface markers showed a dose-dependent upregulation [13].

Another work from the same research group (Zhu et al. 2016) compared polysaccharides isolated from *Ganoderma lucidum* (GLP) with other herbs to determine the immunoactivities on innate immune response, applying bone marrow-derived DC. DCs incubated with salt eluent of GLP 45 μg/mL for 24 h showed similar immuno-potentiation ability on DC maturation through the increased expression of CD40, MHCII, CD80, and CD86 compared to 0.1 μg/mL LPS, elongated protrusion (Fig. 1.2), and increased level of nitric oxide (NO). Interestingly, blockage of NO by an iNOS inhibitor (S-methylisothiourea sulfate, SMT) significantly decreased

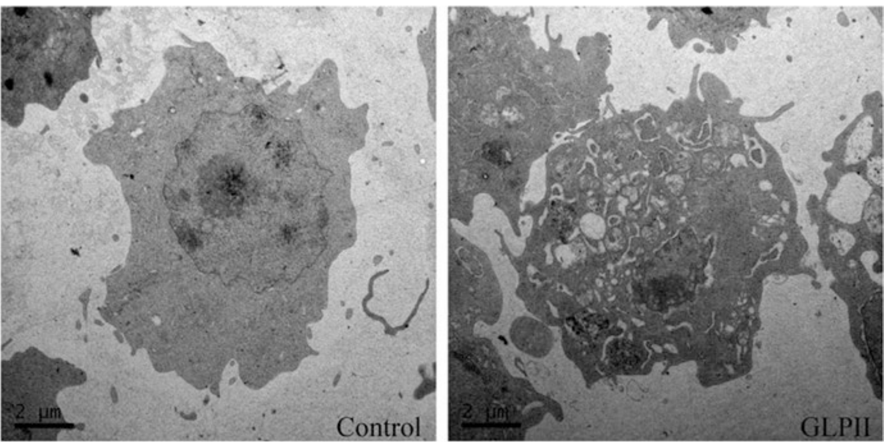

Fig. 1.1 Morphology changes of Raw264.7 cells by GLPII. Cells were treated with culture medium, 40 μg/mL GLPII and 0.1 μg/mL LPS (positive control). Transmission electron microscopy of non-treated (left) and 40 μg/mL GLPII-treated Raw264.7 cells (right). (Reproduced with permission from Ref. 13)

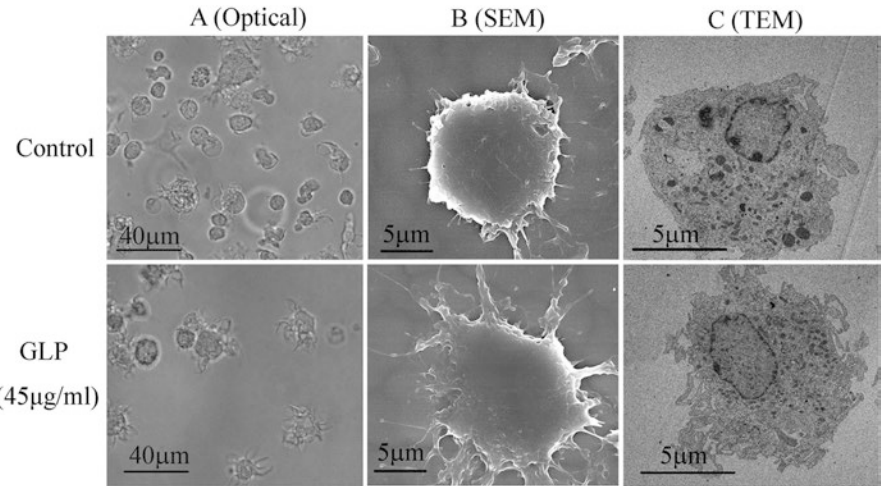

Fig. 1.2 Morphological changes of DC by GLP. Cells were treated with culture medium or 45 μg/mL GLP and imaged by (**a**) optical microscopy, (**b**) scanning electron microscopy, and (**c**) transmission electron microscopy. (Reproduced with permission from Ref. 14)

CD40/MHCII but not CD80/CD86 expression induced by GLP, indicating that NO was partially involved in DC maturation. Immature DCs keep the capability to capture antigens, while after maturation, DCs start to process and present antigen. The phagocytic ability expressed by FITC-dextran uptake in cells evaluated by flow cytometry reduced significantly in GLP-treated DC compared to the non-treated

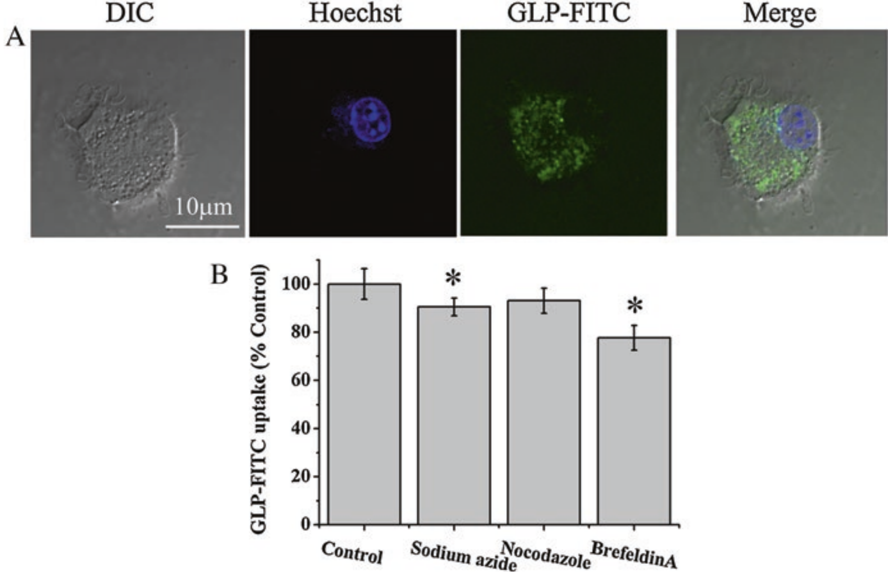

Fig. 1.3 Endocytosis of GLP-FITC that can be blocked by endocytic inhibitors. (**a**) Endocytosis revealed by confocal microscopy. Cells were incubated with 180 μg/mL GLP-FITC for 24 h. Differential interference contrast (DIC) showed the morphology of DC with elongated dendrites. GLP-FITC was found to distribute in the cells as a punctate pattern (green). Hoechst staining represents the cell nuclei (blue). (**b**) Inhibition of GLP-FITC endocytosis. Endocytic inhibitors sodium azide (0.65 mg/mL), nocodazole (10 μg/mL), and brefeldin A (10 μg/mL) were added with 180 μg/mL GLP-FITC for 1 h. Cells were collected and washed and analyzed by flow cytometry and normalized to GLP-FITC-treated sample. $*0.01 < p < 0.05$ was considered statistically significant difference from control. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.). (Reproduced with permission from Ref. 14)

control group. Moreover, GLP labeled with FITC can be detected to enter cells in a dose- and time-dependent manner (Fig. 1.3), and the confocal images revealed a punctate distribution in the cytoplasm, which can be blocked by different types of endocytic inhibitors (sodium azide and brefeldin A) as well as the DC maturation markers, revealing that the cellular uptake of GLP may also play an important role in DC maturation [14].

1.1.2 *Effect of Ganoderma on Mononuclear Phagocyte System*

Mononuclear phagocyte system (MPS), also known as macrophage system, is a major class of antigen-presenting cells that play a key role in the induction and regulation of specific immune responses. In the early 1980s, we observed the effect of the ethanol extract isolated from the fruiting body of *G. lucidum*. The results showed

that both *G. lucidum* extract and *G. lucidum* polysaccharide D6 could significantly increase the ability of mouse peritoneal macrophages to phagocytose chicken red blood cells. The percentage of phagocytosis and phagocytic index were significantly higher than those of the control group [15]. We have also found that polysaccharides extracted from the fruiting bodies and mycelia of *G. lucidum* can restore the reduced carbon particle clearance rate of mice reduced by injection of hydrocortisone. Moreover, we found that GL-PS (final concentration of 0.2, 0.8, 3.2, or 12.8 μg/mL) can significantly promote the activity of peripheral exudate cells (PEC) devouring neutral red (NR) in vitro [6].

Further studies demonstrated that IL-1 and TNF-α productions significantly increased in mouse peritoneal macrophages treated with *Ganoderma* polysaccharides [16]. Berovic et al. (2003) reported that a polysaccharide isolated from *G. lucidum*, which was mainly composed of β-D-glucan, could induce TNF-α synthesis in primary cultures of human peripheral blood mononuclear cells [17]. Zhang and Lin (1999) found that the addition of *G. lucidum* polysaccharides B (GL-B), which were extracted from the fruiting body of *G. lucidum* with lower molecular weight of 6900–9100 (25–400 μg/mL), to the in vitro macrophages culture media resulted in a significantly increased TNF-α mRNA expression in a concentration-dependent manner [18]. Following the administration of *G. lucidum* extract at 5, 10, or 20 g (crude material)/kg by forced stomach tube feeding, Zhang and Lin (1999) also found that TNF-α mRNA expression in the peritoneal macrophages was also increased markedly [19].

Ji et al. (2007) reported a proteoglycan component (GLIS) purified from the fruiting body of *G. lucidum*, which could significantly enhance the proliferation of mouse bone marrow macrophages and RAW264.7 cells in a dose-dependent manner. GLIS-treated (5, 10 μg/mL) RAW264.7 macrophages were found enlarged and formed pseudopodia by microscopic examination, induction of cellular respiratory burst activity, and also increased levels of IL-1β, IL-12p35, and IL-12p40 gene expression, while IL- 6, IL-18, and TNF-α mRNA showed no significant effect [20].

Lv et al. (2016) use high content imaging system to quantify the immunostimulation ability of one major fraction of *G. lucidum* polysaccharide, GLPII. GLPII-treated (40 μg/mL) Raw264.7 cells for 24 h significantly increased cell metabolic activity and changed the morphology of Raw264.7 cells toward dendritic-like cells (DC). Within 24 h, the sizes of cells became larger with extended dendritic pseudopods compared to untreated group. Cell irregularity increases quickly within 0–10 h and then gradually reached a plateau after 30 h. Raw264.7 cell differentiation by GLPII was also in a concentration-dependent manner; at the same time, the 30 h plateau increased with the concentration. It indicated that polysaccharides may induce a strong metabolic activity during cell differentiation instead of cell proliferation [21]. S-Methylisothiourea sulfate (SMT), an inducible nitric oxide synthase (iNOS) inhibitor, was used to inhibit NO production. The phagocytic ability of cells was significantly reduced especially for APSII- and GLPII-treated cells. In conclusion, NO production seemed to be very crucial for promoting phagocytic ability of macrophages. Flow cytometry was used to examine phenotypic changes on Raw264.7 cells by polysaccharides. Typical phenotypic maturation surface markers

involved MHCII, CD40, CD80, and CD86. After treatment with different concentrations of APSII and GLPII, all these cell surface markers showed a dose-dependent upregulation. In addition, this work proved for the first time that NO production is strongly correlated to enhanced phagocytic ability of macrophages induced by polysaccharides.

Wei et al. (2007) found that *G. lucidum* polysaccharide (GLPS) enhanced not only the expression of TLR4 and CD14 on the surface of macrophage J774A.1 but also LPS binding activity and endocytosis, resulting in a reduced adhesion time constant and increased the force constant of binding interaction [22].

Yue et al. (2013) found that the *Ganoderma sinense* (GS) hot water extract 400 μg/mL can significantly stimulate the proliferation of peripheral blood mononuclear cells (PBMC). The stalk polysaccharide (50–400 μg/mL) of the GS fruiting body can activate PBMC in a concentration-dependent manner; induct production of TNF-α, IL-10, and TGF-β; and increase the percentage of $CD14^+$ monocytes [23].

Zhang et al. (2013) also found that the antitumor activity of *Ganoderma tsugae* polysaccharide (PSG-1) does not directly kill CT26 cells, but inhibits its proliferation by activating peritoneal macrophages. The spleen weight index and thymus index of tumor-bearing mice are both increased, with a raised production of TNF-α, IL-1β, and NO [24]. Further studies found that PSG-1 acts toward TLR4 and activates NF-κB via the p38 MAPK signaling pathway, thereby stimulating TNF-α production. Similar results were obtained by Li et al. (2011) [25].

Watanabe et al. (2011) reported that lucidenic acid-rich extract isolated from antlered form of *G. lucidum* (*G. lucidum* AF, 10, 50 μg/mL) induces TNF-α production in monocytic THP-1 cells and synergizes with LPS to induce TNF-α production [26]. Notably, *G. lucidum* AF enhanced LPS-induced phosphorylation of p38 mitogen-activated protein kinase (MAPK), while it suppressed LPS-induced phosphorylation of c-Jun N-terminal kinase (JNK) MAPK. Use of p38 inhibitor and JNK inhibitor demonstrated that synergistic effect of the extract lucidenic acid-A and lucidenic acid-F may work by modulating p38 and JNK MAPKs, respectively.

Chang et al. (2009) reported that oral administration of *G. lucidum* extract (3, 6 mg/kg) for 28 consecutive days decreased the spleen weight and enhanced the phagocytosis of peripheral blood mononuclear cells and the activity of NK cells in leukemia mice. *G. lucidum* extract treatment group can alleviate the decrease of CD3 and CD19, inhibit the increase of Mac-3 and CD11b, promote the proliferation of ConA-stimulated spleen cells, and have no significant effects on LPS stimulation [27].

Hsu et al. (2011) applied dynamic gene expression profiling to study human monocyte (THP-1) cells treated with *G. lucidum* polysaccharide F3 and showed that the cell differentiation was enhanced, including expression changes of differentiation marker molecules including CD11b, CD14, CD68, and MMP-9, cell adhesion, cell cycle arrest, nitroblue tetrazolium reduction, and myeloperoxidase expression changes, which may be mediated by caspase cleavage and p53 activation [28].

Cheng et al. (2010) applied chip technology to compare the effects of ethanol extracts of *G. lucidum* (GL) and *G. sinense* (GS) on human monocyte (THP-1) gene

expression. After the culture of 0.2% of GS and GL extract with THP-1 monocytes for 24 h, the number of differential expressed genes was 1189 for GS extract and 629 for GL extract. The action pathway of GS is mainly focused on inflammation and immune response, and GL increases gene expression involved in macromolecular metabolism [29]. Furthermore, neither of them inhibited the monocyte development. GL extract has no significant effect on the gene of NF-κB pathway, while GS extract increases multiple key genes in this pathway.

Macrophages can polarize into M1 phenotype (classically activated macrophages) or M2 phenotype (alternatively activated macrophages) in response to different microenvironmental signals, which are involved in many different pathogeneses. In general, M1 is responsible for pathogen clearance, inflammation, and tumor-suppressing response, while the M2 performs immunosuppressive and tumor-promoting functions. It has been recognized that improvement on M1 polarization, instead of M2 polarization, may be health beneficial [30]. Sun et al. (2017) revealed that *G. lucidum* polysaccharide peptide (Gl-PS) might have the potential to promote macrophage M1 polarization induced by LPS. The level of M1 stimulating factor IL-6, IL-12, and TNF-α stimulated by LPS was upregulated in a dose-dependent manner after treatment of Gl-PS. In contrast, the level of M2 stimulating factor arginase I and IL-10 was reduced by Gl-PS, demonstrating the potential of Gl-PS to promote M1 polarization versus M2 [31].

1.1.3 Effect of Ganoderma on the Natural Killer Cells

Natural killer (NK) cells are considered to be part of the innate defense system. NK cells, in contrast to cytotoxic T cells, have the ability to kill certain tumor cells in vitro without prior sensitization. In addition, NK cells display Fc-receptors for IgG and are important mediators of antibody-dependent cell-mediated cytotoxicity. In some cases, NK cells have spontaneous cytotoxic activity on target cells, participating in the occurrence of hypersensitivity reactions and autoimmune diseases.

A number of reports indicated that water extracts isolated from *G. lucidum* fruiting bodies or *G. lucidum* polysaccharides could enhance activity of NK cells in in vivo experiments. Chien et al. (2004) reported that a fucose-containing glycoprotein fraction (F3) (10–100 μg/mL), isolated from the water-soluble extracts of *G. lucidum*, was applied to human umbilical cord blood mononuclear cells (MNCs) in vitro. After 7 days of culture, cell phenotypic analysis by flow cytometry showed that $CD14^+CD26^+$ monocytes/macrophages, $CD83^+CD1a^+$ dendritic cells, and $CD16^+CD56^+$ NK cells increased by 2.9%, 2.3%, and 1.5%, respectively, indicating that F3 quantitatively influenced NK cell activities. They also found that F3 is not harmful to human cells in vitro; and after F3 treatment, NK cell-mediated cytotoxicity was significantly enhanced by 31.7% at effector/target cell ratio (E/T) 20:1, but was not altered at E/T 5:1 [32].

Zhu et al. (2007) designed an immunosuppressive animal model by intraperitoneal injection of cyclophosphamide (Cy) 300 mg/kg. After 24 h, mice were injected

intraperitoneally (i.p.) once daily with *G. lucidum* polysaccharide (Gl-PS, 2.5, 25, 250 mg/kg), respectively, for 7 consecutive days. In Cy-treated mice, compared to vehicle, low-dose Gl-PS accelerated recovery of bone marrow cells, red blood cells, and white blood cells, as well as splenic natural killer cells and natural killer T cells, and enhanced T- and B-cell proliferation responses on day 8, cytotoxic T lymphocyte (CTL) activity on day 5, as well as NK cell and lymphokine-activated killer (LAK) cell activity on days 7–9. Furthermore, it promoted phagocytosis and cytotoxicity of macrophages on day 12. The above beneficial effects induced by the low-dose Gl-PS treatment did not result in any side effects [33].

Wang et al. (2002) found that the fucose-containing *G. lucidum* polysaccharide peptide component (F3) (10 μg/mL) promoted mRNA of IL-1, IL-2, and IFN-γ in mouse spleen cells by RT-PCR. Proteomic analysis indicated that this dose of F3 caused about 50% change in the proteomics of mouse spleen cells. The same concentration of crude extract of *G. lucidum* (10 μg/mL) could not stimulate the production of cytokines, indicating that F3 is the main active component of *G. lucidum*, which is related to the activation of immune cells; F3 induces high levels of IFN-γ expression, suggesting that F3 may activate NK cells [34].

1.2 *Ganoderma* Enhances Specific Immunity and the Possible Mechanisms

1.2.1 Ganoderma Enhances Cellular Immunity

Cellular immunity is a sensitized small lymphocyte-mediated immune response produced by the differentiation and proliferation of T lymphocytes. There are at least two kinds of effector cells for cellular immunity, namely, T helper (Th) cells and T killer (Tc) cells, which is also called cytotoxic T cells (CTL). Th cells may also indirectly kill target cells by releasing cytokines. The influence of *G. lucidum* on cellular immune function is an important aspect of its immunomodulatory effects. A series of investigations from our laboratory demonstrated that the cell-mediated immune function was also enhanced by *G. lucidum*. The mRNA expression and production of IFN-γ were significantly increased in the T lymphocytes [18].

Lei et al. (1993) found that *G. lucidum* polysaccharide GL-B (50–800 μg/mL) can promote mixed lymphocyte reaction (MLR) stimulated by allotype antigens in a concentration-dependent manner in mice and can reverse the inhibition of MLR by low-dose cyclosporine (0.01 μg/mL) to near-normal level. GL-B can also partially antagonize the suppressive effect of hydrocortisone on MLR [35].

Further, Lei and Lin (1992) used anti-L_3T_4 and anti-Lyt2 monoclonal antibodies and indirect fluorescent immunoassay to detect the number of T-cell subsets in mixed lymphocyte cultures. The results indicated that GL-B 200 μg/mL significantly increased the recovery of total T cells and the recovery of $L_3T_4^+$ cells and

Lyt2+ cells, and the percentage of Lyt2+ cells was also significantly increased. Mouse $L_3T_4^+$ cells belong to Th cells, and this result indicates that GL-B can promote Th cell proliferation. While T suppressor (Ts) cells and CTL both carry Lyt2+ antigen, the exact meaning of GL-B promoting Lyt2+ cell proliferation remains to be studied. The plaque reduction assay for T-cell activity also showed that GL-B significantly enhanced the function of CTL and increased its killing activity by 100% at a concentration of 200 μg/mL. The killing activity indicates that at least some of the Lyt2+ cells increased by GL-B belong to CTL cells [36].

In co-culture of human T cells and non-T cells in vitro, lymphocyte proliferation occurs, which is called autologous mixed lymphocyte reaction (AMLR). The mouse spleen contains a variety of immune cells. T cells are located in the thymus-dependent region; B cells are located in the non-thymus-dependent region; and macrophages are present in the interstitium. When in a spleen cell suspension, these cells are mixed together and can be used to produce AMLR. Lei and Lin (1993) found that GL-B (100–800 μg/mL) promoted AMLR in mouse spleen cells, but its effect was much weaker than ConA and LPS, but similar to thymosin. GL-B promoted the spontaneous proliferation of mouse spleen cells, which may be inhibited by cyclosporine A by 93.2%. Since cyclosporine mainly acts on T cells, GL-B acts mainly on T cells. Daily intraperitoneal injection of GL-B 50 and 100 mg/kg for 4 consecutive days can also promote the spontaneous proliferation of mouse spleen cells. If the macrophages in the splenocytes are partially removed, the spontaneous proliferation ability of the splenocytes is significantly reduced, and the ability to secrete IL-2 is also weakened, and the proliferative effect of GL-B on splenocytes is also attenuated. After the addition of peritoneal macrophages, the spontaneous proliferation of spleen cells and the ability to secrete IL-2 were restored, and the proliferative effect of GL-B was also restored. The results further demonstrate that the proliferative effect of GL-B on spleen cells is a promoting effect on AMLR [35].

Zhang and Lin (1999) observed the effects of *G. lucidum* extract on ConA-induced lymphocyte proliferation and MLR by means of serum pharmacology, and similar results were obtained. The mice were intragastrically administered with *G. lucidum* extract 5, 10, and 20 g (raw material)/kg for 10 days. Then the drug-containing serum was prepared and used in in vitro study compared with the *G. lucidum* extract dilutions. The results showed that both the serum containing *G. lucidum* and *G. lucidum* extract (50~200 μg/mL) can significantly promote ConA-induced lymphocyte proliferation and MLR in a dose-dependent manner. The results revealed that *G. lucidum* extract may contain active ingredients that promote the above two reactions, which are effective in vitro and absorbable [18].

Kohguchi et al. (2004) studied the immuno-potentiating effects of the antler-shaped fruiting body of *G. lucidum* (Rokkaku-Reishi, RR) in mice. BALB/c mice were administered orally with suspension of dried powder made from RR for 3 days at a dose of 50 or 500 mg/kg, and IFN-γ production by splenocytes in response to LPS was examined on day 4. The oral administration of 500 mg/kg of RR resulted in a significant increase in IFN-γ and IL-12 production. It suggests that splenic macrophages were activated by RR administration. Furthermore, 500 mg/kg of RR

administered for 14 days resulted in a significant increase in IFN-γ production by splenocytes in response to both LPS and ConA. These results suggest that not only splenic macrophages but also T cells were activated by the long-term treatment with RR in mice. The results suggest that the oral administration of RR resulted in Th1-associated immuno-potentiating activities in vivo [37].

Since *G. lucidum* polysaccharide can promote T-cell uptake [^{3}H]TdR in mixed lymphocyte culture or ConA-induced spleen cell culture, it indicates that it can promote DNA synthesis of T cells. To observe the mechanism by which *G. lucidum* polysaccharides promote T-cell DNA synthesis, Lei and Lin (1991) observed the effect of GL-B on DNA polymerase α activity of spleen lymphocytes in MLR model. After GL-B was co-cultured with spleen lymphocytes for 3 days, spleen cells were collected, and the activity of DNA polymerase α in the cells was measured. The results showed that GL-B could significantly enhance the activity of DNA polymerase α in mouse spleen lymphocytes while promoting MLR at a concentration of 62~250 μg/mL. However, in the cell-free system, the above concentration of GL-B has not only enhanced the activity of the enzyme, but has a slight inhibitory effect. This opposite result suggests that at the cellular level, GL-B may induce DNA polymerase α synthesis through some indirect mechanism, thereby increasing its activity [38].

Xiao et al. (1994) used microscopic spectrophotometer to measure the content of mitochondria, ATPase, DNA, and RNA in spleen cells cultured in vitro and observed the ultrastructure of spleen cells by electron microscopy and studied the effect of GL-B on it. The results showed that GL-B (50~200 μg/mL) with spleen cells co-cultured for 24 h can significantly increase the mitochondrial mitochondria, ATPase, DNA, and RNA content. Under the action of GL-B, the nucleus of spleen cells became larger, the cytoplasm was more abundant, and the degree of cytoplasm increased was greater than that of nucleus. It is known that mitochondria are vital for intracellular energy metabolism. Therefore, it is conceivable that GL-B promotes synthesis of RNA and protein by enhancing energy metabolism, which in turn promotes DNA synthesis and cell division and proliferation [39].

Zhao et al. (2018) explored the immunomodulating effect of *Ganoderma lucidum* extract (GLE) in the tumor treatment process. GLE (0.011, 0.023, 0.046 g injected intraperitoneally once a day for 28 consecutive days) could effectively increase the number of peripheral blood NK cells and the $CD4^+$ and $CD4^+/CD8^+$ T cells. The mRNA profiles of GLE-treated and GLE-untreated Hepa1-6-bearing C57BL/6 mice were detected, and 302 differential expressed (DE) mRNAs were identified. Then six kernel mRNAs were screened from the established protein-protein interaction (PPI) network and the Cluster-PPI network, including CD3G, GNAS, MAP 3K8, NGFR, PRKG2, and PTPN22. Quantitative RT-PCR and Western blot analysis indicated that six mRNAs have had statistically significant differences between the GLE-treated and GLE-untreated mice. Among them, PTPN22 were reported to enhance the efficacy of antitumor T-cell responses. Furthermore, based on the KEGG-Target network, four immune-related pathways were screened and verified by Western blot that GLE treatment might suppress Jak/Stat signaling pathway

(reduce the Jak3 expression level and tyrosine phosphorylation of Stat1, 3, 5, and 6), T-cell receptor signaling pathway (downregulated p-Lck and p-Zap70), and PI3K/Akt/mTOR signaling pathway. Serum cytokine detection results demonstrated that the levels of IFN-γ, IL-1β, IL-4, IL-9, IL-12, RANTES, and TNF-α were increased in the GLE group [40].

1.2.2 Ganoderma Enhances Humoral Immunity

Humoral immunity is an antibody-mediated immunity. The antibody is produced by plasma cells differentiated from B lymphocytes (B cells). B lymphocytes play a key role in humoral immune response. LPS-induced spleen lymphocyte proliferation in mice is an in vitro experiment to value the immune function of B lymphocytes in the body. It can non-specifically stimulate B lymphocyte transformation in vitro and further proliferation and differentiation. The binding of LPS to the membrane directly activates PKC, induces B lymphocytes to express IL-2 receptor, and enables B cells to produce responsive proliferation and/or immunoglobulin secretion to IL-2, similar to the reaction of antigen-activated B lymphocytes in vivo. By measuring the effect of drugs on LPS-induced B lymphocyte proliferation in vitro, the effect on the humoral immune function of the body can be observed.

The plaque-forming cell (PFC) response is a specific method to examine the effect of medicine on the animal's humoral immune function. Our research group found that *G. lucidum* polysaccharide components BN_3A, B, C, and D (with molecular weight of 16,200, 22,400, 24,500, and 31,600, respectively) 5 mg/kg intraperitoneal injection for five continuous days can significantly increase the PFC response in SRBC-immunized mice. The result indicated that *G. lucidum* polysaccharide could increase antibody (IgM) production [41].

The exciting results from our research group showed that polysaccharides isolated from *G. tsugae* fruiting body (10~100 mg/kg) by gavage for 4 days can significantly increase the number of anti-SRBC PFC in cyclophosphamide-suppressed mice. Further result showed that *G. tsugae* polysaccharides can restore immunosuppression in cold water-stressed mice. The mice were forced to swim in cold water at 14 ± 1 °C for about 5 min every day. After 8–10 days of repetition, anti-SRBC PFC response of mouse was significantly reduced due to stress. *G. tsugae* polysaccharides can completely antagonize the stress-induced decrease of anti-SRBC PFC response [42]. Our study also found that intraperitoneal injection of *G. lucidum* polysaccharide GL-B (25~100 mg/kg) daily for 4 days can significantly enhance the proliferative response of mouse spleen cells to LPS stimulation. The spleen cell proliferation response in GL-B 100 mg/kg group increased by 84.8% compared with the control group. This result showed that GL-B can enhance the sensitivity of B lymphocytes to LPS stimulation [35].

Cao et al. (1993) also found that the production of hemolysin antibody (IgM) was enhanced in mice intraperitoneally injected with GLP (molecular weight

80,000) 0.05 and 0.1 mg. When the dose was increased to 2 and 4 mg per dose, an inhibitory effect can be seen [43].

Zhang et al. (2002) reported that a bioactive immunomodulating substance (GLIS) isolated from the fruiting body of *G. lucidum* can stimulate the proliferation, activation, and differentiation of B lymphocyte, enlarge expressed CD71 and CD25 expression on the cell surface, and show an increase in the secretion of immunoglobulin. Furthermore, the activation of B lymphocytes by GLIS did not depend on the activation of T lymphocytes; it was associated with stimulating the expression of PKCα and PKCγ in B lymphocytes by GLIS directly. However, GLIS did not influence the $[Ca^{2+}]i$ of lymphocytes. According to these results, it suggested that GLIS may be a new B-cell-stimulating factor [44].

The mechanisms underlying the activation of B cells by F3 were unclear. Lin et al. (2006) found that F3 can induce the activation of mouse spleen B cells and the differentiation of IgM secretory plasma cells, which is dependent on F3-mediated Blimp-1 expression. However, in human peripheral blood B lymphocytes, F3 could not induce the activation of B cells. However, it can induce Blimp-1 mRNA expression and enhance antibody secretion. This function of F3 is dependent on TLR4/TLR2; also, the p38 mitogen-activated protein kinase (p38 MAPK) signaling pathway is involved in the expression of Blimp-1 mRNA. At the same time, signaling through the ERK, p38 MAPK, JNK, and IKK complexes is involved in F3-mediated immunoglobulin secretion. Thus, the different mechanisms of activation of F3 in mouse and human B cells may be due to the presence of a Blimp-1 regulatory site in the human CD86 promoter region [45].

1.2.3 *Ganoderma* Promotes the Production of Immune Cytokines

Unactivated $CD4^+$ T cells are activated and differentiate into Th1 and Th2 subpopulations, which play a supporting and immunomodulatory role, also known as helper T (Th) cells. IL-2 and IL-12 induced the differentiation of unactivated $CD4^+$ T cells into Th1; IL-4 and IL-13 induced differentiation into Th2. Th1 mainly secretes IL-2, IFN-γ, and TNF-β and promotes cellular immune response; Th2 mainly secretes IL-4, 5, 6, 10, and 13, promotes B-cell proliferation, and converts to IgE and IgG1 and IgA production. Immunocytokines are small molecular peptides synthesized and secreted by immune cells, such as interleukin (IL), interferon (IFN), tumor necrosis factor (TNF), and colony-stimulating factor (CSF). Immunocytokines have a wide range of effects, in addition to affecting the immune system, but also affect the hematopoietic system, nervous system, endocrine system, and cardiovascular system. They not only affect physiological functions but also cause pathological reactions. The synthesis and secretion of immune cytokines are also affected by drugs. *G. lucidum* affects immune function by affecting the synthesis and secretion of immune cytokines.

In mixed lymphocyte culture, T helper (Th) cells are stimulated by allogeneic antigens, and proliferative reactions and synthesis of IL-2 are produced in synergy with IL-1 secreted by macrophages. Lei and Lin (1992) investigated the action of *G. lucidum* polysaccharide B (GL-B) at a final concentration of 200 μg/mL added to the reaction system. A two-way reaction was found: In the first 24 h of culture, GL-B can promote the synthesis and secretion of IL-2 by spleen cells and gradually reduce the synthesis and secretion of IL-2 after 24 h. When the fixed culture time was 12 h and the concentration of GL-B was changed, it was found that GL-B promoted the synthesis and secretion of IL-2 by spleen cells in a concentration-dependent manner. Since GL-B does not promote proliferative effects on IL-2-dependent HT2 cells and alloantigen or ConA-activated spleen cells, it means that GL-B does not promote IL-2 receptor expression in activated cells. In addition, in mouse peritoneal exudate cells (mainly macrophages) co-cultured with GL-B for 24 h, IL-1 in supernatant and peritoneal exudation increased significantly, indicating that GL-B can promote synthesis and secretion of IL-1 in peritoneal exudate cells [46].

Recently, Wang et al. (2012) found that *G. lucidum* spore polysaccharides (Gl-BSP) (50, 100, and 200 mg/kg) by intragastric administration significantly inhibited S180 sarcoma growth in mice, but Gl-BSP had no direct cytotoxic effects on S180 sarcoma cells and PG cell. However, the serum of mice treated with Gl-BSP can promote the proliferation of mouse spleen lymphocytes induced by ConA or LPS in vitro, enhance the cytotoxicity of NK phagocytic activity of macrophages, and increase percentage of $CD4^+$ or $CD8^+$ T lymphocytes. Gl-BSP (200 mg/kg) elevated serum IFN-γ, TNF-α, and NO in S180-bearing mice, whereas neutralization with anti-TNF-α and/or anti-IFN-γ apparently diminished S180 or PG cell lines growth inhibition induced by *Gl*-BSP-treated serum. This finding suggests IFN-γ and TNF-α in the serum may also play an important role for antitumor effect of Gl-BSP [47].

Chang et al. (2009) studied the results which showed that *G. lucidum* extracts (3 and 6 mg/kg) were administered orally for 14 consecutive days in a dose-dependent manner to promote ConA- or LPS-stimulated spleen cell proliferation in BALB/c mice. Compared with the control group, *G. lucidum* extract (3 and 6 mg/kg) by intraperitoneal injection significantly increased the phagocytosis of macrophages; the dose of 6 mg/kg group significantly enhanced the activity of natural killer cells. The results of flow microsphere analysis and flow cytometry showed that the expression of IL-6 and IFN-γ increased ($p < 0.001$) in *G. lucidum* extract treatment group [48].

Ji et al. (2011) found that *G. lucidum* proteoglycan (GLIS) can enhance the proliferation of bone marrow macrophages (BMM) in a dose-dependent manner. Microscopic examination revealed that the volume and formed pseudopods of BMM cells increased in GLIS-treated group. GLIS treatment significantly increased the production of NO, induced the increase of cellular respiratory burst activity, and increased the gene expression of IL-1β, IL-6, IL-12p35, IL-12p40, IL-18, and TNF-α, also TNF-α, IL-1β, and IL-12 protein levels. The experimental results suggest that GLIS activates the immune system by regulating the production of cytokines [49].

Yoshida et al. (2012) reported that *G. lucidum* water extract can activate DC cells and promote production of TNF-α and IL-23. *G. lucidum* extract activates DC cells, generates a large amount of IL-23, enhances IL-23p19 transcription, and continues phosphorylation of ERK1/2 (p44/p42). IL-23p19 promoter activity detection and MEK inhibitor U0126 reverse inhibition assay results also support the MEK-ERK signaling pathway is involved in induction, and the activity of inducing JNK and IκBα is similar to LPS [50].

Fan et al. (2018) evaluated the protective effect of combined fungal polysaccharides from *Cordyceps sinensis* and *Ganoderma atrum* on colon immune dysfunction induced by cyclophosphamide in mice. Then, *C. sinensis* polysaccharides (CSP) and *G. atrum* polysaccharides (PSG) were combined or separated administered for the next 7 days. CSP was partial to inhibiting Th17 cytokines (IL-17 and IL-21), and PSG 180 mg/kg was partial to elevating Treg cytokines (IL-10 and TGF-β3) stimulated by CP treatment and significantly reduced the expression of MyD88, revealing a suppression on TLR signaling. Moreover, PSG promoted secretory immunoglobulin A (sIgA) secretion and significantly activated transcription factor RORγt, along with a slight activation of Foxp3 resulting in a mildly elevated the ratio of Foxp3/RORγt compared with CP-treated group, which was related Treg/T helper 17 (Th17) balance. In conclusion, combined fungal polysaccharides contribute to the reinforcement of the Treg/Th17 balance and inhibition of pro-inflammation activities [51].

Tsai et al. (2012) reported that two components of *G. lucidum* polysaccharides were obtained by acid hydrolysis: peptidoglycan GLPS-SF1 (molecular weight about 20 kDa, glucose/mannose about 4:1) and oligosaccharide GLPS-SF2 (molecular weight is about 7 kD). Both of these components can induce CD69 on the surface of monocytes, T lymphocytes, and NK cells in human peripheral blood mononuclear cells (hPB-MNCs) and promote the production of Th1 type cytokine IL-12 by hPB-MNC and promote IL-2, TNF-α, and IFN-γ expression. GLPS-SF1 induced production of CD80/CD86 costimulatory molecules, IL-12, and TNF-α in $CD14^+$ monocyte. In normal mouse macrophages (HeNC2), GLPS-SF2 can activate and promote proliferation of T lymphocytes and NK cells and induce production of IL-2 and IFN-γ [52].

Pi et al. (2014) reported that *G. formosanum* polysaccharide (PS-F2) can stimulate DC cells to produce pro-inflammatory cytokines in vitro, including TNF-α, IL-6, and IL-12/IL-23 p40. PS-F2 can also stimulate DCs; express mature marker molecules CD40, CD80, CD86, and MHC II; and increase T lymphocyte and IFN-γ expression in cultured mouse spleen cell. The experimental results suggested that PS-F2 can promote the production of specific antibodies stimulated by ovalbumin (OVA), promote the expression of IFN-γ, and induce OVA-specific CTL cells to protect mice from tumor cell attack. Therefore, PS-F2 can induce Th1 adaptive immune response [53].

Habijanic et al. (2015) obtained five fractions of extracellular and cell wall polysaccharides by extraction, ethanol precipitation, and purification by ion exchange and gel and affinity chromatography from *G. lucidum* submerged liquid substrate cultivation. The results showed that fungal cell wall polysaccharides were stronger innate

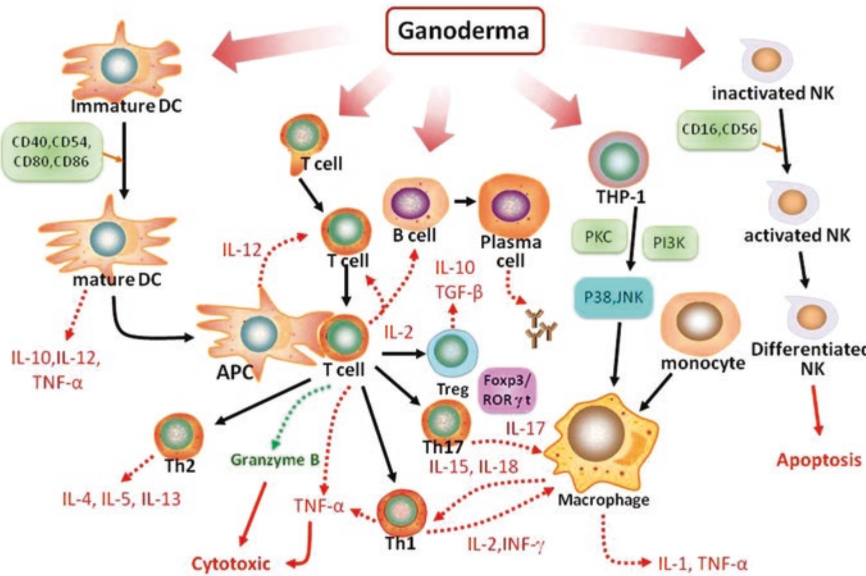

Fig. 1.4 The immunomodulatory effects of *Ganoderma* on various immune cells

inflammatory cytokine inducers, while extracellular polysaccharides demonstrated a higher capacity to modulate cytokine responses of IONO+PMA induced production of IL-17. The results indicate that *G. lucidum* polysaccharides enhance Th1 response with high levels of IFN-γ and IL-2 and display low to no impact on IL-4 production. A similar pattern was observed at regulatory cytokine IL-10. All of the polysaccharide fractions tested induced IL-17 production at different concentration levels [54].

A summary of the immunomodulatory effects of GLPS on various immune cells and possible mechanisms were shown in Fig. 1.4. GLPS stimulates B-cell proliferation and activation, promotes T cell releasing TNF-α and INF-γ, increases immature DC activation and maturation, enhances macrophage differentiation and maturation, and sensitizes NK cell-mediated cytotoxicity.

1.3 Possible Receptors and Signaling Cascade Pathway Triggered by *Ganoderma*

1.3.1 Toll-Like Receptors and Signaling Cascade Pathway

Due to the complex structure of polysaccharides, the monosaccharide composition and glycoside bond connections were affected by different sources, extraction sites, and extraction methods. So the binding of receptors of immune cells and the signal transduction pathways triggered by *G. lucidum* polysaccharides are also different.

Pattern recognition receptor (PRR) on the membrane of DC or macrophage, which combines pathogen-associated molecular pattern (PAMP) on the surface of pathogenic organisms, is the key to initiating innate immune response. At present, it is believed that *G. lucidum* polysaccharides contain different glycoside bonds such as (1 → 3), (1 → 4), and (1 → 6)-β-glucans and (1 → 4), (1 → 6)-α-glucans, linked glucans, or polysaccharide-peptide complexes formed by protein binding. β-Glucan mainly exists in the cell walls of fungi and bacteria and can be recognized by the innate immune system (Fig. 1.5).

Toll-like receptors (TLRs) are a class of innate immune-related pattern recognition receptors (PRRs), which play an important defensive role against a variety of microbes. Activation of TLR receptor can induce the secretion of immunostimulatory cytokines, leading to an enhanced immune response based on a cytotoxic T-cell response. As well known, TLR4 is mainly expressed in macrophages, recognizing LPS, or some endogenous heat shock proteins such as HSP69 and HSP70. Studies by Shao et al. (2004) have shown that fluorescein-labeled *Astragalus* polysaccharide (fl-APS) binds human and mouse macrophages in a TLR4-dependent manner. Interestingly, flow cytometry analysis showed that *G. lucidum* polysaccharide (Gl-PS) with an average molecular weight of 5.849×10^5 can competently inhibit the binding of fl-APS with mouse peritoneal macrophages. This result suggests that Gl-PS is able to bind directly with TLR4 receptor on the surface of macrophage membrane. Furthermore, Gl-PS can significantly induce IL-1β production by peritoneal macrophages from BALB/c but not C3H /HeJ mice; by contrast, LPS can stimulate IL-1β secretion by peritoneal macrophages from the both. Since there is a base mutation in the TLR4 gene of C3H/HeJ mice, resulting in an amino acid in the cytoplasmic region of TLR4 receptor mutated from proline to histidine, which makes TLR4 lose its signal transduction function, the result suggests that Gl-PS

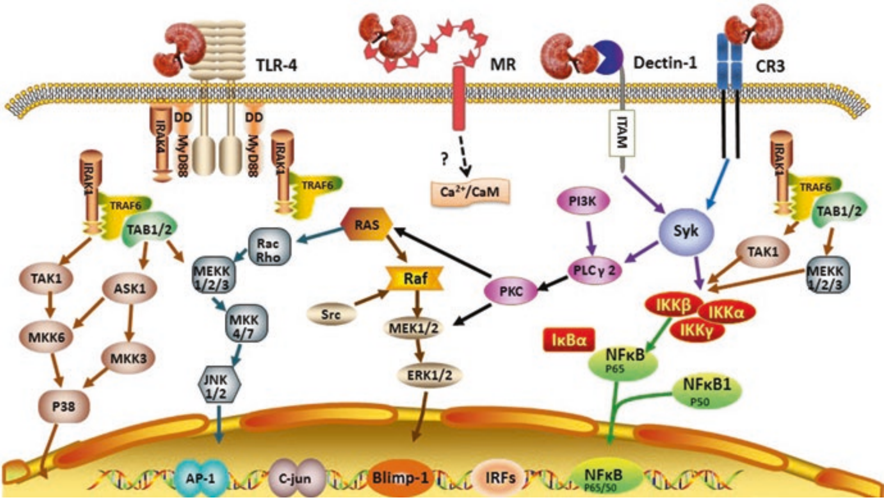

Fig. 1.5 Possible receptors and signaling pathways triggered by *Ganoderma*

activated macrophages via TLR4. Also, a unique 31 kDa serum protein and two intracellular proteins (ribosomal protein S7 and a transcriptional co-activator) are identified that can bind with Gl-PS in co-precipitation experiments. In addition, it was found that Gl-PS could significantly promote the proliferation of purified mouse B cells rather than T cells, and this activity could be blocked by rabbit anti-mouse Ig antibody. Comparing the response of spleen cells from Balb/c and C3H/HeJ mice to Gl-PS, similar to LPS, Gl-PS could significantly stimulate the proliferation of spleen cells from Balb/c mice, but not from C3H/HeJ mice. The result indicated that TLR4 participated in the activation and proliferation of B cells by Gl-PS [55].

Similarly, Wang et al. (2012) found that PS-F2, an extracellular heteropolysaccharide component extracted from *G. lucidum* culture liquid, could induce macrophages to produce TNF-α. The TNF-α production from bone marrow-derived macrophages of C3H/HeJ mice (TLR4 mutant) was significantly lower than that from C3H/HeN mice (TLR4 wild type). It suggested that TLR4 was involved in the activation of macrophages induced by *G. lucidum* polysaccharide [56].

Hsu et al. (2004) demonstrated that TLR4, but not complement receptor type 3, is a putative receptor of the extract of *G. lucidum* polysaccharides (EORP), mediating the consequent immunomodulating events associated with IL-1 gene expression. They have found that the EORP differentially modulates the protein kinase (PK)-mediated signal transduction pathways associated with inflammatory cytokine IL-1. In human macrophages and murine macrophage J774A.1 cells, EORP was found to upregulate IL-1 secretion and precursor of IL-1 (pro-IL-1) as well as IL-1-converting enzyme expression. Specifically, EORP rapidly stimulates protein tyrosine kinase-mediated phosphorylation, followed by induction of PKs and activation of the mitogen-activated protein kinase (MAPK) signaling pathway (ERK, JNK, and p38). These findings establish that EORP induces cytokine expression via TLR4-modulated PK signaling pathways [57]. Batbayar et al. (2011) also found that the extract of β-glucan isolated from *G. lucidum* could induce the expression of Toll-like receptors (TLR2, TLR4, and TLR6) and secretion of cytokines GCSF, IL-6, and TNF-α in RAW264.7 macrophages of mice. If LPS was added, the expression of TLR4 and TLR6 and the secretion of these cytokines could be further increased. The production of IL-1, IL-6, and iNOS was decreased after the administration of NF-κB inhibitors [58].

The immune response triggered by TLRs is mainly mediated by activation of the transcription factor NF-κB. TLR4 is responsible for signal transduction and activates NF-κB signal transduction pathway. Mouse TLR4 consists of a leucine-rich extracellular domain, a single transmembrane region, and an intracellular signal transduction region (TIR) that is highly homologous to the IL-1 receptor cytoplasmic region. Chen et al. (2004) found that TLR4 molecules on macrophages are involved in the activation of macrophages by *G. lucidum* polysaccharides. *G. lucidum* polysaccharide (F3) binds to TLR4 on the macrophage membrane, and TLR4 dimerizes recruit the downstream adaptor protein (adaptor) MyD88, which is

interacted with TIR. MyD88 is a 35 kDa protein with two functional domains. The C-terminus has homology with the Toll cytoplasmic domain and can interact with the homologous domain in the TLR. The N-terminus is a death domain (DD), which may react with DD in the IL-1 receptor-associated kinase (IRAK). MyD88 is combined with IRAK, which has serine/threonine protein kinase activity. The autophosphorylated IRAK can be combined with soluble tumor necrosis factor-related factor 6 (TRAF6) to form a complex. Then oligomerized TRAF6 activates transforming growth factor-β activating kinase (TAK-1), a mitogen-activated protein kinase. To sum up, the signal transduction pathway of *G. lucidum* polysaccharide (F3) binding to TLR receptor on macrophage membrane is as follows: protein-tyrosine kinase PTK (Sac)/PLCγ1/protein kinase C/mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK1)/extracellular signal-regulated kinase (ERK), PTK(Src)/Rac1/PAK(pp21-activated kinase)/p38 mitogen-activated protein kinase (p38 mitogen-activated protein kinase), and PIK/Rac1/PAK/JNK (c-Jun N-terminal kinase), thereby inducing cytokine IL-1 production. Moreover, the role of F3 in activating macrophages to produce IL-1 cannot be inhibited by endotoxin antagonists such as polymyxin B, suggesting that this process is not due to contamination by LPS [59]. Zhang et al. (2013) found that *G. tsugae* polysaccharide (PSG-1) 50, 100, and 200 mg/kg can inhibit the proliferation of splenocytes stimulated by ConA and LPS in S180 tumor-bearing mice, increase the phagocytic activity of macrophages, and promote the cytokine TNF-α, IL-1β, and NO production. PSG-1 upregulates TLR4 expression on the cell membrane surface in a dose-dependent manner and promotes nuclear translocation of p65 subunit and degradation of IκBα by NF-κB. In addition, PSG-1 also increased ERK1/2, JNK1/2, and p38 phosphorylation of the MAPK signaling pathway, thereby upregulating cytokine expression [60]. Lin et al. [7] showed treatment of human monocyte-derived dendritic cells (DC) with *G. lucidum* polysaccharides (PS-G) resulted in enhanced T-cell stimulatory capacity and increased T-cell secretion of IFN-γ and IL-10. Neutralization with antibodies against TLR4 inhibited the PS-G-induced production of IL-12 p40 and IL-10, suggesting a vital role for TLR4 in signaling DC upon incubation with PS-G. Further study showed that PS-G was able to augment IκB kinase and NF-κB activity and also phosphorylation of IκBα and p38 MAPK. Furthermore, inhibition of NF-κB by helenalin and p38 MAPK by SB98059 prevented the effects of PS-G in the expression of CD80, CD86, CD83, CD40, CD54, and HLA-DR and production of IL-12 p70, p40, and IL-10 in various degrees. Taken together, PS-G can effectively and rapidly induce the significant activation and maturation of human DC by the NF-κB and p38 MAPK pathways [61]. Hasnat et al. (2015) reported a protective effect of *G. lucidum* grown on germinated brown rice (GLBR) against colitis via inhibition of MAPK phosphorylation and NF-κB activation. GLBR suppressed the production of nitric oxide (NO) and prostaglandin E2 (PGE2) in LPS-stimulated macrophages and decreased the expression of COX-2, TNF-α, iNOS, IL-1β, IL-6, and IL-10 mRNAs. GLBR also inhibited activation of p38, ERK, JNK, MAPKs, and NF-κB [62].

1.3.2 Other Receptors

The role of non-Toll-like PRRs in natural immunity is also indispensable. They can either cooperate with TLR or play a separate role. Dectin-1 is a kind of non-Toll-like PRR which attracts much attention in recent years. It belongs to NK cell receptor-like C-type agglutinin. It is a type II transmembrane receptor with molecular weight of about 28 kD. It participates in the process of phagocytosis and killing of immune effector cells by recognizing fungi and induces the body to produce a series of cytokines and chemokines, thus participating in the natural immune response of the body against fungal pathogen. Dectin-1 can recognize β-(1 → 3) or β-(1 → 6) glucans from a variety of fungi and plants by its extracellular C-type lectin-like region (CTLD) and bind polysaccharide molecules in a non-calcium-dependent manner, without recognizing monosaccharides or other linked polysaccharides. Dectin-1 was detected in the spleen, thymus, lung, and small intestine of mice by Sung et al. [63]. The expression of IL-6 and TNF-α in RAW264.7 cells induced by LPS could be promoted by the action of β-glucans (GLG) extracted from *G. lucidum*, but GLG alone could not increase the levels of IL-6 and TNF-α. It suggests that Dectin-1 receptor needs to activate signal transduction with CD14 to initiate immune response. Guo et al. (2009) prepared RAW264.7 cells expressing wild-type Dectin-1 and signal transduction deficiency Dectin-1-38, which were incubated with *G. lucidum* spore polysaccharide (GSG) 100 μg/mL for 24 h, respectively. The results showed MAPK- and Syk-dependent TNF-α and IL-6 secretion in murine resident peritoneal macrophages. The production of TNF-α in stable-expressing wild-type Dectin-1 cells increased significantly. The expression of TNF-α could not be increased after signal deletion. Therefore, complete Dectin-1 can mediate the immune activation of *G. lucidum* polysaccharides [64]. Hsu et al. (2009) also confirmed that Dectin-1 could specifically bind the β-glucan component in *G. lucidum* extract by ELISA based on innate immune receptor-Fc fusion protein [65].

In addition, β-D-glucan can also activate immune effector cells (lymphocytes, macrophages, and NK cells) by binding to macrophages and other receptors on lymphocyte membranes, such as complement receptor 3 (CR3), macrophage mannose receptor, DC-SIGN, and langerin, αMb2 integrin, and CD11b/CD18. Zhu and Lin (2005) found that anti-CR3 antibody blocked (60~70%) *G. lucidum* polysaccharide-induced proliferation and cytotoxicity of cytokine killer cells in receptor blocking experiments, which suggest that polysaccharide-specific receptor CR3 may be one of the binding pathways of Gl-PS and mediate some of its immune activities [66].

In research of Wang et al. (2012), besides the TLR4 pathway, the production of TNF-α stimulated by PS-F2 could be antagonized by Dectin-1 antibody and CR3 antibody, suggesting that Dectin-1 and CR3 are involved in the activation of macrophages. This was also demonstrated by a “LPS tolerance” test. Pre-stimulated with LPS or PS-F2 for 5 h, RAW264.7 cells were subjected to secondary stimulation with LPS or PS-F2. LPS-exposed macrophages failed to show a further TNF-α production after the second LPS challenge. However, subsequent PS-F2 stimulation

could further increase the production of TNF-α in either LPS or PS-F2 pretreated cells, which may be due the activation of Dectin-1 and CR3 [56].

Spleen tyrosine kinase (Syk) is a common downstream signaling molecule of Dectin-1 and CR3. The cytoplasmic tail of Dectin-1 contains an immunoreceptor tyrosine-based activation motif (ITAM), which is transformed into a Src family kinase. A succession of phosphorylation of tyrosine, ITAM motifs activation by enrichment of phosphorylation, and Syk activation causes intracellular signaling cascades, such as MAPK phosphorylation and NF-κB activation, ultimately leading to various cell-specific responses. Guo et al. (2009) found that Dectin-1 in GSG (a water-soluble polysaccharide extracted from the spores of *G. lucidum*)-activated macrophages can be immunoprecipitated by anti-myc antibody [67]; Wang et al. (2012) found that TNF-α production in PS-F2-stimulated macrophages can be specifically inhibited by the Syk kinase inhibitor piceatannol. And Syk kinase inhibitors also block I-κB degradation and ERK phosphorylation, but do not affect the phosphorylation of p38 and JNK [56]. So the downstream signaling cascades of macrophage activation and TNF-α production may involve Syk, JNK, p38, ERK, and NK-κB.

Mannose receptor (MR), as one of C-type lectin family members, recognizes the carbohydrate structures through carbohydrate recognition domains (CRDs) in their extracellular carboxy-terminal domains. MR is present in macrophages, dendritic cells, specific lymphocytes, etc. Li et al. (2017) reported that treatment of a *Ganoderma atrum* polysaccharide (PSG-1) on mouse peritoneal macrophages was found to increase the expression of MR and the levels of TNF-α and IL-1β in culture supernatant significantly in a dose-dependent manner. Addition of mannan (a MR inhibitor) remarkably attenuated the PSG-1-induced increase of phagocytosis in macrophages and concentrations of IL-1β, but no significant effect on TNF-α production. Furthermore, treatment of macrophages with LPS significantly decreased MR expression, while addition of different concentrations of PSG-1 may elevate the expression of MR compared to the LPS group. Similarly, in pretreatment with anti-MR antibody, the inhibitory effect of PSG-1 on LPS-induced increases of the phagocytic ability and IL-1β secretion were partly reduced, as well as the promoting effect of PSG-1 on LPS-induced IL-10 secretion. There is no influence on TNF-α production suggesting that the other receptors may be also involved in PSG-1-triggered immunomodulatory effects. Further studies demonstrated the coordination of MR and TLR4 in PSG-1-mediatd host immune response via the NF-κB signaling pathway [68].

Although we have found some experimental evidence about the receptor-signaling events related to the roles of *Ganoderma* polysaccharides, we still do not know the exact signaling pathways involved [69]. The binding of *G. lucidum* polysaccharides with different receptors may trigger the activation of intracellular signal transduction pathways to different degrees, and different signal transduction pathways may overlap partially. In some way, it increases the complexity of elucidating the precise mechanism of *Ganoderma* polysaccharides' immunomodulatory effects. These effects may be due to the different signaling pathways mediated by various components of *Ganoderma* through different receptors, which is summarized in Fig. 1.5.

1.4 Anti-allergic Effect of *Ganoderma* and Its Possible Mechanism

Some other studies showed that *G. lucidum* also could downregulate the excessive immune function. It appears that the cytokine-modulating effect of *G. lucidum* polysaccharides would be tissue-specific. Our original study found that the fermentation concentrate of *G. lucidum* can significantly inhibit the passive cutaneous anaphylaxis reaction induced by ovalbumin (OVA) antiserum and tetanus antiserum. We observed the effect of different fractions extracted from *G. lucidum* on releases of histamine and allergic slow reactive substances (SRS-A) in guinea pig lung tissue actively sensitized by OVA and tetanus toxoids. The results showed that the *G. lucidum* fermentation concentrate can significantly inhibit the release of histamine and SRS-A, with the intensity proportional to the concentration of *G. lucidum* [70]. *G. lucidum* 600 mg/kg i.p. for 3 days can significantly inhibit Forssman cutaneous vasculitis (FCV) and alleviate their general symptoms of Forssman systemic shock (FSS) in guinea pigs. It was also found that *G. lucidum* at 300 or 600 mg/kg i.p. for 3 days could decrease the skin swelling of reversed cutaneous anaphylaxis (RCA) in rats; thus even by oral administration, *G. lucidum* at 800 mg/kg i.g. for 6 days appeared to exert the same effect [71]. *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 [72].

The active ingredients and mechanism of anti-allergic effect of *G. lucidum* have not yet been fully elucidated. Airway inflammation and Th2 response play central roles in the pathogenesis of allergic asthma. Chen and Lin (2007) investigated the anti-inflammatory effects of triterpenoid extracts of *G. tsugae* on airway responses and regulatory effects on Th2 responses in vivo. BALB/c mice were sensitized intraperitoneally and challenged with OVA to induce asthma. After treated with either triterpenoid-rich extracts (TRE) of *G. tsugae* for 2 weeks, TRE significantly decreased bronchial airway hyperresponsiveness (AHR) and reduced the total infiltrating leukocytes and eosinophils, as well as the levels of inflammatory mediators, such as IL-4, IL-5, and eotaxin, in bronchoalveolar lavage fluid. In vitro experiments showed that TRE suppressed IL-5 secretions from OVA-stimulated splenocytes, but did not affect the cell number of splenocytes. Although OVA-specific IgE levels did not change significantly, OVA-specific IgG1 levels, another Th2-related antibody, were found lower in TRE treatment [73].

Pi et al. (2014) found that a polysaccharide fraction purified from the submerged culture broth of *Ganoderma formosanum* (PS-F2) can suppress Th2-mediated bronchial inflammation and the development of AHR in a murine model of allergic asthma. BALB/c mice were sensitized by repeated immunization with chicken OVA and alum, followed by intranasal challenge of OVA to induce acute asthma. PS-F2 administration at the course of OVA sensitization and challenge effectively prevented AHR development (airway responses to methacholine presented as the ratio of the lung resistance), reduced OVA-specific IgE and IgG1 production, and inhib-

ited bronchial inflammation (Fig. 1.6) and Th2 cytokine (IL-4, IL-5, and IL-13) production. However, PS-F2 does not inhibit extravasation of plasma, suggesting that its site of action may be not related to mast cells and histamine receptors [74].

An anti-asthma herbal medicine intervention ASHMI, composed of extracts from three traditional Chinese medicinal herbs, Lingzhi (*Ganoderma lucidum*), Kushen (*Sophora flavescens*), and Gancao (*Glycyrrhiza uralensis*), in the percentages of 62.5, 28.1, and 9.4, respectively (each capsule contained 0.3 g dried aqueous extract, equivalent to extracts of a mixture of the raw herbs Lingzhi 1.6 g, Kushen 0.75 g, and Gancao 0.25 g), is systematically studied both in vivo and in vitro. Jayaprakasam et al. (2013) compared the activity of ASHMI with the three individual components extracts of Lingzhi (L), Kushen (K), and Gancao (G) in vitro. The results demonstrated that ASHMI can synergistically inhibit Th2 cytokine secretion by murine memory Th2 cells (D10.G4.1) and eotaxin-1 production by human lung fibroblast (HLF-1) cells, with the mean 25% inhibitory concentration (IC_{25}) values of 30.2, 263, 123.2, and 100 mg/mL (for IL-5); the mean 50% inhibitory concentration (IC_{50}) values of 158.5, 239.9, 446.7, and 281.8 mg/mL, respectively, for IL-4; and IC_{50} values of 38.1, 33.1, 100, and 158.5 mg/mL, respectively, for eotaxin-1 [75]. Further in vivo study showed that ASHMI treatment was efficacious in a neutrophil-predominant murine model of ragweed (RW) asthma, with markedly reduced AHR, mucous production, and neutrophilic inflammation (Fig. 1.7), via modulation of innate chemokines (TNF-α, IL-8, and IL-17 levels decreased), reduction of TH2 responses (reduced IL-5 and IL-13), and suppression of NF-κB activation (decreased pIκB and increased HDAC2 expression) [76]. Chronic oral ASHMI administration abrogated AHR in OVA-induced murine asthma models and was associated with suppression of airway eosinophilic inflammation, decrease of plasma histamine and leukotriene releasing following allergen challenge, and reduction of Th2 cytokine production. Otherwise, these effects persisted for at least 8 weeks post-therapy [77]. As an herbal medicine, ASHMI™ has received approval of US Food and Drug Administration (USFDA) for phase I and II clinical trials (IND No. 71526) for treating asthma. In the controlled phase I study of adult patients with allergic asthma, a total of 20 subjects (8 subjects on placebo and 12 on ASHMI, receiving 2, 4, and 6 capsules twice a day for 1 week) completed the study. The results showed that no serious AEs among subjects at all doses were detected, as well as adverse effects on liver, kidney, and adrenal function. Similarly, it reduced symptom scores and decreased β2-agonist use. But no statistically significant effects in Th1/Th2 responses were observed possibly due to the small sample size and short duration [78]. Wen et al. (2005) reported a randomized, double-blind, placebo-controlled clinical study performed in China to compare the efficacy, safety, and immunomodulatory effects of ASHMI treatment (4 capsules, three times a day, for 4 weeks) in patients with moderate-severe, persistent asthma with prednisone therapy. ASHMI significantly reduced serum Th2 cytokine levels (IL-5 and IL-13) and increased IFN-γ levels, suggesting an immunomodulatory effect, but not overall immune suppression [79]. The same author (2012) observed the efficacy and safety of a similar Chinese herbal medicine compound prescribed, the decoction of raw herbs Lingzhi 20 g, Kushen 4 g, and Gancao 3 g (74%, 15%, and 11%,

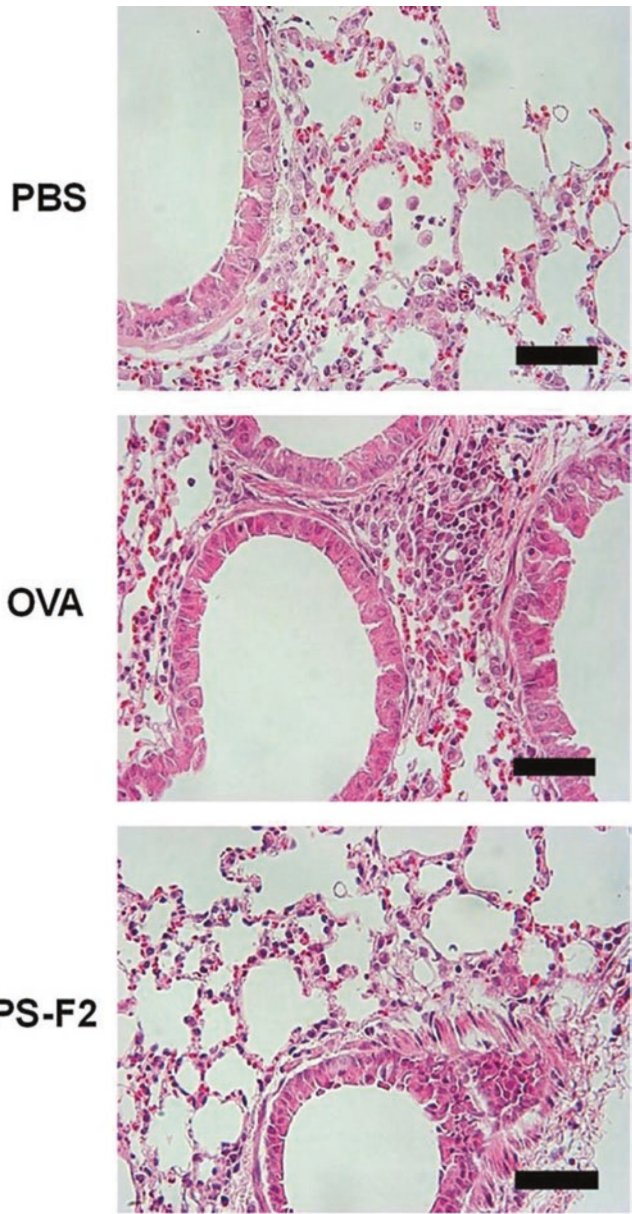

Fig. 1.6 PS-F2 treatment attenuates inflammatory cell infiltration of the airways. Mice were immunized with OVA + alum or PBS on days 0, 10, and 20. Mice also received treatment with PS-F2 or PBS on the indicated days. All animals were challenged i.n. with OVA on day 27. On day 28, lung sections were prepared, stained with hematoxylin and eosin, and photographed under light microscopy at × 400 magnification (scale bar = 50 μm). Prominent infiltrates of inflammatory cells are present in OVA group mice but not in PBS and PS-F2 group mice. (Reproduced with permission from Ref. 74)

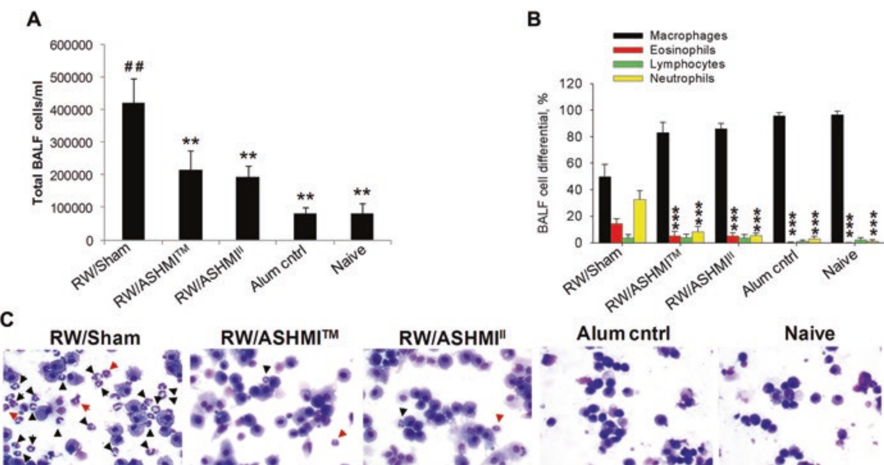

Fig. 1.7 Treatment with antiasthma simplified herbal medicine intervention (ASHMI) or refined ASHMI (ASHMIII) decreased neutrophil-eosinophil airway inflammation. (**a**), Total number of bronchoalveolar lavage fluid (BALF) leukocytes. (**b**), Percentage of BALF macrophages (black), eosinophils (red), lymphocytes (blue), and neutrophils (yellow). (**c**), Representative illustration of neutrophil predominance among granulocytes (black arrows point to neutrophils; red arrows point to eosinophils) in BALF from ragweed (RW)/sham mice, which was markedly reduced compared with RW/ASHMI- and RW/ASHMIII-treated mice. A and B, Data expressed as mean (SD). n = 5 mice per group. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. (Reproduced with permission from Ref. 76)

respectively), once a day for 12 weeks on patients with bronchial asthma in China. A total of 552 patients with asthma were involved in the randomized, placebo-controlled clinical study, and no abnormal parameter was reported. In the conventional therapy plus the decoction group, the clinical symptoms, sign scores, level of IgE and percentage of eosinophilic cells (EC%) of the patients after treatment showed significant improvement than that of placebo group [80]. Zheng et al. (2016) evaluated the influences on pulmonary function and immune function by a conventional therapy plus the same decoction once a day for 12 weeks in patients with bronchial asthma. The results showed a total effective rate of 94% compared with 80% of the control group with a conventional therapy alone. It increased lung function (forced expiratory volume in 1 s, FEV1), reduced peripheral blood $CD4^+$ cells and $CD4^+/CD8^+$, and increased $CD8^+$ cells, revealing a promotion on patients' immune function [81]. This decoction might be useful as a supplementary therapy for asthmatic patients. Aside from polysaccharide, ganoderic acid C1 was found in suppression of TNF-α produced by peripheral blood mononuclear cells from asthma patients [82].

Jan et al. (2011) generated monocyte-derived dendritic cells (MDDCs) from asthmatic children allergic to house dust mites and stimulated with the related

allergen, Der p 1, for 24 h in the presence or absence of *G. lucidum* polysaccharide (PS-G). The results showed that PS-G induced maturation of MDDCs with increased expressions of CD80, CD86, CD83, and HLA-DR and a reduced expression of CD1α on the cell surface. After incubation with PS-G and Der p 1, MDDCs produced higher amounts of IL-12 p40, IL-12 p70, IL-6, IL-23, and IL-10 than Der p 1-pulsed DCs. Particularly, PS-G induced a large amount of IL-12 p40 in DCs from healthy compared to allergic donors. IL-12 is a heterodimeric cytokine produced by activated macrophages, neutrophils, and dendritic cells. Endogenous IL-12 p40 selectively inhibits AHR and airway remodeling in an asthma model. The induction of IL-12 p40, IL-12 p70, and IL-10 secretion by PS-G may regulate the immune balance toward Th1 and Th1/Th2 immunity. Furthermore, when naïve autologous T cells were co-cultured with Der p 1-pulsed MDDCs, production of Th1 cell cytokine (INF-γ) was highly increased. Naïve T cells stimulated by MDDCs pulsed with Der p 1 failed to produce proliferation of T cells but increased the production of Th2 type cytokine (IL-5), whereas the addition of PS-G to Der p 1 induced a significant proliferation of T cells and a significantly reduced production of IL-5 similar to that observed with PS-G alone. The production of other Th2 cell cytokines (IL-4, IL-13) and Th17 cytokines (IL-17A) was not affected [83]. Although some studies have shown that Th17 cytokines are associated with a variety of allergic diseases such as contact dermatitis and psoriasis, elevated IL-17 concentrations have also been found in the lung and blood of allergic asthma patients and have been linked to the severity of asthma, and the results suggest that whether Th17 cells play a special role in asthma is still controversial.

The above results suggest that *G. lucidum* extract exerts anti-allergic effects by attenuating Th2 response rather than overall immunosuppression, but its mechanism and target of the active ingredient have not yet been fully elucidated.

1.5 *Ganoderma* Improves Immune Dysfunction by Various Causes and the Possible Mechanisms

The active ingredients of *G. lucidum* can enhance the immune function of normal mice; on the other hand, it can significantly restore the immune function caused by aging, stress, and drugs (including immunosuppressive drugs such as cyclosporine A, cyclophosphamide, fluorouracil, mitomycin C, cytarabine, etc.) [84]. Moreover, chronic inflammation can be correlated well with the onset of a plethora of autoimmune disorders, such as systemic lupus and polymyalgia, rheumatic and other diseases like inflammatory bowel diseases, and cardiovascular disorders. Ideal drugs should effectively inhibit the development of chronic inflammation without interfering in normal homeostasis. *G. lucidum* has been found to possess the immunomodulating and immuno-potentiating capabilities and has been applied in these immune dysfunction diseases.

1.5.1 Ganoderma Improves Immune Function Decline Due to Aging

Declining immune function is one of the most obvious features of aging. In fact, since the beginning of adolescence, the thymus is progressively degraded. The thymus-controlled T-cell function and its ability to produce cytokines are associated with an increase in age, which is the main cause of poor immune function in the elderly. Besides, B-cell function regulated by the bone marrow and its ability to secrete immunoglobulin also decreased. Modern research has shown that the decline in immune function caused by aging can be delayed or partially restored. Among the many measures and drugs to prevent the decline of immune function, *G. lucidum* has been proved to be effective. A series of studies demonstrated that *G. lucidum* polysaccharide could restore the reduced PFC response, ConA-induced lymphocyte proliferation response, and MLR to near the normal levels in aged mice, indicating that *G. lucidum* polysaccharide could significantly regain the decreased humoral and cellular immune functions due to aging. At the same time, *G. lucidum* polysaccharides can also increase the declined production of IL-2 and enhance the activity of DNA polymerase α in aged mice, which is the most important molecular biological basis for its recovery of senile immune function [35].

1.5.2 Ganoderma Improves Immunosuppression Caused by Stress

Significant stress-induced immunosuppression was observed when the mice were forced to swim in cold water for 5 min every day for 10 days; delayed skin allergic reaction (DCH) induced by dinitrochlorobenzene (DNCB) in mice was reduced by about 50%. When a simultaneous daily administration of *G. tsugae* polysaccharides (50, 100 mg/kg), the DCH reaction could be maintained at a near-normal level. It is indicated that the *G. tsugae* polysaccharide can antagonize the inhibition of cellular immune function induced by stress [85]. Shi et al. (2012) reported that mice were subjected to 5% weight-bearing swimming training, 6 days a week for 4 weeks, simulating a long-term high-intensity exercise model and simultaneously administering *G. lucidum* polysaccharide 50, 100, and 200 mg/kg. The results showed that long-term high-intensity exercise caused a significant decrease in phagocytosis of peritoneal macrophages and also a decreased secretion of NO and IL-1β. *G. lucidum* polysaccharide (GLP) could increase peripheral blood leukocytes (WBC) and the absolute value of neutrophils (NEUT), enhance the phagocytosis of the macrophages and plaque-forming cells and thus reverse the immune function impairment to near-normal level in a dose-dependent manner [86].

Zhang et al. (2008) reported effect of *G. lucidum* capsules on T lymphocyte subsets in football players on “living high-training low.” Forty male football players

were randomly assigned to four groups: control (living at sea level), LHTL1, LHTL2, and LHTL3. The three LHTL groups had stayed in normobaric hypoxic (O_2 15.4%) rooms to simulate an altitude of 2500 m for 4 weeks. The four groups trained together at sea level. LHTL1, LHTL2, and LHTL3 groups were orally administrated with placebo, *G lucidum* 10 capsules/day and *G lucidum* 20 capsules/day, respectively, for 6 weeks (2 weeks of before treatment with *G. lucidum* and 4 weeks of following treatment with *G. lucidum*). T lymphocyte subsets were detected by flow cytometry. The results showed that LHTL could affect T lymphocyte subsets significantly as a result of the two simultaneous stimuli of physical activity and exposure to hypoxia. The ingestion of *G lucidum* in the LHTL3 group could help to ameliorate the reduction in the $CD4^+/CD8^+$ ratio in LHTL training [87].

1.5.3 Ganoderma Improves Immunosuppression Caused by Drug

It is well known that patients with morphine and heroin addiction often have immune dysfunction. Improving the immune function of such patients is part of the comprehensive treatment of detoxification. We found that *G. lucidum* polysaccharide peptide (GLPP) antagonized immunosuppression induced by high concentrations of morphine in vitro. Morphine (0.063~0.5 μmol/mL) can significantly inhibit phagocytosis of peritoneal macrophages, lymphocyte proliferation, and production of IL-1 and IL-2 in mice. While morphine with GLPP is added to the cell cultured media in vitro, an antagonistic effect is observed. GLPP (50~800 μg/mL) can reverse the reduction of phagocytosis of macrophages, lowering of IL-1 and IL-2 production, and decrease of lymphocyte proliferation to the normal level [88]. Further study was performed to explore the influences of GLPP on the immunosuppressive effects of morphine-dependent mice induced by repeated morphine administration. The mice were successively subcutaneously injected with morphine 20, 30, 40, and 50 mg/kg twice a day (8:00 a.m., 16:00 p.m.), to establish a stable morphine body dependence model after 4 days of injection. GLPP were administered alone or in combination with morphine at a dose of 50 mg/kg. The results showed that the phagocytic function of peritoneal macrophages, the ability to induce TNF and IL-1, delayed allergic reaction (DTH) and hemolysis ability of plague-forming cell (PFC), ConA- or LPS-induced lymphocyte proliferation reaction, and mixed lymphocyte culture reaction were significantly inhibited in morphine-dependent mice. GLPP reversed the inhibitory effects of morphine to normal levels. The authors also found that GLPP's immune enhancement effect on morphine-dependent mice was significantly greater than that on normal mice and speculated that GLPP's antagonism effects may be achieved through regulation of neural-endocrine-immune networks [89].

At present, chemotherapy is still one of effective methods for clinical treatment of cancer. But chemotherapy has different degrees of toxic and side effects. For

example, leukopenia often occurs after chemotherapy in particular neutropenia, thrombocytopenia, etc. The serious reduction of the body's immune function or even immune function defects greatly limited the use of chemotherapy drugs and affected the drugs' therapeutic effect. Therefore, finding a means to alleviate the immunosuppressive effect of chemotherapy drugs is of great significance. Cyclophosphamide, a chemotherapy drug, is an alkylation-based immunosuppressant that inhibits the immune response and causes damage to the liver and antioxidant enzymes. Zhu et al. (2007) found that in cyclophosphamide-induced immunosuppressive mice, a low-dose GLPS (2.5 mg/kg, intraperitoneal injection once a day) could promote restoration of bone marrow cells, red blood cells, white blood cells, spleen NK cells, and natural killer T cells. Also, T- and B-cell proliferative responses, cytotoxic T lymphocyte activity, and NK cell and lymphokine-activated killer (LAK) cell activities were also enhanced [33].

We found that *G. lucidum* polysaccharide (Gl-PS) (0.2, 0.8, 3.2, or 12.8 μg/mL) can significantly enhance the lymphocyte proliferation ratio of mouse MLC response induced by ConA or LPS and can functionally antagonize the inhibition on mouse MLC by the immunosuppressant cyclosporine A (CsA) or mitomycin C (mitomycin, Mit) or the antitumor drug etoposide (VP-16) in vitro. These results suggest that in combination with antitumor drugs, *G. lucidum* can antagonize the immunosuppressive effects of antitumor drugs, which helps to enhance efficacy and reduce adverse reactions [90]. Similarly, Nonaka et al. (2008) observed that the antlered form of *G. lucidum* had a moderate recovery effect on body weight, NK cell activity, IFN-γ, and cytotoxic T-cell (CTL) activity in cyclophosphamide-immunosuppressed mice and increased production of IL-4 [91]. Li et al. (2011) reported that the combination of *G. atrum* polysaccharide (PSG-1, 25 mg/kg, p.o.) can increase the spleen index and thymus index, inhibit tumor growth, and prolong the survival time in cyclophosphamide-treated S180 tumor-bearing mice. Further study revealed that PSG-1 increased the antitumor activity of CTX via mitochondrial apoptotic pathways (increase of apoptosis, activation of caspase-3 and caspase-9 activities, loss of Bcl-2 from mitochondria, and Bax translocation); meanwhile, increased ConA- and LPS-stimulated lymphocyte proliferation elevates the reduced serum TNF-α and IL-2 levels to some degree [92].

1.5.4 Other Diseases with Dysregulated Immune Responses

Inflammatory bowel disease (IBD) is a chronic disorder caused by dysregulated immune responses to intestinal microorganisms that occurs within all or parts of the intestinal tract. A report demonstrated that ganoderic acid C1 may inhibit the secretion of TNF-α and other inflammatory cytokines including IFN-γ and IL-17A by

PBMCs and inflamed intestinal biopsy tissue from Crohn's disease patient [93]. Dysregulated immune responses are involved in the pathogenesis of dextran sulfate sodium (DSS)-induced colitis. Wei et al. (2018) revealed that *G. lucidum* polysaccharides (GLP, 100 mg/kg, i.g. for 2 weeks) can regulate the intestinal immunological barrier functions in mice and attenuate symptoms of DSS-induced acute colitis. Meanwhile, GLP markedly suppressed the secretions of TNF-α, IL-1β, IL-6, IL-17A, and IL-4, as well as significantly affected populations of Th17 cells, B cells, NK cells, and NKT cells in the lamina propria lymphocytes (LPL). The percentage of $B220^{+}CD3^{-}$ cells in LPL significantly increased in GLP-treated mice, while $CD3^{+}B220^{-}$ and $NK1.1^{+}$ $CD3^{-}$ cells (NK) significantly decreased; the percentages and absolute numbers of $CD4^{+}CD44^{+}$, NKT ($NK1.1^{+}$ $CD3^{+}$), and IL-17A-producing $CD4^{+}$ T cells in LPL were significantly lower in the GLP-treated acute colitis group. That is, GLP can promote the proliferation of B cells; regulate Th1, Th17, and Th2 cell responses; and inhibit the proliferation of NK cell and NKT cells in LPL of colon, so as to maintain intestinal homeostasis and regulate the intestinal immunological barrier functions in mice with DSS-induced colitis [94].

1.6 Summary and Perspectives

As mentioned above, a number of studies have proved that *Ganoderma* possess superior extensive immunomodulation properties and deeply elucidated the mechanism involved in the cellular and molecular regulation on immune components, as well as the signal pathways, including promoting the function of APC, humoral immunity, and cellular immunity. Unlike "Western medicine" with one single active chemical substance, the multitarget pharmacological activities of *Ganoderma* arise from a mixture of active ingredients with different chemical composition and configuration and physical properties, such as polysaccharides, triterpene, etc. Although it is difficult to correlate the structure and activity of complex polysaccharides, some possible relationships can be inferred. It has been reported that most of the *Ganoderma* polysaccharides show the same basic β-glucan structure with different types of glycosidic linkages [95]. Some structural features such as β-1,3-linkages in the main chain of the glucan are needed for immunomodulating activities [96]. However, a study of the relationship between structure and efficacy of *G. lucidum* is needed in the future.

In addition, the pharmacological results from in vivo experiments are more important than those from in vitro experiments. More in vivo studies and randomized controlled clinical trials with placebo should be performed to further elucidate the mechanisms of the immunomodulating and immuno-potentiating capabilities of *Ganoderma*, and the molecular mechanism should be studied intensively.

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Chapter 2
Antitumor Effect of *Ganoderma* (Lingzhi) Mediated by Immunological Mechanism and Its Clinical Application

Zhibin Lin and Lixin Sun

Abstract The antitumor effect of *Ganoderma* (Lingzhi) is closely related to immunoregulation. Based on our research and other references, this article discussed the antitumor effect of *Ganoderma* mediated by immunological mechanism, including promoting the function of mononuclear-macrophages and natural killers; promoting M1-type macrophage polarization vs M2-type; promoting maturation and differentiation of dendritic cells, increasing its antigen presentation, activating lymphocytes and increasing cytotoxicity of cytotoxin T lymphocyte; promoting production of cytokines; and inhibiting tumor escape from immune surveillance. Also, clinical studies with immunological indexes were reviewed.

Keywords *Ganoderma* · Lingzhi · Polysaccharides · Triterpenes · Immune · Tumor

2.1 Introduction

Ganoderma (Lingzhi) is a famous traditional Chinese medicine in China. More than 2000 years ago, *Ganoderma* was published in *Shen Nong Ben Cao Jing* (*Shennong's herbal classic*) and listed as effective and non-toxic drug. Since 2000, the Chinese pharmacopoeia listed fruiting body of *Ganoderma lucidum* (Leyss. ex Fr.) Karst (Chi Zhi) and *Ganoderma sinensis* Zhao, Xu et Zhang (Zi Zhi) as the legal traditional Chinese medicine [1].

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Modern research of phytochemistry and pharmacology shows that *Ganoderma lucidum* contains polysaccharides, triterpenes, sterols, and small molecular proteins and has a wide range of pharmacological effects such as immunoregulation, antitumor effect, protection from radiotherapy- and chemotherapy-induced injuries, sedative and hypnotic effect, anti-cardiac ischemic effects, cerebral hypoxia-reoxygenation injury avoidance, hypotensive effect, blood lipid regulation, hypoglycemic effect, DNA polymerase activity enhancement, nucleic acid and protein syntheses, hypoxia tolerance, anti-oxidation and free radical scavenging effect, anti-aging, chemical and immunological liver injury prevention, anti-gastric ulcer, etc. Toxicological study demonstrates that *Ganoderma lucidum* has extremely low toxicity [2–4]. In addition *Ganoderma* could be used to treat bronchitis, hyperlipidemia, neurasthenia and insomnia, hepatitis, diabetes, cancers, aging, etc. [5].

Present article reviewed the antitumor effect of *Ganoderma* mediated by immunological mechanism and its clinical uses basing on our studies and literatures.

2.2 Antitumor Effect of *Ganoderma* by Immunological Mechanism

2.2.1 Discovery on Antitumor Effect of Ganoderma

Ito H et al. (1977), Kim BK et al. (1980), Miyazaki T et al. (1981), Mizuno T et al. (1984), Lee SS et al. (1984), Sone Y et al. (1985), Maruyama H et al. (1989), Furusawa E et al. (1992), Ma L et al. (1995), and Cao QZ et al. (2004) successively reported that the extract or polysaccharide isolated from fruiting body of *G. lucidum* administrated by i.p. or i.g. can inhibit the growth of xenograft tumor in mice [6–16]. It was considered that antitumor activity of *G. lucidum* in vivo may be "host-mediated," which regulates the body's immunity [17].

To prove this hypothesis, since the 1990s we investigated the antitumor experiments of *G. lucidum* in mice in vivo. In our research results, it was found that the water extract from fruiting body of *G. lucidum* (GLE), *G. lucidum* polysaccharide B (GL-B), *G. lucidum* polysaccharide peptide (GLPP), and broken spore polysaccharide of *G. lucidum* (Gl-BSP) significantly inhibited growth of sarcoma-180, human lung cancer (PG), and Lewis lung cancer in mice in vivo. But they have no inhibitory effect on S180 sarcoma, human leukemia (HL-60), and PG cells, even at the very high concentration such as 400 mg/L of *G. lucidum* polysaccharides by directly being added to the cultured medium in vitro. The results suggest that *G. lucidum* extract and polysaccharide may be enhancing the body's antitumor immunity but do not have direct cytotoxic effect on tumor [18–22].

2.2.2 Antitumor Effect of Ganoderma Mediated by Host Immunity Assayed with Serologic Pharmacology Method

The serologic pharmacological experiment is one of the methods to study the pharmacology of traditional Chinese herb. The serum of the experimental animals was obtained after they were given extracts or components of traditional Chinese herb, and then pharmacological experiments with this serum were carried out in vitro. It is possible that the serum contains traditional Chinese herb or its active metabolites and may also contain endogenous active substances produced under the action.

Using serologic pharmacological method, after addition of *G. lucidum* extract (GLE)-treated serum to S-180 culture media, the results showed that GLE-treated serum could inhibit proliferation of S-180 cells and induce their apoptosis in vitro. Similarly, *G. lucidum* polysaccharide B (GL-B)- or *G. lucidum* polysaccharide peptide (GLPP)-treated serum also inhibited proliferation of HL-60 cells and induced apoptosis in vitro [18–21]. These results suggest that GLE-, GL-B-, or GLPP-treated serum may have the substances with antitumor 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 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 the immune system in vivo. Therefore, according to the results mentioned above, GLE- or GL-B-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 activities of TNF-α in serum treated with GLE (crude material) at 5, 10, and 20 g/kg or CL-B at 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 GLE or GL-B was increased three- to sevenfold or four- to eightfold, respectively [18–20].

In order to simulate the result of serologic pharmacology in vitro. The next step is to study the effect of *G. lucidum* polysaccharides on cytokine production by T lymphocytes and macrophages and the effect of GL-B-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 GL-B for 12~72 h, which were called macrophage culture medium with GL-B (GL-B-M-CM) and T-lymphocyte culture medium with GL-B (GL-B-T-CM). At the dose of 50, 100, and 200 μg/mL, GL-B-MCM or GL-B-T-CM significantly inhibited the HL-60 cell proliferation and induced cell apoptosis in vitro. Moreover, there is a positive correlation between the level of TNF-α in GL-B-M-CM or IFN-γ in GL-B-TCM and the antitumor effect of GL-B-M-CM or GLB-T-CM [19, 20]. Similar results were also found where at the dose of 12.5, 50, and 200 μg/mL, the macrophage culture medium with polysaccharides isolated from

mycelia of *G. lucidum* inhibited proliferation of HL-60 cells and induced apoptosis significantly, with an increased TNF-α level in the cultured supernatant [21, 22].

The subsequent results showed that the addition of *G. lucidum* polysaccharides (50~200 μg/mL) to the in vitro macrophage or T-lymphocyte culture media resulted in a significantly increased TNF-α and IFN-γ mRNA expression in a concentration-dependent manner. Following the administration of the GLE (crude material) at 5, 10, and 20 g/kg by forced stomach tube feeding, TNF-α and IFN-γ mRNA expression was increased markedly [18–20]. These results indicate that the water extract or the polysaccharide fraction of *G. lucidum* could induce TNF-α and IFN-γ mRNA expression in vitro and in vivo.

Wang SY et al. (1997) reported that the polysaccharides of *G. lucidum* (*PS-G*) were isolated from fresh fruiting bodies and used to promote cytokine production by human monocytes-macrophages and T lymphocytes. Results had shown that the levels of IL-1b, tumor TNF-α, and IL-6 in macrophage cultures treated with PS-G (100 μg/mL) were increased by 5.1-, 9.8-, and 29-fold. In addition, the release of IFN-γ from T lymphocytes was also greatly promoted in the presence of *PS-G* (25~100 μg/mL). Furthermore, the mononuclear cell-conditioned media with PS-G (*PSG*-MNC-CM) were found to suppress the proliferation and clonogenicity of both the HL-60 and the U937 leukemic cell lines. DNA labeling and gel electrophoresis showed that treatment with *PSG*-MNC-CM markedly induced leukemic cell apoptosis. Flow cytometric analysis revealed that *PSG*-MNC-CM treatment resulted in a significant increase in the apoptotic population both in the HL-60 and U937 cells. In addition, 40~45% of the treated leukemic cells were triggered to differentiate into mature monocytic cells expressing CD14 and CD68 surface antigens. However, *PS-G* alone had no such effects even at a higher dose of 400 μg/mL. This finding suggests that the antitumor activity of *PSG*-MNC-CM was derived from the elevated levels of cytokines. Antibody neutralization studies further revealed that the antitumor cytokines in the *PSG*-MNC-CM were mainly of TNF-α and IFN-γ [23]. Li et al. (2000) found that *Ganoderma* polysaccharide (GLB7) could increase IL-1 and TNF-α mRNA expression levels in cultured mouse macrophages in vitro. The activities of IL-1α and TNF-α in the supernatants were enhanced markedly in GLB7-treated cultures [24].

Wang et al. (2012) found that broken spore polysaccharide (Gl-BSP) extracted from spore of *G. lucidum* (50, 100, and 200 mg/kg) by intragastric administration significantly inhibited S180 sarcoma growth in mice. Gl-BSP did not inhibit sarcoma-180 and PG cell proliferation in vitro when added directly to the cultured medium; but Gl-BSP (50, 100, and 200 mg/kg)-treated serum of S180-bearing mice markedly inhibited S180 or PG cell proliferation in vitro. We also found that IL-2, IFN-γ, and TNF-α in serum were undetectable in normal mice. Only IFN-γ in serum was detectable in S180-bearing mice. The serum levels of IL-2, IFN-γ, and TNF-α were markedly increased in S180-bearing mice administrated with *Gl*-BSP (200 mg/kg), compared with that in S180-bearing control mice administrated with physiological saline. To determine whether the growth inhibition in sarcoma-180 cells or PG cells induced by *Gl*-BSP 200 mg/kg-treated serum was related to the cytokines released from the immune system activated by *Gl*-BSP in S180-bearing

mice. Serums collected from mice in all groups were preincubated with one or two cytokine- neutralizing antibodies before addition to cell cultures. Neutralization with anti-TNF-α apparently diminished S180 or PG cell lines growth inhibition induced by *Gl*-BSP 200 mg/kg-treated serum, and a similar result was obtained by use of anti-IFN-γ. Blocking effect was noted in the combination of anti-TNF-α and anti-IFN-γ, which reduced the inhibition rate from 55.6% or 45.1% (before neutralization in S180 or PG cell lines) down to 11% or 15.8%, respectively. However, the cytokine antibodies used above did not completely block growth inhibition induced by the *Gl*-BSP 200 mg/kg-treated serum of S180-bearing mice in S180 or PG cell lines, suggesting other bioactive products in the serum, such as NO, and complements may also play a role.

Further research also found that *Gl*-BSP promoted the splenic lymphocyte proliferation induced by Con A or LPS, enhanced natural killer cell (NK cell) cytotoxic activity, augmented the percentage of neutral red phagocytosis by macrophages, and increased the percentage of the $CD4^+$ and $CD8^+$ subset in S180-bearing mice. These findings suggest that the antitumor activity of *Gl*-BSP may be mainly related to the activation of the immune response of the host organism by stimulation of NK cells, T cells, and macrophages [25].

2.2.3 Recent Studies on Immunological Antitumor Mechanism of Ganoderma

Recently, Su et al. (2018) found that the extract extracted from the sporoderm-breaking spores of *G. lucidum* (ESG) effectively inhibited 4 T1 breast tumor xenograft growth, accompanied with a significant necrosis in the tumor tissue, whereas ESG had not directly cytotoxicity in in vitro experiment. Flow cytometry analysis revealed that ESG could significantly increase both Tc subpopulation and the ratio of Tc/Th in peripheral blood of the tumor-bearing mouse; similar promotion on Tc and the ratio of Tc/Th was also found in tumor-infiltrating lymphocyte. Moreover, ESG evidently downregulated the two immune checkpoints, programmed cell death protein-1 (PD-1, in the spleen) and cytotoxic T-lymphocyte antigen-4 (CTLA-4, in the tumor), suggesting that ESG could effectively restore the T cell paradigm by recovering the exhaustion status via suppressing the co-inhibitory checkpoints. By 16S rRNA gene sequence analysis on the fecal microbiota, it was found that ESG would remodel the overall structure of the samples from tumor-bearing mice toward that of the normal counterparts, including 18 genera in 5 phyla, together with regulations on several genes that are responsible for signaling pathways involved in metabolism, cellular processes, and environmental information processing. Collectively, this study demonstrated that ESG would serve as a natural anticancer adjuvant via a restoration on the exhausted Tc, highlighting important clinical implications for the treatment of breast cancer [26].

Zhao et al. (2018) found that *Ganoderma lucidum* extract (GLE) effectively inhibited tumor growth without hepatic/renal toxicity and bone marrow suppression

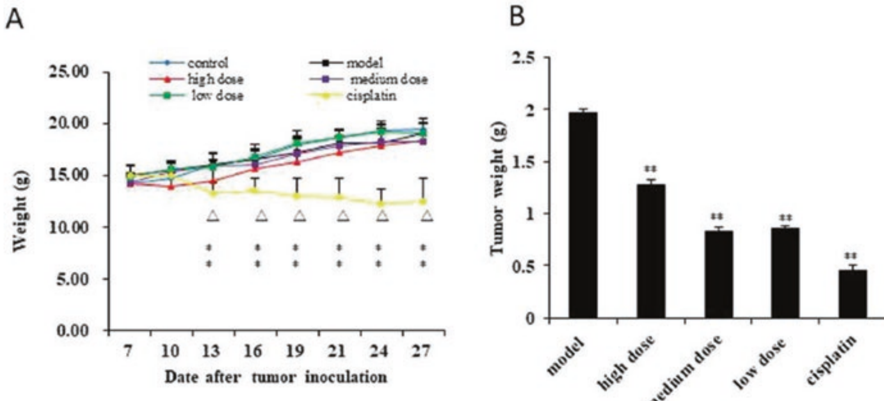

Fig. 2.1 GLE effectively inhibited tumor growth in vivo. (**a**) The weight of mice was significantly reduced in cisplatin group from the 10th day ($P < 0.01$) compared with those in model group and control group. The difference was not statistically significant in GLE treatment group. (**b**) The average tumor weight in GLE treatment and cisplatin groups decreased significantly compared with those in model group ($P < 0.01$). Representative results of three independent experiments are shown. Error bars, SD; $*P < 0.05$; $**P < 0.01$, versus control values; $\Delta P < 0.05$; $\Delta\Delta P < 0.01$, versus model values. (Reproduced with permission from Ref. [27])

and might be enhancing immunological function in Hepa1-6-bearing C57 BL/6 mice (Figs. 2.1 and 2.2). The serum cytokine protein chip showed that GLE significantly regulated the expression levels of serum immune cytokines such as IFN-γ, IL-1β, IL-4, IL-9, IL-12, RANTES, and TNF-α in tumor-bearing mice (Table 2.1). Basing on the mRNA profiles of GLE-treated and GLE-untreated mice, 302 differentially expressed mRNAs were identified and 6 kernel mRNAs were identified from the established protein-protein interaction (PPI) network. Quantitative RT-PCR and Western blot analysis indicated that six mRNAs have had statistically significant differences between the GLE-treated and GLE-untreated mice. Furthermore Western blot and cytokine detection results demonstrated that GLE suppressed growth and proliferation of tumors by the Jak-STAT signaling pathway, T cell receptor signaling pathway, and PI3K-Akt signaling pathway. This study indicated that GLE may have potential immunomodulating effect in the tumor treatment process [27].

Wang et et al. (2018) was designed to identify and characterize the antitumor action and influence of the immune system of GL-PS extracted from fruiting body of *G. lucidum* in glioma-bearing rats. Results showed that GL-PS significantly inhibited glioma growth and prolonged survival of rats. Meanwhile GL-PS also increased the concentration of serum IL-2, TNF-α, and IFN-γ and enhanced the cytotoxic activity of natural killer cells and T cells, promoting the functional maturation of dendritic cells. Therefore, GL-PS may be potentially useful as part of the treatment regimen to regulate host immune responses and increase the antitumor effects of immunotherapy for glioma [28].

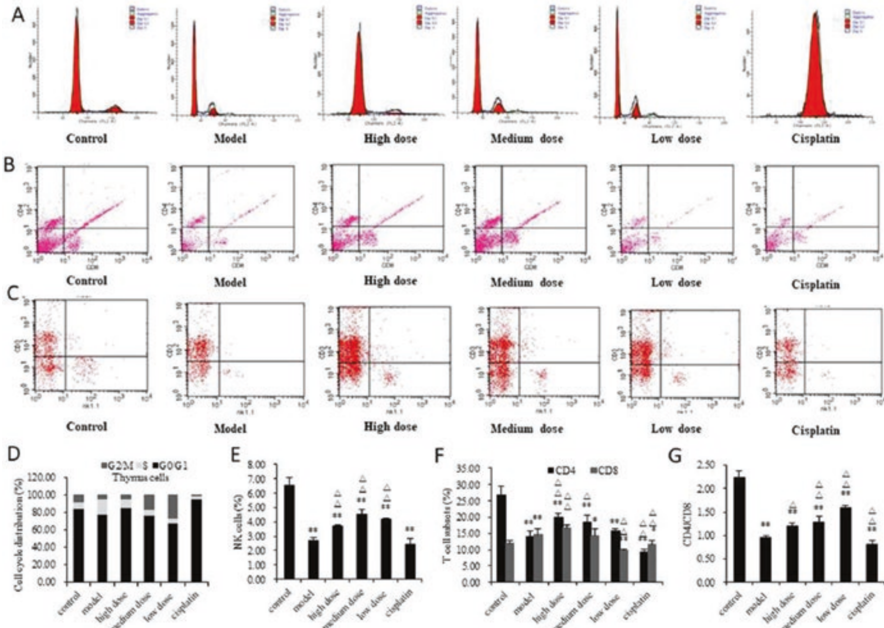

Fig. 2.2 Effect of GLE on immunostimulatory activity of tumor-bearing mice was determined. (**a**, **d**) GLE improved the suppression of thymus cells in tumor-bearing mice. Cell cycle distribution was analyzed by FACS, in order to detect the effect of GLE on the cell cycle progression of thymus. (**b**, **f**, **g**) Effects of GLE on T cell subsets in peripheral blood in tumor-bearing mice were detected. The lymphocyte subsets were detected by flow cytometry. The lymphocyte subset analysis included $CD3^+$ (T lymphocyte), $CD4^+$ (T-helper cells), and $CD8^+$ (T-suppressor cells). Representative results of three independent experiments are shown. (**c**, **e**) Effects of GLE on NK cell activity in tumor-bearing mice. The percentage of NK cells were quantified by FACS. Error bars, SD; $*P < 0.05$; $**P < 0.01$, versus control values; $\Delta P < 0.05$; $\Delta\Delta P < 0.01$, versus model values. (Reproduced with permission from Ref. [27])

2.2.4 Ganoderma Promotes the Maturation and Differentiation of Dendritic Cells and Enhances Their Functions

Dendritic cells (DCs) are antigen-presenting cells (APC) and can highly and effectively uptake, process, and present antigen. Mature DC is highly expressed major histocompatibility complex, MHC)-I-antigen peptide complex–and MHC-II-antigen peptide complex, and B7-1/CD80, B7-2/CD86, CD40 and LFA-3/CD58 co-stimulating molecules, which bond with T lymphocytes and activate T lymphocytes.

Cao and Lin (2002, 2003) firstly found that *G. lucidum* polysaccharides (*Gl*-PS) at the concentrations of 0.8, 3.2, and 12.8 mg/L could increase the co-expression of CD11c and I-A/I-E molecules on cultured murine bone marrow-derived DC surface,

Table 2.1 The changes of serum cytokine concentration in mice after GLE treatment ($x \pm s$, n = 3) [Ref. 27]

Cytokine	Control	Model	GLE
GM-CSF	25.39 ± 1.74	22.89 ± 0.71	29.11 ± 5.04
IFN-γ	138.511 ± 5.00	116.72 ± 1.23*	184.65 ± 4.35$^{**,\Delta\Delta}$
IL-1a	82.77 ± 5.30	74.31 ± 1.72	132.8 ± 41.64
IL-1b	143.02 ± 6.79	136.32 ± 39.36	176.27 ± 31.94$^{\Delta}$
IL-2	260.75 ± 13.80	161.26 ± 13.52*	333.71 ± 101.76
IL-3	5.00 ± 0.14	5.92 ± 2.57	6.31 ± 0.88
IL-4	1.87 ± 0.07	1.33 ± 0.43	6.10 ± 0.89$^{*,\Delta}$
IL-5	7.06 ± 0.46	16.01 ± 2.92*	13.75 ± 3.85
IL-6	21.17 ± 0.16	41.98 ± 8.56	42.28 ± 7.59*
IL-9	312.20 ± 0.34	111.04 ± 2.32**	390.26 ± 0.46$^{**,\Delta\Delta}$
IL-10	271.02 ± 66.32	283.57 ± 38.61	314.09 ± 45.92
IL-12	105.30 ± 26.37	54.90 ± 3.48	110.72 ± 17.74$^{\Delta}$
IL-13	26.29 ± 0.02	44.61 ± 9.57	27.82 ± 2.21
IL-17	7.57 ± 0.29	8.82 ± 3.48	11.02 ± 0.86*
KC	0.54 ± 0.38	5.61 ± 2.17*	1.02 ± 0.31
MCP-1	589.63 ± 36.40	315.41 ± 46.02	613.42 ± 124.53
M-CSF	118.30 ± 25.68	127.09 ± 21.10**	123.21 ± 27.69
RANTES	3.58 ± 0.42	7.45 ± 0.33**	15.68 ± 1.77$^{*,\Delta}$
TNFa	851.19 ± 68.10	803.139 ± 36.36	1129.72 ± 85.82$^{\Delta}$
VEGF	33.65 ± 0.94	48.54 ± 6.44	33.69 ± 4.53

Note: Data in Table 4 was based on Log-Log Regression Standard Curves. *$P < 0.05$; **$P < 0.01$, versus control values; Δ$P < 0.05$; ΔΔ$P < 0.01$, versus model values

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. *Gl*-PS 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 [29]. Cultured murine bone marrow-derived DCs were pulsed with P815 tumor cell lysates and co-incubated with or without various concentrations of *Gl*-PS (0.8, 3.2, or 12.8 mg/L) at the same time. P815-specific CTL were induced by spleen lymphocytes stimulated with mature DC. Non-adherent cells and culture supernatants were harvested on day 5 for analysis of specific cytotoxicity with lactate dehydrogenase (LDH) activity assay, mRNA expression of IFN-γ and granzyme B with RT-PCR assay, and protein expression of IFN-γ and granzyme B with ELISA or Western blot assay, respectively. *Gl*-PS could promote the specific cytotoxic effects of CTL induced by DC pulsed with P815 lysates during the stage of antigen presentation, which may be effective in eliminating tumor escape from immunity. Result found three concentrations of *Gl*-PS promoted LDH activities released into culture supernatants ($P < 0.01$). It also increased mRNA expression of IFN-γ in CTL (*Gl*-PS 12.8 mg/L vs RPMI medium 1640, $P < 0.05$) and granzyme B in CTL ($P < 0.01$). Protein production of IFN-γ in culture supernatants ($P < 0.05$)

and protein expression of granzyme B in CTL (*Gl*-PS 12.8 mg/L vs RPMI medium 1640, $P < 0.05$) were also augmented by *Gl*-PS [30].

Lin et al. (2005) investigated the effects of *G. lucidum* polysaccharide (PS-G) with a branched (1 → 6)-β-D-glucan moiety on human monocyte-derived dendritic cells (DCs). Treatment of DC with PS-G (10 g/L) resulted in the enhanced cell-surface expression of CD80, CD86, CD83, CD40, CD54, and human leukocyte antigen (HLA)-DR, as well as the enhanced production of interleukin (IL)-12p70, p40, and IL-10 and also IL-12p35, p40, and IL-10 mRNA expression, and the capacity of endocytosis was suppressed in DC. In addition, treatment of DC with PS-G resulted in enhanced T cell-stimulatory capacity and increased T cell secretion of IFN-γ and IL-10. Neutralization with antibodies against Toll-like receptor (TLR)-4 inhibited the PS-G-induced production of IL-12 p40 and IL-10, suggesting a vital role for TLR-4 in signaling DC upon incubation with PS-G. Study also showed that PS-G was able to augment inhibitor of kB (IkB) kinase and nuclear factor (NF)-kB activity and also IkB and p38 mitogen-activated protein kinase (MAPK) phosphorylation. Further, inhibition of NF-kB by helenalin and p38 MAPK by SB98059 (p38 MAPK inhibitor) prevented the effects of PS-G in the expression of CD80, CD86, CD83, CD40, CD54, and HLA-DR and production of IL-12p70, p40, and IL-10 in various degrees. Taken together, these results demonstrate that PS-G can effectively promote the activation and maturation of immature DC [31].

LZ-8 is a protein derived from the fruiting body of *G. lucidum* and has antitumor and immunomodulatory activity. Lin et al. (2009) first used the yeast *Pichia pastoris* protein expression systems for producing the immunomodulatory protein LZ-8 and investigated the immunomodulatory effects of rLZ-8 on human monocyte-derived DCs. Treatment of DC with rLZ-8 (10 μg/ml) resulted in the enhanced cell-surface expression of CD80, CD86, CD83, and HLA-DR, as well as the enhanced production of IL-12 p40, IL-10, and IL-23, and the capacity of endocytosis was suppressed in DCs. In addition, treatment of DCs with rLZ-8 resulted in an enhanced naive T cell-stimulatory capacity and increased naive T cell secretion of IFN-γ and IL-10. Neutralization with antibodies against TLR4 inhibited the rLZ-8-induced production of IL-12 p40 and IL-10 in DCs. rLZ-8 can stimulate TLR4- or TLR4/MD2-transfected HEK293 cells to produce IL-8. These results suggested an important role for TLR4 in signaling DCs upon incubation with rLZ-8. Further study showed that rLZ-8 was able to augment IKK, NF-kB activity, and also IkB and MAPK phosphorylation. Further, inhibition of NF-kB by helenalin prevented the effects of rLZ-8 in the expression of CD80, CD86, CD83, and HLA-DR and production of IL-12 p40 and IL-10 in various degrees. Immunization with OVA/rLZ-8 (500 μg, i.p.) showed that the anti-OVA IgG2a, IFN-γ, and IL-2 were increased significantly compared with OVA alone in BALB/c mice. These experiments demonstrated that rLZ-8 can effectively promote the activation and maturation of immature DCs, preferring a Th1 response, suggesting that rLZ-8 may possess a potential immune-regulating effect [32].

Chan et al. (2007) found that purified polysaccharides from *G. lucidum* mycelium could induce human PBMC proliferation and phenotypic and functional matu-

ration of DCs with significant IL-12 and IL-10 production. Polysaccharides of *G. lucidum* spore and barley were both rather weak immunostimulator in vitro. In general, all these polysaccharides did not polarize T cells into either Th1 or Th2 or regulatory T cells, except for crude spore polysaccharides-treated DCs which could suppress T cell proliferation with IL-10 production. This study revealed the polysaccharides of different sources have different immune potency and effect on human immune cells [33]. Chan et al. (2008) used in vitro culture model with leukemic monocytic cell lines THP-1 and U937 as monocytic effector cells for proliferation responses and DCs induction. Result showed that GL-PS (100 μg/mL) alone induced proliferative response on both THP-1 and U937 cells but only THP-1 transformed into typical DC morphology when stimulated with GL-PS plus GM-CSF/IL-4. The transformed THP-1 DCs had significantly increased expression of HLA-DR, CD40, CD80, and CD86 though not as high as the extent of normal monocyte-derived DCs. They had similar antigen-uptake ability as the normal monocyte-derived DCs positive control. However, their potency in inducing allogeneic T cell proliferation was also less than that of normal monocyte-derived DCs. These findings suggest that GL-PS could induce monocytic leukemic cell differentiation into DCs with immunostimulatory function. The possible clinical impact of using GL-PS in patients with monocytic leukemia (AML-M4 and M5) deserved further investigation [34].

Meng et al. also found that *Ganoderma lucidum* polysaccharide (GLP) increased CD40, CD86, and MHC expression by DCs and promoted phenotypic maturation of DC in mice. GLP can downregulate activity of acid phosphatase and decrease phagocytosis, enhance antigen presentation, and increase IL-12 production in DCs [35].

Yoshida et al. (2012) investigated the effects of *G. lucidum* and its principal ingredient, β-glucan, on the activation of dendritic cells and the differentiation of Th17 cells. *G. lucidum* extracts as well as purified β-glucan (Curdran) activated DCs and caused them to produce large amounts of IL-23. β-Glucan also enhanced and sustained the transcription of IL-23p19. The MEK-ERK signaling pathway positively regulates IL-23p19 transcription in β-glucan-stimulated DCs. In a mixed leukocyte reaction, *Ganoderma lucidum* extracts-stimulated DCs preferentially induced Th17 cells. Furthermore, orally administrated *G. lucidum* extracts increased the percentages of Th17 cells and the transcription levels of antimicrobial peptides. The results show that *G. lucidum* extracts and β-glucan activate DCs to produce large amounts of IL-23, which induces Th17 differentiation both in vitro and in vivo [36].

Yue et al. (2013) showed that the polysaccharide-enriched fraction of *Ganoderma sinense* (GS) hot water extract (400 μg/ml) exhibited significant stimulatory effects on PBMC proliferation. When the fruiting bodies of GS were divided into pileus and stipe parts and were separately extracted, the GS stipe polysaccharide-enriched fraction (50~400 μg/ml) showed concentration-dependent immunostimulating effects on PBMC. The productions of TNF-α, IL-10, and transforming growth factor-β (TGF-β) were significantly enhanced by this fraction. In addition, the proportion of CD14(+) monocyte subpopulation within the PBMC was specifically increased. The IL-10 and IL-12 productions in monocyte-derived dendritic cells

were significantly enhanced by GS stipe fraction. Results firstly demonstrated the immunostimulatory effects of GS stipe polysaccharide-enriched fraction on PBMC and dendritic cells [37].

Recently, Zhang et al. (2019) reported that immunoactive polysaccharide from *G. lucidum* was incorporated with gold to form nanocomposite (GLP-Au). GLP-Au efficiently induced dendritic cell (DC) activation by increase of CD80/CD86/CD40/MHCII, decrease of phagocytic ability and acid phosphatase activity, and increased cytokine transcription. GLP-Au strongly promoted the proliferation of $CD4^+$ and $CD8^+$ T cells via DC. GLP-Au combined with doxorubicin inhibited 4 T1 tumor growth and metastasis in mice. This study indicated nanoformulation not only promoted stability and prolonged activation of *G. lucidum* polysaccharides but also increased the efficacy of chemotherapeutic drug and reduced its toxicity [38].

2.2.5 *Ganoderma Enhances the Activity of Immune Cells to Kill Tumor Cells*

2.2.5.1 *Ganoderma* Enhances the Activity of Mononuclear Macrophages

Lin et al. (1980) discovered that both *G. lucidum* extract and *Ganoderma* polysaccharide D6 extracted from fruiting body of *G. lucidum* can markedly improve mice phagocytic ability of peritoneal macrophages in mice [39]. Later, Gao and Yang (1989), Gu et al. (1990), Cao et al. (2003), and Tang et al. (2004) reported that *Ganoderma applanatum* polysaccharide, *Ganoderma capense* extract, Gl-PS, and *G. lucidum* spore polysaccharides can effectively promote phagocytosis of macrophage and production of IL-1 in mice [40–43].

Hsu et al. (2003) found that the polysaccharide component with a branched (1 – > 3)-beta-D-glucan (PS-G) isolated from *G. lucidum* is able to enhance phagocytic activity and migration of human primary neutrophils and neutrophilic-phenotype cells differentiated from all trans-retinoic acid-treated HL-60 cells. Exposure of neutrophils to PS-G time dependently caused increases in protein kinase C (PKC), p38 mitogen-activated protein kinase (MAPK), hematopoietic cell kinase (Hck), and Src-family kinase (Lyn) activities. PS-G is also able to inhibit spontaneous and Fas-induced neutrophil apoptosis, and this effect on neutrophils primarily relies on activation of Akt-regulated signaling pathways. All these results demonstrate PS-G enhance non-specific immune function and further provide evidence to strengthen the beneficial remedy of *G. lucidum* in human to enhance the defense system [44, 45].

Chien (2004) found that when human umbilical cord blood (hUCB) mononuclear cells (MNCs) were treated with a fucose-containing glycoprotein fraction isolated from the water-soluble extracts of *G. lucidum* (F3) (10~100 μg/mL) for 7 days, the population of $CD14^+CD26^+$ monocyte/macrophage, $CD83^+CD1a^+$ dendritic cells, and $CD16^+CD56^+$ NK-cells were 2.9, 2.3, and 1.5 times higher than those of the untreated controls ($p < 0.05$). However, T cell growth was slightly inhibited, and

CD3 marker expression decreased approximately 20% in the presence of higher concentrations of F3 (100 μg/mL). B-cell population has no significant change. After F3 treatment, NK-cell-mediated cytotoxicity was significantly enhanced by 31.7% ($p < 0.01$) at effector/target cell ratio (E/T) 20:1 in vitro [46].

Tang QJ et al. showed that the active glycoprotein GLIS with relative molecular weight is about 2×10^6 extracted from *G. lucidum* which containing more than 90% of carbohydrates which composed of 8 monosaccharides, mainly galactose, glucose, and mannose. GLPS can stimulate the secretion of TNF-α and IL-1 and production of NO in macrophages. GLIS also enhanced the phagocytic function of mouse macrophages on latex particles. The above effect of GLIS on macrophages in tumor-bearing mice was significantly stronger than that in normal mice [47].

Zhang et al. (2010) showed that after treatment with GLIS, spleen-derived B lymphocytes from tumor-bearing mice were activated, proliferated, and produced a large number of immunoglobulins. Bone marrow-derived macrophages from tumor-bearing mice also were activated after exposure to GLIS, and they produced important immunomodulatory substances, such as IL-1β, TNF-α, and NO. GLIS markedly increased phagocytosis of macrophages, and very importantly, it markedly raised the macrophage-mediated tumor cytotoxicity. Treatment with GLIS caused an inhibition of sarcoma S180 tumor growth by 60% in mice [48].

Wei et al. (2007) reported the trapping of LPS-coated polystyrene particles via optical tweezers and measured its interaction with murine macrophages (J774A.1 cells) for cells pretreated with extract of *G. lucidum* polysaccharides (EORP) vs those without EORP treatment. Results indicate that the cellular affinity for LPS increases when the macrophage is pretreated with EORP. EORP not only enhances J774A.1 cell-surface expression of TLR4 and CD14, two receptors on macrophages, as well as LPS binding and phagocytosis internalization but also reduces the adhesion time constant and increases the force constant of the binding interaction [49].

Watanabe et al. (2011) investigated that triterpenes-rich extract isolated from antlered form of *G. lucidum* (AF) induces TNF-α production in monocytic THP-1 cells. Furthermore, the AF also synergized with lipopolysaccharide (LPS) to induce TNF-α production in THP-1 cells, suggesting an immunostimulatory role of AF. Notably, AF enhanced LPS-induced phosphorylation of p38 mitogen-activated protein kinase (MAPK), while it suppressed LPS-induced phosphorylation of c-Jun N-terminal kinase (JNK) MAPK. p38 inhibitor suppressed TNF-α production, while JNK inhibitor enhanced TNF-α production, implying that synergistic effect of the extract may work by modulating p38 and JNK MAPKs. Lucidenic acids A, F, and D were purified from AF, and lucidenic acids A and F were identified as modulators of JNK and p38, respectively [50].

Zhang et al. (2012) found that *G. atrum* polysaccharide (PSG-1) did not kill CT26 cells directly but inhibited the proliferation of CT26 cells via the activation of peritoneal macrophages. PSG-1 significantly suppressed the tumor growth in CT26 tumor-bearing mice. Treatment with PSG-1 significantly increased the phagocytosis of macrophages and the production of TNF-α, IL-1β, and nitric oxide via TLR4-dependent signaling pathways, then activated NF-κB, and stimulated TNF-α production [51].

Huang et al. (2016) reported that *G. atrum* polysaccharide (PSG-1) can promote expression of TNF-mRNA and production of macrophages at a concentration-dependent manner in S180 tumor-bearing mice. This effect is mediated by NF-kB, PI3K/Akt, and MAPK signaling pathways and inhibited by NF-kB, PI3K/Akt, and MAPK-specific inhibitors [52].

As well known, macrophages that are divided into M1 phenotype (classically activated macrophages) or M2 phenotype (alternatively activated macrophages), with their different roles, display distinct cytokine profiles: M1 preferentially produces TNF-α, IL-6, and IL-12; conversely, M2 generates more IL-10 and arginase. The M1 are involved in the inflammatory response, pathogen clearance, and antitumor immunity. In contrast, M2 perform immunosuppressive and tumor growth-promoting functions, although M2 have some possible physiological functions such as homeostasis [53].

Sun et al. (2017) reported that *G. lucidum* polysaccharides (Gl-PS) might have the potential to promote macrophage M1 polarization by lipopolysaccharide (LPS). The polarization of M1 phenotype macrophages was induced by LPS. It was shown that the IL-12, TNF-α, and IL-6 mRNA detecting by qRT-PCR in the M1 phenotype macrophages induced by LPS for 24 h were higher in the wells treated with Gl-PS than that in the control wells. The ELISA assay showed that the levels of IL-12, TNF-α, and IL-6 in the wells treated with Gl-PS were higher than that in the control wells (Fig. 2.3a, c, d). But the IL-10 mRNA in the M1 type macrophages was lower in the wells treated with Gl-PS than that in the control wells (Fig. 2.3b). As shown by Western blot assay, after the induction with LPS for 24 h, the arginase I in the

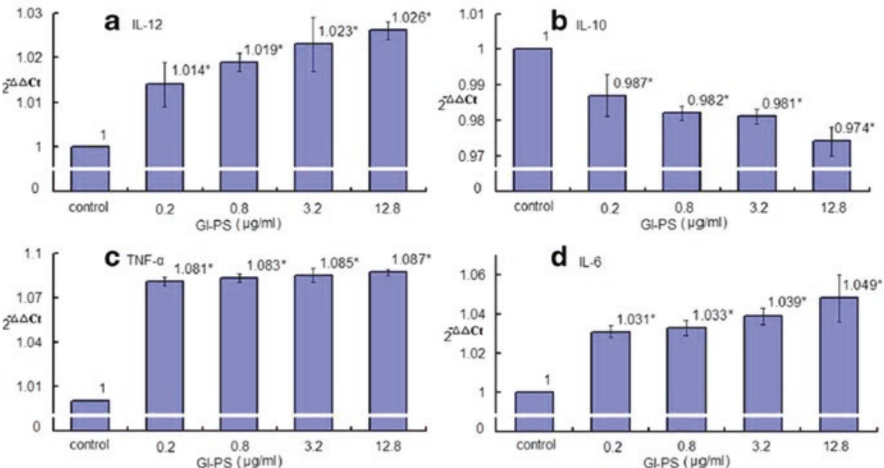

Fig. 2.3 The mRNAs of the cytokines detected by qRT-PCR in the macrophages**.** The macrophages were polarized by LPS (M1 polarization) in the presence of Gl-PS for 24 h. The mRNAs of the cytokines were detected by qRT-PCR. Error bars indicate standard deviation of the means. Asterisks indicate Dunnett *t* test p values <0.05 after one-way ANOVA, compared with the control. (n = 3). (Reproduced with permission from Ref. [54])

M1-type macrophages was much lower in the wells treated with Gl-PS than that in the control wells (Fig. 2.4). The results indicate that the Gl-PS may have the potential to promote M1 polarization vs M2. Consequently, Gl-PS may improve the immunological responses by promoting M1 polarization vs M2 to defend against pathogenic organism and cancer [54].

Wang et al. (2015) showed that oral administration of mushroom beta-glucan purified from mycelium extract of *Ganoderma lucidum* or *Antrodia camphorata* significantly increase the mRNA expression of IL-12 and IFN-γ; in tumor tissues and decrease serum TGF-β concentration and mRNA expressions of IL-6, IL-10, COX-2, and TGF-β in the tumor microenvironment. The study suggests the efficacious effect of *Ganoderma lucidum* or *Antrodia camphorata* beta-glucan for ameliorating the immune suppression in the tumor microenvironment. Increased M1 phenotype of tumor-associated macrophages and attenuated M2 phenotype of tumor-associated macrophages could be achieved by ingesting mushroom polysaccharides [55].

Li et al. (1999, 2000) investigated the action of the *Ganoderma* polysaccharide fraction GLB7 on intracellular $[Ca^{2+}]i$ in macrophages using laser scanning confocal microscope imaging of the calcium fluorescent indicator dye Fluo3/AM and found that GLB7 (20 μg/ml) could increase $[Ca^{2+}]i$ in murine peritoneal macrophages; the average percentage of increase in $[Ca^{2+}]i$ was (248 ± 18)%. The increase in $[Ca^{2+}]i$ induced by GLB7 was due to the influx of extracellular Ca^{2+} and intracellular Ca^{2+}

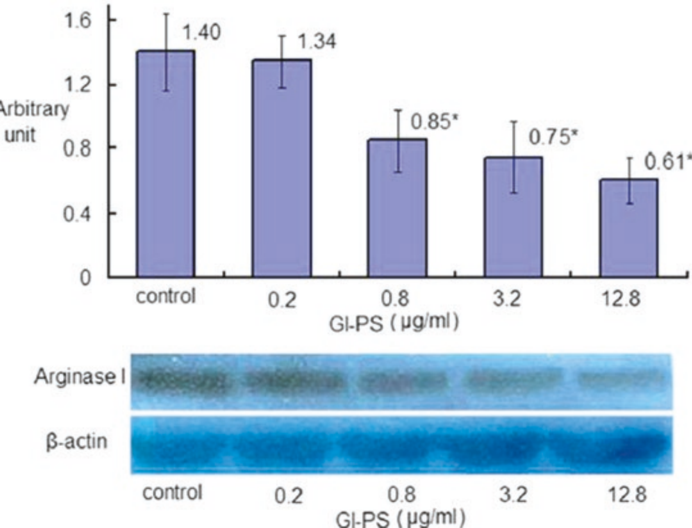

Fig. 2.4 The arginase I detected by Western blot in the macrophages. The macrophages were polarized by LPS (M1 polarization) in presence of Gl-PS for 24 h. The arginase I was detected by Western blot. Error bars indicate standard deviation of the means. Asterisks indicate Dunnett *t* test p values <0.05 after one-way ANOVA, compared with the control (n = 3). (Reproduced with permission from Ref. [54])

release through both IP3-sensitive and IP3-insensitive Ca^{2+} stores; depolarization in macrophage membrane did not affect the increase in $[Ca^{2+}]i$. The increase of intracellular $[Ca^{2+}]i$ may be associated with GLB7 enhancing macrophage function. GLB7 (40 mg/L) can increase activity of PKC and induce plasmalemma translocation in murine peritoneal macrophages. GLB7 antagonized the inhibition effect of PKC inhibitor staurosporine on PKC in murine peritoneal macrophages [56, 57].

Tert-butyl-hydroperoxide (tBOOH), as a membrane-permeant oxidant, has been extensively used as a model of oxidative injury in different cells in vitro. You and Lin (2002) found that *G. lucidum* polysaccharide peptide (GLPP) had protective effect on oxidative damage induced by tBOOH or alloxan in mouse peritoneal macrophages in vitro and in vivo. Under the light microscope or electron microscope, it was found that administration of GLPP can increase the survival rate of macrophages and improve morphological change of macrophages by oxidative injury with tBOOH, such as inhibiting membranous degeneration and necrosis and protecting the membrane microvilli and organelle such as mitochondria (Fig. 2.1). GLPP also can restore depressing mitochondrial membrane potential injured by tBOOH in mouse peritoneal macrophages [58] (Fig. 2.5).

You and Lin (2004, 2005) used confocal microscope with fluorescent probe, 2′,7′-dichlorodihydrofluorescein diacetate (DCHF-DA) to detect the effects of GLPP on the free radical formation in mice peritoneal macrophages. Results showed that DCHF-DA exhibited low fluorescence in cells of the control group and fluorescence apparent increased in cells of oxidant injury group, no matter whether they were injured by alloxan or by tBOOH. But GLPP given in vivo or in vitro could decrease the fluorescence in macrophages. The result of time series scans of confocal microscope revealed that GLPP lowered the fluorescence in macrophage over time. It took effect immediately after GLPP were given. PMA greatly increased the fluorescence in macrophage. After treatment with GLPP, the increased fluorescence by PMA was lowered over time and GLPP took effect immediately as well. The results showed that DCHF exhibited low fluorescence in the control cells. The injury cells by alloxan or by tBOOH gave a bright signal. But GLPP lowered the fluorescence signal to darker. It indicated that GLPP could decrease free radical in mouse peritoneal macrophages. The results of time series scan with confocal microscopy showed that GLPP could lower the free radical in mouse peritoneal macrophages immediately. No matter the cells were at rest state or at the respiratory burst state, GLPP took effect immediately. The results indicate that GLPP have the free radical scavenging activity. It could scavenge not only the free radical directly in mouse peritoneal macrophages at rest state but also the decreased free radical in mouse peritoneal macrophages by pathological cause [59, 60].

Cheng et al. (2010), using microarray technology, compared the ethanol extracts of two different lingzhi (*G. lucidum, GL*; and *G. sinense, GS*) for their effects on gene expression profile in human monocytic cells. Results suggest that at best approximately 25% of target genes are common to the two lingzhi: functionally ranging from cell development, negative regulation of cellular process, and cellular protein metabolic process to signal transduction and transcription (Table 2.2). The pathways mediated by *GS* focus on inflammation and immune response, whereas

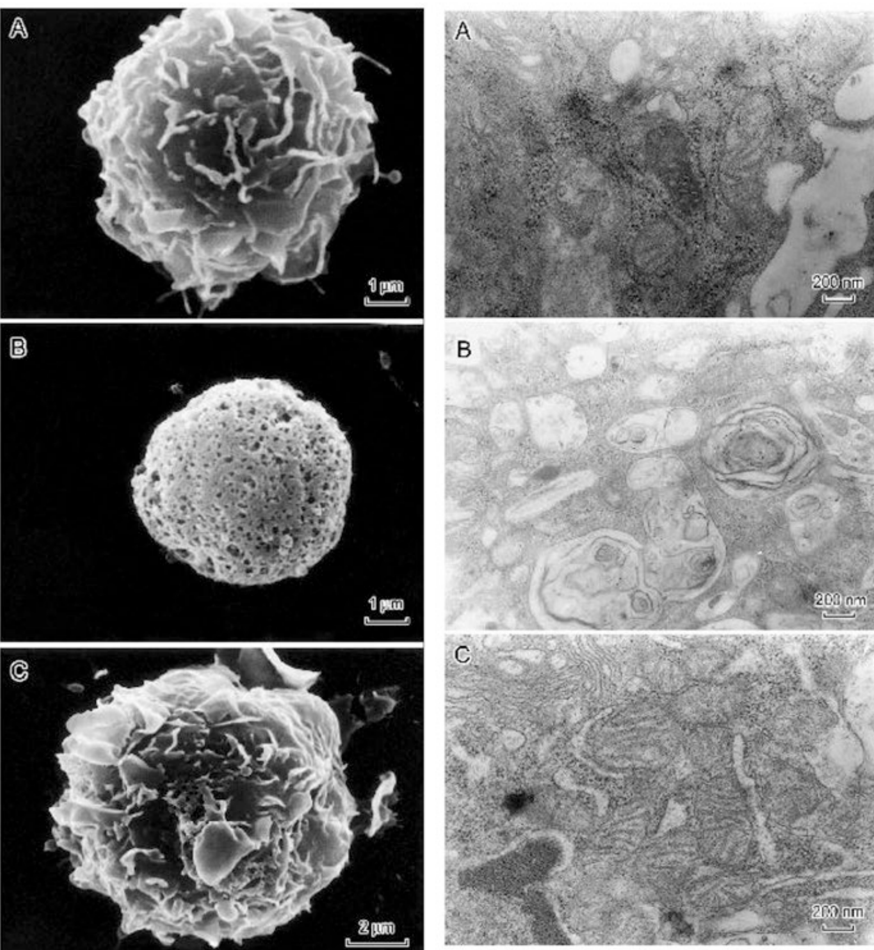

Fig. 2.5 Electron microscopy of macrophage. ***Left***: A macrophage incubated with tBOOH (0.1 mmol/L) for 24 h was observed under the scan electron microscope. (**a**): Long microvilli were observed in control group (×11,000). (**b**): Membrane of macrophages became smooth in the tBOOH-treated group (×11,000). (**c**): A few of microvilli of macrophages were slightly short in GLPP-treated group (×8000). ***Right:*** Structure of microphages incubated with tBOOH (0.01 mmol/L) for 24 h was observed under transmission electron microscope. (**a**): The structure of mitochondria was normal in control group (×30,000). (**b**): Structure of mitochondria became stratified in the tBOOH-treated group (×25,000). (**c**): The cristae of mitochondria was slightly disorganized or unchanged in GLPP-treated group (×30,000). (Reproduced with permission from Ref. [58])

Table 2.2 Top 20 common genes (10 upregulated and 10 downregulated) affected by both purple *lingzhi* (*G. sinense*) and red *lingzhi* (*G. lucidum*). Ref. [61]

Gene Symbol	Gene Name	Possible Function	Fold change	
			G. sinensis	*G. lucidum*
PLA2G7	Phospholipase A2, group VII	Degradation of inflammatory platelet-activating factor (PAF)	14.5	3.1
ADAMDEC1	ADAM-like, decysin 1	Dendritic cell maturation	12.6	1.5
CXCLI	Chemokine (C-X-C motif) ligand 1	Leukocyte trafficking; immune response; inflammatory response	11.0	1.5
BIRC3	Baculoviral IAP repeat-containing 3	Antiapoptosis	9.7	1.5
IL8	Interleukin 8	Inflammatory response; angiogenic factor	9.6	1.5
IL1B	Interleukin 1, beta	Inflammatory response; cell proliferation; differentiation; apoptosis	8.2	1.4
SLAMF8	SLAM family member 8	Lymphocyte activation	6.4	1.4
CLEC4E	C-type lectin domain family 4, member E	Inflammation and immune response	6.4	1.4
CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1	Drug metabolism; synthesis of cholesterol, steroids, and other lipids	6.1	3.9
CXCL10	Chemokine (C-X-C motif) ligand 10	Stimulation of monocytes, natural killer, and T-cell migration	5.6	1.4
ZP1	Zona pellucida glycoprotein 1 (sperm receptor)	Unknown	0.7	0.6
PLA2R1	Phospholipase A2 receptor 1, 180 kDa	Unknown	0.6	0.7
BX097190	BX097190 Soares placenta Nb2HP	Unknown	0.6	0.7
NAPSB	Napsin B aspartic peptidase pseudogene	Unknown	0.6	0.7
ENST00000361200	Unknown messenger RNA	Unknown	0.5	0.7
KIAA0746	KIAA0746 protein	Unknown	0.5	0.7
XI5674	Human pTR5 mRNA for repetitive sequence	Unknown	0.5	0.6
GPA33	Glycoprotein A33 (transmembrane)	Colon cancer antigen; function unknown	0.4	0.6
DEFB1	Defensin, beta 1	Antimicrobial peptide	0.4	0.7
SERPINB10	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 10	Unknown	0.4	0.7

GL modestly increases levels of expression for genes involved in macromolecule metabolism. Furthermore, ethanolic extracts of both *GL* and *GS* do not inhibit monocytic cell growth. The extract of *GL* does not have significant effect on the genes in the nuclear factor kappa B (NF-kB) pathway (an important inflammation pathway), whereas the extract of *GS* can increase multiple key genes in the NF-kB pathway. Altogether, these results suggest that the common mode of action for lingzhi is complex; and different species of *Ganoderma* can modulate different pathways in human cells [61].

2.2.5.2 *Ganoderma lucidum* Increase the Cytotoxic Activity of Natural Killer Cells

Natural killer cell (NK) is an important immune cell in the body. Antiviral infection is associated with immunoregulation and, in some time, NK has spontaneous cytotoxic activity on various target cells and is involved in hypersensitivity reaction and occurrence of autoimmune diseases.

Won et al. (1989) reported that treatment with alcohol-insoluble components (GL-AI) extracted from *Ganoderma lucidum* by i.g., i.p., or i.v. could strongly enhance the cytotoxic activity of spleen NK cells in C3H/HeN mice [62].

Won et al. (1992) observed that effects of the water-soluble extract of *Ganoderma tsugae* mycelium (GT), its alcohol insoluble subfraction (GTI), and its alcohol-soluble subfraction (GTS) on splenic natural killer (NK) cell activity and blood interferon (IFN) production were assessed in mice. Intraperitoneal administration of GT (4~200 mg/kg) or GTI (1~50 mg/kg), but not GTS, augmented the NK cytotoxic activity in a dose-dependent manner in C3H/HeN mice. This augmentation of splenic NK cytolytic activity was not mouse-strain-dependent. The blood IFN titers of mice were also elevated after i.p. doses of GTI. The GTI-induced blood IFN was reduced by either IFN-($\alpha + \beta$) antiserum or IFN-γ monoclonal antibody in vitro. The treatment with antiserum neutralizing IFN-($\alpha + \beta$) resulted in a 70% reduction of GTI-induced IFN, while monoclonal antibody against mouse IFN-γ moderately neutralized the GTI-induced IFN (50%). These results demonstrated that both the splenic NK activity and blood IFN [IFN($\alpha + \beta$) and IFN-γ] titers are elevated by *Ganoderma tsugae* mycelium extracts in mice [63].

Ning et al. (2004) found that *Ganoderma lucidum* polysaccharide intraperitoneal injection (1 mg/d) for 7 consecutive days significantly increased NK cell activity and lymphocyte proliferation and serum levels of TNF-α and IL-2 in tumor-bearing mice [64].

Zhu et al. (2007) found that mice were injected intraperitoneally (i.p.) once daily with low-dose (2.5 mg/kg), intermediate-dose (25 mg/kg), and high-dose (250 mg/kg) of *Ganoderma lucidum* polysaccharides (*Gl*-PS), respectively, for 7 consecutive days at 24 h after i.p. injection of an immunosuppressing antitumor agent cyclophosphamide (Cy, 300 mg/kg). In Cy-treated mice, compared to vehicle, low-dose *Gl*-PS accelerated recovery of bone marrow cells, red blood cells, and white blood cells, as well as splenic natural killer cells and natural killer T cells, and

enhanced T and B cell proliferation responses on day 8, cytotoxic T-lymphocyte activity on day 5, as well as NK cell and lymphokine-activated killer cell (LAK) activity on days 7~9. Furthermore, it promoted phagocytosis and cytotoxicity of macrophages on day 12. The above beneficial effects induced by the low-dose *Gl*-PS treatment did not result in any side effects. These results demonstrate the efficacious effects of low-dose *Gl*-PS treatment for enhancing the activity of immunological effector cells in immunosuppressed mice and may provide a basis for applying *Ganoderma* as an efficacious adjacent immunopotentiating therapy against cancer chemotherapy-induced immunosuppression [65].

Chang et al. (2009) reported that crude extract of *G. lucidum* (GL) (3 and 6 mg/kg, by oral administration) increased the survival rate of WEHI-3 leukemic BALB/c mice and decreased the percentage of leukemia cells in the spleens of mice before they were injected with WEHI-3 cells. GL enhanced percentages of CD3 and CD19, but decreased the percentages of Mac-3 and CD11b markers, suggesting that differentiation of the precursor of T and B cells was promoted but macrophages were inhibited. GL decrease the weight of spleens in WEHI-3 leukemic BALB/c mice as compared with control mice. GL promoted phagocytosis by macrophage from peripheral blood mononuclear cell (PBMC) and also promoted natural killer cell activity. These findings indicate that crude extract of *G. lucidum* can promote immune response in WEHI-3 leukemic BALB/c mice [66].

Tsai et al. (2012) reported that the acid-hydrolyzed fragments of *Ganoderma lucidum* polysaccharides (GLPS) obtained by Smith degradation were separated by size-exclusion chromatography into two major water-soluble fractions: peptidoglycans (GLPS-SF1) and oligosaccharides (GLPS-SF2). Both fractions induced CD69 in human peripheral blood mononuclear cells (hPB-MNCs). GLPS-SF1, with a molecular weight of around 20 kDa, were heterogeneous peptidoglycans composed of glucose/mannose (4:1) that exhibited biological activities with Th1 cytokines IL-12, IL-2, TNF-α, and IFN-γ in hPB-MNCs and stimulated macrophage cytokine expression via Toll-like receptor 4 (TLR4) signaling. For GLPS-SF2, with a molecular weight of around several kD, its sugar sequence was elucidated by mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy as [-α-1,4-Glc-(β-1,4-GlcA) (3)-](n). This oligosaccharide displayed specific immune property with low monocyte induction and greatly stimulated cell activation and proliferation of NK and T cells. This oligosaccharide isolated from *G. lucidum* polysaccharides with internal glucuronic acids/glucose repeat unit in a 3:1 ratio may be responsible for the active stimulation of NK and T cells [67].

2.2.5.3 *Ganoderma lucidum* Increase the Activity of Cytokine Induced Killer Cells

Cytokine-induced killer (CIK) cells have been shown to generate effector cells with higher proliferative capacity, increased cytotoxicity, antagonized multidrug resistance of tumor cells, and fewer side effects than lymphokine-activated killer (LAK) cells.

To study the effects of *Ganoderma lucidum* polysaccharides (*Gl*-PS) on the proliferation and the antitumor activity of cytokine-induced killer (CIK) cells and to make use of CIK cells as a means to investigate the interactions between *Gl*-PS and cytokines. We prepared CIK cells by using the standard protocol as a positive control. Experimental groups also underwent the standard protocol, except that *Gl*-PS (400 mg/L or 100 mg/L) was added and the dose of anti-CD3 and interleukin-2 was reduced by 50% and 75%, respectively. For negative controls, *Gl*-PS in the experimental protocol was replaced with soluble starch or methylcellulose (400 mg/L or 100 mg/L). CIK cell proliferation, cytotoxicity, and phenotype were determined by using the Trypan blue exclusion method, MTT assay, and flow cytometry. Our research results showed that by synergizing cytokines, *Gl*-PS (400 mg/L or 100 mg/L) could decrease the amount of cytokine in lymphokine-activated killer (LAK) cell and CIK cell culture, but had no significant effect on the proliferation, cytotoxicity, or phenotype of LAK cells, or CIK cells induced by cytokines at higher doses alone, in which CIK cells expanded about 80-fold, $CD3^{+}NK1.1^{+}$ cells, expanded by more than 15%. The cytotoxicity of CIK cells in experimental groups was 79.3% ± 4.7%, 76.9% ± 6.8% versus the positive control 80.7% ± 6.8% against P815 ($P > 0.05$) and 88.9% ± 5.5%, 84.7% ± 7.9% versus the positive control 89.8% ± 4.5% against YAC-1 ($P > 0.05$). The activity of *Gl*-PS could mostly be blocked by anti-CR3.Therefore, the effect of *Gl*-PS on CIK cells is possibly mediated primarily through complement receptor type 3 [68]. Further, we also made use of CIK cells as a means to investigate interaction between Gl-PS and cytokines and explore mechanism of Gl-PS acting on proliferation and antitumor activity of CIK cells. The results suggested that Gl-PS (400 or 100 mg/ml) promoting CIK cells proliferation and cytotoxicity were relevant to enhancing IL-2, TNF production, and protein and mRNA expression of granzyme B and perforin in CIK cells through synergizing cytokines in decreasing doses of IL-2 and anti-CD3 by 75 and 50% and maybe were irrelevant to nitric oxide (NO). These results confirmed that Gl-PS was a promising biological response modifier (BRM) and immune potentiator [69].

2.2.6 *Ganoderma lucidum Antagonize Evasion of Tumor Cells*

Tumor cells may evade the immunosurveillance of the host by mechanisms, such as MHC class I downregulation, antigen loss, antigen modulation, or the expression of inhibitory molecules. For antigen presentation, intracellular peptide antigens bind to MHC class I (such as H-2 K and H-2D in mouse), and tumor antigens are generally intracellular proteins. Deficient costimulatory molecules (such as B7-1 and B7-2) on tumor cells also contribute to tumor cells evasion from immunosurveillance, because T cell activation needs costimulatory signal as second signal [70].

2.2.6.1 *Ganoderma lucidum* Promote the Generation of MHC-1 Molecules and Synergistic Stimulus Factors

Sun et al. (2011, 2012) found that *Ganoderma lucidum* polysaccharide (Gl-PS) may antagonize tumor immune evasion by ameliorating the lowered ability of tumor cells to activate immune effector cells. B16F10 cell is a MHC class I-deficient melanoma cell line derived from C57BL mice. In the B16F10 cells, the mRNAs and proteins of H-2K, H-2D, B7-1 and B7-2 were augmented statistically in some concentration of Gl-PS with RT-PCR assay and flow cytometry assay. Treated with some concentration of Gl-PS, B16F10 cells more strongly induced lymphocyte activation according to their statistically stronger induction on lymphocyte proliferation (200 and 600 μg/mL Gl-PS), CD69 (200, 400 and 600 μg/mL Gl-PS) and FasL (400 μg/mL Gl-PS) expression, and IFN-γ production (400 μg/mL Gl-PS) compared with the controls. The antitumor cytotoxicity of spleen lymphocytes as effector cells (activated with PHA) against B16F10 cells was statistically augmented after treatment of these B16F10 cells with Gl-PS at concentrations of 200, 400, and 600 μg/mL [71–73].

2.2.6.2 *Ganoderma lucidum* Inhibit the Secretion of Immunosuppressive Cytokines

Sun et al. (2011, 2014) found that Gl-PS may decrease the immunosuppressive cytokines produced by tumor cells and antagonize their immunosuppressive effects. TGF-β1, IL-10, and VEGF are three of the cytokines commonly secreted by many cancer cells. In B16F10 cell culture supernatant (B16F10-CS), the levels of the three cytokines were all statistically elevated compared with those in the culture supernatant of fibroblast prepared from C57BL/6 mouse (FB-C57BL/6-CS). The mRNAs and proteins of TGF-β1, IL-10, and VEGF were statistically decreased by Gl-PS at 0.2, 0.8, 3.2, and 12.8 μg/mL (except 0.2 μg/mL for IL-10 mRNA and both IL-10 and VEGF proteins) in B16F10 melanoma cells and by Gl-PS at 0.2, 0.8, 3.2, and 12.8 μg/mL (except 0.2 μg/mL for both mRNA and protein of IL-10) in LA795 lung carcinoma cells according to RT-qPCR and ELISA assay. After 72 h induction with PHA, the proliferation ratio, granzyme B and perforin (two important molecules in the granule-mediated pathway to kill target cells), in splenic mononuclear lymphocytes of C57BL/6 mice, and the proliferation ratio in the mixed lymphocyte reaction (MLR) were markedly reduced by B16F10-CS whereas ameliorated statistically by Gl-PS at 0.2, 0.8, 3.2, and 12.8 μg/mL (except 0.2 and 0.8 μg/mL for granzyme B). After being activated by PHA for 72 h, the mRNAs and proteins of IL-2, IFN-γ, and TNF-α in the lymphocytes, compared with the control, were statistically suppressed by B16F10-CS; nevertheless, according to RT-PCR and Western blot assay, Gl-PS at concentrations of 0.8, 3.2, and 12.8 μg/ml (except 0.8 and 3.2 μg/mL for IFN-γ protein and 0.8 μg/mL for both mRNA and protein of TNF-α) ameliorated the suppression fully or partially [74, 75].

The plasma of cancer patients, such as lung cancer, may also contain immunosuppressive factors with immunosuppressive effects on lymphocytes. Sun et al. (2015) showed that incubating with lung cancer patient plasma, the CD69 expression (24 h incubation), cell proliferation (72 h incubation), and the expression of granzyme B and perforin (48 h incubation) in mononuclear lymphocytes, upon PHA stimulation in vitro, were inhibited substantially compared with controls, as detected by flow cytometry, MTT, and Western blot assay, while *Gl*-PS at concentrations of 0.2, 0.8, 3.2, and 12.8 μg/ml (except 0.2 and 0.8 μg/mL for CD69, and 0.2 μg/mL for granzyme B) antagonized these inhibitions [76].

Moreover, the macrophages were suppressed by B16F10-CS as well. It was reported that the viability ratio, phagocytic activity, NO production, TNF-α production in peritoneal macrophages, and the activity of the TNF-α in the culture supernatant of peritoneal macrophages of C57BL/6 mice, activated by LPS, were reduced markedly after 24 h incubation with B16F10-CS, as detected by MTT assay, neutral red uptake assay, Griess assay, Western blot assay, and cytotoxicity assay on L929 cells, respectively, while treatment with 12.8 μg/mL of Gl-PS (besides 3.2 μg/mL for viability ratio and 0.8, 3.2 μg/mL for phagocytic activity) showed antagonism against the reduction [77] .

2.2.7 Research of Ganoderma on Tumor-Associated Antibodies

As well known, carbohydrate-based vaccines have shown therapeutic efficacy for infectious disease and cancer. Liao et al. (2013) reported a crude extract fraction of water-soluble and L-fucose (Fuc)-containing polysaccharides (F3) with high molecular mass (>100 kDa) isolated and characterized from *Ganoderma lucidum*. F3 (120, 240 mg/kg, per day, for 28 days) exhibited a significant inhibition against the growth of Lewis lung carcinoma (LLC1) cells in a dose-dependent manner in LLC1-bearing mice. However, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay results revealed that F3 (<200 μg/mL) had no significant effect on LLC1 cell viability in vitro. In further study, a Fuc-enriched polysaccharide fraction (FMS) with 3.5 kDa of molecular mass was purified from F3. Methylation analysis indicated that FMS is based on a 1,4-mannan backbone with side chains at the C3 position and a 1,6-α-galactan branched at the C2 position and is highly decorated with terminal Fuc. FMS could inhibit the growth of LLC1 cells in vivo. FMS induced antibodies against LLC1 cells, with increased antibody-mediated cytotoxicity and reduced production of tumor-associated inflammatory mediators, such as monocyte chemoattractant protein-1 (MCP-1) and granulocyte colony-stimulating factor (G-CSF). The mice pretreated with FMS showed a significant increase in the peritoneal B1 B-cell population, suggesting FMS-mediated anti-glycan IgM production. Furthermore, the glycan microarray analysis of FMS-induced antiserums displayed a high specificity toward tumor-associated glycans,

with the antigenic structure located in the nonreducing termini (i.e., Fucα1-2Galβ1-3GalNAc-R, where Gal, GalNAc, and R represent, respectively, D-galactose, D-N-acetyl galactosamine, and reducing end), typically found in Globo H and related tumor antigens. The composition of FMS contains mainly the backbone of 1,4-mannan and 1,6-galactan through the Fucα1-2Gal, Fucα1-3/4Man, Fucα1-4Xyl, and Fucα1-2Fuc linkages (where Man and Xyl represent D-mannose and D-xylose, respectively), underlying the molecular basis of the FMS-induced IgM antibodies against tumor-specific glycans. These findings are also consistent that the host immune function enhanced by *Ganoderma* polysaccharides offer potential prospect for the immunotherapy of Globo H-positive lung cancer patients [78].

2.2.8 Antitumor Effect of Ganoderma and "Fu Zheng Gu Ben"

In the 1970s, basing on pharmacological and clinical research of lingzhi, we proposed the working hypothesis to probe into the mechanism of "Fu Zheng Gu Ben" as a therapeutical principle of traditional Chinese medicine (TCM) and suggested "Fu Zheng Gu Ben" effect of lingzhi may be related with (a) enhancing function of vital organs and system of body; (b) reducing the damage of various pathogenic factors to the body; (c) improving the regulation of neuro-endocrine-immune network; and (d) strengthening homeostasis of body [79].

According to the theory of TCM, health and disease belong to the conflict status between good (zheng) and evil (xie). In general, health is due to the fact that "vital Qi exists in the body and evil cannot be disturbed." The disease is "the place of evil gather together, its Qi must empty", but curing disease does not necessarily mean to completely eliminate external evil; as long as the positive qi exists, evil cannot be disturbed [80, 81].

Ganoderma lucidum and its active component *G. lucidum* polysaccharide can neither inhibit tumor cell proliferation nor induce tumor cell apoptosis in vitro, but have obvious antitumor effect in vivo. To explore its mechanism of action, we studied *G. lucidum* using serum pharmacological method and cellular-molecular pharmacological technology on the basis of antitumor experiments in vivo. It has been proven that *G. lucidum* extract *or* polysaccharides have immunomodulatory activity, including enhancing the maturation and function of dendritic cells; increasing activities of macrophages, natural killer cells (NK), and cytotoxic T cell; modulating humoral and cellular immunity; and promoting production of antitumor cytokines, such as TNF, IFN, and IL-2, antagonizing immune escape of tumor cells. Therefore, the antitumor efficacy of *Ganoderma* extract, or the polysaccharides, was mainly a result of their immunomodulating activity. In addition, a series of researches also prove that *G. lucidu*m and its active ingredients have protective effect on toxicity of chemotherapy and/or radiotherapy including gastrointestinal tract damage, liver and kidney toxicity, and bone marrow injuries [3, 4].

Through these effects, *G. lucidum* supports the vital Qi and realizes the existence of the vital Qi, and evil (tumor) cannot be disturbed. Research results could expound the essence of "Fu Zheng Gu Ben (supporting the healthy energy, strengthening and consolidating body resistance)," including normalization of immune response and improving organ damage induced by pathogenic factors and restoring the homeostasis of body. This conclusion is consistent with our working hypothesis on the mechanism of "Fu Zheng Gu Ben" and provides an example for the pharmacological researches of TCM using integrative model of TCM theory with modern science and technology [80, 81].

2.3 *Ganoderma* Could Improve the Immune Function of Cancer Patients with Radiotherapy and Chemotherapy

This section reviews the results from clinical investigation of *Ganoderma* on cancer patients who has improvement of immunological indexes after treatment with *Ganoderma* preparation.

2.3.1 Characteristics of the Therapeutic Effect of Ganoderma lucidum on Tumor

Clinical trials on patients with cancers of the esophagus, stomach, colon, lung, liver, bladder, kidney, prostate, and uterus showed desirable effects when *Ganoderma* preparations were administered in combination with chemotherapy or radiotherapy. Observed therapeutic improvements associated with the side effects induced by chemotherapy or radiotherapy included (a) reduction of myelosuppression, such as leucopenia and thrombocytopenia; (b) attenuation of gastrointestinal injury, such as anorexia, nausea, vomiting, and diarrhea; (c) enhancement of patient's immunological functions of anti-infection and anticancer; and (d) improvement of cancer patient's physical fitness and quality of life. The results clearly demonstrated adjuvant efficacy of Lingzhi in treating cancer patients by strengthening the immunity and decreasing toxic side effects caused by drugs or radiotherapy [5].

2.3.2 Clinical Reports of Cancer Patients Treated with Ganoderma

Yan et al. (1998) investigated the clinical efficacy of *Ganoderma* oral liquid (an extract of the *G. lucidum* fruiting body) combined with chemotherapy in 56 cases of advanced non-small cell lung cancer. The 29 male and 27 female patients averaged

56.2-year-old. The patients were diagnosed with highly suspected clinical manifestations and epidemiological characteristics of lung cancer, confirmed by chest radiology and computed tomography (CT) tests and histopathology or cytological examination with primary non-small cell lung cancer. The patients were unable or unwilling to be subjected to surgery or had postoperative intrapulmonary recurrence that had disseminated. They were otherwise in generally good condition with Karnofsky score greater than 60 and an expected survival time more than 3 months. All patients had their lesion shown on X-ray, CT, or magnetic resonance imaging (MRI) for size determination. All 56 lung cancer patients were in II to IV stage, including 26 in II and III stages and 30 in III and IV stages. There were 32 cases of lung adenocarcinoma, 15 cases of squamous cell carcinoma, 7 cases of squamous adenocarcinoma, and 2 cases of large cell carcinoma.

The patients were randomly divided, 35 in treatment group (*Ganoderma* oral liquid + chemotherapy) and 21 in the control group (chemotherapy only). Before the treatment, the Karnofsky scores of the treatment and the control groups averaged 60.5 and 70, respectively, with no significant difference in their disease conditions. Three times a day, 20 ml of *Ganoderma* oral liquid was administered for 1 month. Prescription of cisplatin (DDP) plus vindesine (VDS) for chemotherapy was applied in the following manner: for the treatment group, 40 mg DDP and 500 ml saline by intravenous drip for 5 days, followed by a 20-day interval before the second monthly treatment [Note: VDS dosage and application were not specified in the report]. At the same time, *Ganoderma* solution was orally given to the patients in the treatment group. The treatment efficacy was evaluated after 2 consecutive monthly treatments. Short-term curative effect was classified based on the WHO objective evaluation criteria of solid tumor as complete remission (CR), partial remission (PR), minor remission (MR), stable disease (SD), MR + SD for no change (NC), and progressive disease (PD). The effective rate (CR + PR) was the remission rate (RR). Change in patient's quality of life, based on Karnofsky score, was classified as "improvement" when the score increased less than 10, "stabilization" when no change occurred, and "decline" when reduced more than 10. Counts on red blood cells (RBC), white blood cells (WBC), hemoglobin (HGB), platelets (PLT), T lymphocytes, and T-lymphocyte subsets were determined. Data from the patients, who completed the two monthly treatments, were used for statistical analysis. Those from the patients who discontinued or died during the course were counted as invalid (PD).

The results are as follows: 2 CRs (5.7% of the total), 21 PRs (60%), 9 NCs (25.71%), 3 PDs (8.57%), and 23 CR + PR (65.71%) from the treatment group and 1 CR (4.76%), 8 PRs (38.14%), 10 NCs (47.62%), 2 PDs (9.52%), and 9 CR + PR (42.85%) from the control group. Comparing RR of the treatment and the control groups, there was a significant difference ($P < 0.01$).

The Karnofsky scores showed 24 "improvements" (68.57%), 7 "stabilizations" (20%), and 4 "declines" (11.43%) in the treatment group and 9 "improvements" (42.85%), 8 "stabilizations" (38.10%), and 4 "declines" (19.05%) in the control group. The improvement rate in the treatment group, 68.57%, was significantly higher than that in the control group (42.85%) ($P < 0.01$). Meanwhile, the life quality improvement rate in the treatment group was greater than the remission rate

suggesting that even though patients might not have achieved CR or PR, their quality of life materially improved.

Hematological tests did not show apparent change in patients in the treatment group after the trial. On the other hand, the RBC, WBC, HGB, and PLT counts in the control croup decreased significantly. This indicated that the *Ganoderma* oral liquid lessened the suppression on the bone marrow hematopoietic function induced by the chemotherapy.

T3, T4, and T8 in the treatment group increased significantly after the treatment, whereas these T-cell subsets decreased in the control group, although statistically insignificant. The results suggest that *Ganoderma* oral solution might enhance the cellular immune function in cancer patients [82].

Zhang et al. (2000) reported the clinical effects of *Ganoderma* Tablets (LZT) prepared by water extract of *G. lucidum* on lung cancers. Twenty-nine patients diagnosed as III~IV stage lung cancers with the Karnofsky scores more than 50 were randomly divided into LZT-treated group and control group. 2~4 tablets of LZT (55 mg per tablet) were given to the patients three times a day for 3 months in the treatment group. Placebo was given to the patients in the control group. The effects of LZT on immunoregulation, hemorheologic parameters, Karnofsky scores, and clinical manifestations were observed before and after treatment. Result showed that the level of tumor necrosis factor (TNF) was raised obviously from 17.7 ± 4.3 to 28.7 ± 6.6 pg/ml in LZT group. With the progression of the disease, the level of soluble interleukin-2 receptor (sIL-2R) was increased from 259 ± 275 kU/L to 501 ± 291 kU/L($P < 0.05$) in control group but in LZT group kept stable, i.e., from 321 ± 311 kU/L to 372 ± 267 kU/L ($P > 0.05$). Karnofsky scores of LZT group (68.6 ± 22.1) were significantly higher than that of control group (47.0 ± 22.0) after treatment. Some hemorheologic parameters were decreased in LZT group. Although LZT had not been proved to reduce the lesion directly, it was helpful to improve the whole body state. No severe adverse reactions such as liver and kidney function injuries happened in usual dose. Results indicate LZT could regulate the immunity and improve the hypercoagulative condition [83].

Wang et al. (2014) explored the changes of T cell subsets in peripheral blood of non-small cell lung cancer (NSCLC) patients during chemotherapy with broken *Ganoderma lucidum* spores. According to the inclusion criteria, divide 58 patients diagnosed as III~IV stage NSCLC into 2 groups, 29 cases in test group with Taxol plus cisplatin chemotherapy combined with *Ganoderma lucidum* spores and 29 cases in control group with Taxol plus cisplatin chemotherapy alone; T cell subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$) in the peripheral blood were detected before chemotherapy, after second and fourth cycles of chemotherapy, respectively. Results showed that after treatment, $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ significantly increased and $CD8^+$ significantly decreased in test group; on the contrary, $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ decreased and $CD8^+$ increased in control group. Rate of $CD4^+/CD8^+$ in test group had significant differences compared with that before treatment and after treatment in control group ($P < 0.05$). This finding suggests that *G. lucidum* spores can improve the indexes of T cell subsets in patients with NSCLC cancer during

chemotherapy and enhance the cellular immune function of patients. It is worthy of clinical promotion and application [84].

Lin et al. (2004) compared the effect of *G. lucidum* extract combined with chemotherapy on 114 cases of cancer patients. These patients were confirmed with cancers of the stomach, esophagus, lung, liver, cervix, colon, or bladder by pathological, cytological, and CT examination, based on the *National Guidelines for Diagnosis and Treatment of the Common Cancers in China*. All patients were randomly divided into two groups, i.e., chemotherapy with *G. lucidum* group (CG) (66 cases) and chemotherapy control group (CC) (48 cases). In CC, the FAM chemotherapy was applied, including intravenous injections of 300 mg/m^2 5-flurouracil twice a week and 30 mg/m^2 adriamycin once a week in the first and the fourth weeks and intravenous injection of 3 mg/m^2 mitomycin once a week, for 6 weeks as one course of treatment. After 4 to 5 months, the treatment could be repeated according to the patients' conditions. For the patients in CG, in addition to the same chemotherapy, four capsules of *G. lucidum* fruiting body extract were administered, four times daily for 40 days as one course of treatment.

The results demonstrated that *G. lucidum* extract markedly ameliorated immune suppression induced by chemotherapy. In CG, the natural killer (NK) cell activities in the patients were 51.24 ± 7.9 before and 48.10 ± 7.90 after the treatment, showing no statistically significant difference ($P > 0.05$). On the other hand, those in CC were 51.40 ± 6.62 before and 44.43 ± 7.19 after the treatment, showing a statistically significant difference ($P < 0.05$). As shown in Table 2.3, the CD_3, CD_4, and CD_8 cell subsets in CG changed a little, but in CC, they were significantly depressed by the treatment. Furthermore, both clinical symptoms and quality of life of the patients in CG were improved [85].

Qi YF et al. reported that 200 cases of hospitalized patients with gastric cancer, esophageal cancer, liver cancer, colorectal cancer, pancreatic cancer, gallbladder cancer, bile duct cancer, periampullary cancer, and gastric malignant lymphoma were diagnosed by cytology or pathology (liver cancer for clinical diagnosis); the average quality of life score of Karnofsky scores was greater than 60 points. No cancer treatment was given within 1 month before treatment. Test group (100 cases) oral administration of *Ganoderma* spore powder capsules (0.25 g per capsules), 4 capsules each time, 3 times a day. Control group (100 cases) oral Zhen Qi Fu Zheng granule (15 g per packet), 1 packet each time, 3 times a day. Patients in both groups

Table 2.3 Changes on T-lymphocyte subsets in CG and CC group before and after treatments. Ref [85]

Group	n		CD_3^+	CD_4^+	CD_8^+
CG	66	Before	51.43 ± 6.00	36.57 ± 6.69	31.20 ± 6.90
		After	50.67 ± 6.29	37.10 ± 6.49	30.24 ± 7.60
CC	48	Before	50.99 ± 6.52	37.75 ± 7.40	30.99 ± 6.69
		After	43.38 ± 6.39∗	31.01 ± 6.31∗	26.42 ± 7.15∗

$\bar{x} \pm s$; ∗p<0.05, compared with before and after the treatment in the same group

were given the drug for 4 weeks one course of treatment, not less than two courses of medication per case.

Conventional chemotherapy should be given in both groups at the beginning of each course. FAM was used for gastric cancer, liver cancer, and large intestine cancer and CFP for esophageal cancer. Four weeks was one period; apply two cycles in a row. The curative effect was judged after the course of treatment. The curative effect was judged after the course of treatment. Results showed the following:

1. Short-term objective efficacy: total effective rate (CR + PR) in the test group was 43%, including 3 cases of CR, 40 cases of PR, 45 cases of NC, and 12 cases of PD. The total effective rate of the control group was 33%, including 2 cases of CR and 31 cases of PR, 48 cases of NC, and 19 cases of PD. There were significant differences between the two groups ($P < 0.05$).
2. Change of life quality: Karnofsky score of the test group increased by more than 10 points of the 66 cases, 23 cases had a fluctuation of less than 10 points (stable) on the top and bottom, and 11 cases had a decrease of 10 points. Karnofsky score of control group increased in 49 cases, stabilized in 19 cases, and decreased in 32 cases. There were significant differences between the two groups ($P < 0.05$).
3. Change of body weight: in the test group 68 cases with body weight increase greater than or equal to 1.5 kg, there were 21 cases with lower fluctuation within 1.5 kg (stable) and 11 cases with lower 1.5 kg. In the control group, the body weight increased in 45 cases, stabilized in 26 cases, and decreased in 29 cases. There were significant differences between the two groups ($P < 0.05$).
4. Changes of peripheral blood: peripheral white blood cells and platelets restored to normal were 89 and 92 cases, respectively, and 11 and 8 cases lower than normal at the end of treatment in the test group. In the control group, those who recovered to normal were 93 and 95, respectively, and those who were lower than normal were 7 and 5, respectively. There was no significant difference between the two groups ($P > 0.05$).
5. The immune functional changes: $CD3^+$ cells in the test group were compared with those before and after treatment from $55.35 \pm 7.30\%$ to $67.23 \pm 6.61\%$ ($P < 0.01$), the $CD4^+/CD8^+$ ratio increased from 1.35 ± 0.67 to 1.58 ± 0.44 ($P < 0.05$), and the T-lymphocyte transformation rate increased from $60.19 \pm 8.05\%$ to $65.02 \pm 9.64\%$ ($P < 0.05$). There was no significant change in the above immune indexes before and after treatment in the control group, while the improvement in the above cellular immunological indexes was significantly different between the experimental group and the control group after treatment ($P < 0.05$).

No adverse reaction was observed in the test group. The results showed that *Ganoderma* spore powder capsule could be used as tumor chemotherapy and as an adjuvant therapy drug, have synergistic effect, and reduce toxic action [86].

Zhen et al. (2012) reported the clinical efficacy of *Ganoderma lucidum* spore (GLS) on recurrence after curative resection of hepatocellular carcinoma (HCC). Sixty patients undergoing curative resection of HCC were randomly divided into conventional treatment group and GLS treatment group. GLS-treated group based

on conventional treatment with oral administration of *Ganoderma* spore powder (0.3 g per capsules) with 5 capsules each time, 3 times a day, for 6 months.

After 2 years of follow-up, the disease-free survival (DFS) and overall survival (OS) were compared between the two groups. Results showed that there was no significant difference in age, gender, tumor size, intraoperative blood loss, hospital stay, the proportions of the patients with AFP more than 20 ng/ml, microvascular invasion, tumor satellite nodules, hepatitis B virus infection, cirrhosis, and postoperative anti-virus therapy, between the two groups ($P < 0.05$). The 2-year DFS were 53.3% and 70.0% ($P = 0.034$) and 2-year OS were 60.0% and 83.3% ($P = 0.023$), respectively, in the conventional treatment group and GLS group. There was no significant difference in postoperative complication rate between the two groups ($P = 0.472$), and only one case developed diarrhea due to adverse effect of GLS. These results suggest that GLS treatment is safe and effective to inhibit tumor recurrence and improve DFS and OS of the patients after radical resection for HCC [87].

Zhen et al. (2013) reported the effect of *Ganoderma lucidum* spore (GLS) on the immunological function in the patients of hepatocellular carcinoma (HCC) after operation. A total of 70 HCC patients who underwent hepatectomy in the hepatobiliary and pancreatic surgery were studied prospectively. The informed consents of all patients were obtained, and the ethical committee approval was received. The patients were randomly divided into the conventional therapy group (CT) and *G. lucidum* spore therapy group (GLS) using computer random number table method. There were 35 cases in the CT group, of which 30 were males and 5 were females with the mean age of (51 ± 10) years old. There were 35 cases in the GLS group, of which 28 were males, 7 were females with the mean age of (50 ± 9) years old. Another 35 healthy people who received physical examination were enrolled as the control group. Patients in the CT group began to take compound glycyrrhizin and GIK injection 1 day after hepatectomy. GLS group based on conventional treatment were oral administration of 5 *Ganoderma* spore powder (0.3 g per capsules) capsules each time, 3 times a day, for 1 month. Detections of $CD4^+$, $CD8^+$, and natural killer (NK) cells in peripheral blood were undertaken preoperatively (or when enrolled in the study) and 1, 7, and 28 days after the operation. Results showed that $CD4^+$ cells in HCC patients (34 ± 7%) reduced significantly, compared with the control group (43 ± 7%) ($t = 5.63$, $P < 0.05$). $CD8^+$ cells in HCC patients (30 ± 3%) elevated significantly, compared with the control group (27 ± 3%) ($t = 4.83$, $P < 0.05$). NK cells in HCC patients (13 ± 4%) decreased significantly, compared with the control group (19 ± 5%) ($t = 6.18$, $P < 0.05$). The $CD4^+$, $CD8^+$, and NK cells of patients before the operation were 34 ± 7%, 30 ± 4%, 14 ± 4%, respectively, in the CT group and 34 ± 7%, 31 ± 3%, and 13 ± 4% in the GLS group, and there was no significant difference between them ($t = 0.00, 1.18, 1.05$; $P > 0.05$). The $CD4^+$, $CD8^+$, and NK cells of patients in two groups decreased evidently 1 day after operation compared with the preoperative data ($t = 4.01, 2.69, 2.75$; $P < 0.01$). Table 2.4 shows that compared with the CT group, $CD4^+$ in the GLS group elevated significantly 7 and 28 days after operation, while $CD8^+$ decreased significantly, and the NK cells elevated significantly. The cellular immunological function in patients

Table 2.4 *Ganoderma lucidum* spores (GLS) ameliorated immune function after hepatic resection in patients with hepatocellular carcinoma. (Ref [88])

Group	n	$CD4^+$ (%)				$CD8^+$ (%)				NK(%)			
		0 day	1 day	7 days	28 days	0 day	1 days	7 days	28 days	0 day	1 days	7 days	28 days
CT	35	34 ± 7	29 ± 4	33 ± 5	38 ± 6	30 ± 4	28 ± 4	33 ± 5	29 ± 3	14 ± 4	12 ± 4	15 ± 3	16 ± 4
GLS	35	34 ± 7	30 ± 3	37 ± 4*	42 ± 7*	31 ± 3	28 ± 3	29 ± 3*	27 ± 3*	13 ± 4	10 ± 3	17 ± 3*	18 ± 4*

GLS (0.3 g/capsule) 1.5 g, three times a day for 1 month; $p < 0.05$ vs CT group

with HCC is inhibited before and after the operation. Application of *G. lucidum* spores early after the operation can improve the findings indicate the cellular immunological function in patients with HCC is inhibited before and after the operation. Application of *Ganoderma lucidum* spores early after the operation can improve the cellular immunological function and help to maintain the body immunological balance [88].

Zhou and Zhang reported (2014) that 90 patients with of elderly cervical cancer (squamous cell carcinoma, adenocarcinoma, and adenosquamous carcinoma) were randomized controlled trial. Patients were divided into control group and observation group by numerical table method. General data of two groups of patients such as age, pathological classification, and clinical stage were not statistically different. The control group was given neoadjuvant chemotherapy with BOMP (bleomycin + vincristine + mitomycin + cisplatin) regimen by arterial intervention 3 weeks before surgery. The cervix carcinoma was performed radical resection, including mainly pelvic and iliac lymphadenectomy and extensive uterine resection after neoadjuvant chemotherapy. On the basis of treatment in the control group postoperative, observation group was orally administered 5 *Ganoderma* spore powder (0.3 g per pill) pills per time, 3 times a day, for 28 continuous days. Flow cytometry was used to detect T-lymphocyte subsets in peripheral blood. Vascular endothelial growth factor (VEGF) mRNA was detected by real-time fluorescence quantitative PCR. The results showed that T-lymphocyte subsets, NK cells activity, and VEGF mRNA levels have no significant difference in peripheral blood before treatment in the control group and the observation group ($P > 0.05$). After treatment with *Ganoderma* spore powder, $CD8^+$ were decreased, and both $CD4^+$ and $CD4^+/CD8^+$ ratio and NK cell activity were significantly increased in the observation group compared with the control group ($P < 0.05$). VEGF mRNA expression was significantly decreased. This result suggests that *Ganoderma lucidum* spore can significantly improve the postoperative cellular immune function and reduce VEGF expression in elderly patients with cervical cancer [89].

Zhao et al. (2012) investigated the effectiveness of *Ganoderma lucidum* spore powder on cancer-related fatigue in breast cancer patients undergoing endocrine therapy. Forty-eight breast cancer patients with cancer-related fatigue undergoing endocrine therapy were randomized into the experimental or control group. Patients of experimental group were orally administered with *Ganoderma* spore powder 1000 mg, 3 times a day, for 4 weeks. Functional Assessment of Cancer Therapy: Fatigue (FACT-F), The Hospital Anxiety and Depression Scale (HADS), and European Organisation for Research and Treatment of Cancer Core Quality of Life Questionnaire C30 (EORTC QLQ-C30) questionnaires data were collected at baseline and 4 weeks after treatment. The concentrations of TNF-α and IL-6, immune markers of CRF, and liver and kidney functions were measured before and after intervention. Result showed that experimental group had statistically significant improvements in the domains of physical well-being and fatigue subscale after intervention. These patients also reported less anxiety and depression and better quality of life. Compared with control group, the fatigue, sleep disturbance, and appetite loss of patients in experimental group were improved according to the outcomes of the EORTC QLQ-C30 ($P < 0.01$, $P < 0.01$, and $P < 0.05$, resp.). The mean

serum concentrations of TNF-α and IL-6 in pre- and post-treatments in experimental group were 128.70 pg/mL and 71.89 pg/mL and 62.43 pg/mL and 37.62 pg/mL, respectively. The level of TNF-α and IL-6 in serum was significantly low ($P < 0.01$ and $P < 0.05$, resp.). No serious adverse effects occurred during the study. This pilot study suggests that spore powder of *Ganoderma lucidum* may have beneficial effects on cancer-related fatigue and quality of life in breast cancer patients undergoing endocrine therapy [90].

Two reviews of Cochrane Database summarized that *G. lucidum* could be administered as an alternative adjunct to conventional treatment in consideration of its potential of enhancing tumor response and stimulating host immunity. *G. lucidum* was generally well tolerated by most participants with only a scattered number of minor adverse events. Future studies should put emphasis on the improvement in methodological quality, and further clinical researches on the effect of *G. lucidum* on cancer long-term survival are needed [91, 92].

2.4 Conclusion

Over the years, the academic circles have focused great importance to the antitumor effect of *Ganoderma* and its immunological mechanism. Besides the immunological mechanism of *Ganoderma* against tumor mentioned in this paper, a series of research also found that *Ganoderma* can inhibit tumor angiogenesis [93–99] and tumor cell movement and adhesion [100–104] and inhibit multidrug resistance of tumor cells against antitumor drugs [105–108].

Furthermore, alcohol extract and triterpenoids isolated from fruiting body of *G. lucidum* had direct cytotoxic effect on tumor cells in vitro [109–115]. Most of these results were obtained from cells cultured in vitro, so there is still a need for study in animal models in vivo.

G. lucidum is used as adjuvant therapy with chemotherapy and/or radiotherapy in cancer patients and plays a synergistic role such as prolonging long-term survival, promoting life quality, and regulating immune to normalization. It also reduces the adverse reactions in cancer patients with radiotherapy and/or chemotherapy. These results have been preliminarily confirmed by clinical trial. However, this is only the beginning; many things need to be done, especially for GCP-compliant multicenter, clinical trial of large samples.

There are two thinking ways for antitumor research of *Ganoderma*: one is to find out the effective components and ultimately develop a new antitumor drug; the other one is to study the adjuvant therapeutic mechanism of *Ganoderma* on tumor using combination of traditional Chinese medicine and Western medicine method and to illustrate modern mechanism of “Fu Zheng Gu Ben (supporting the healthy energy, strengthening and consolidating body resistance),” as an important principle of TCM. The latter is more than that of former for us. Our academic thinking and way took over the past years has been proved correct by preclinical research and clinical practice.

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Chapter 3
Cellular and Molecular Mechanism of *Ganoderma* (Lingzhi) Against Tumor

Yu Sun and Lixin Sun

Abstract The anticancer potential of *Ganoderma* (Lingzhi) and its extracts has been widely demonstrated, including antiproliferative and apoptosis inductive, anti-metastatic, antiangiogenic, and multidrug resistance reversional activities, involving a variety of cellular and molecular mechanisms besides antitumor immunology. Intrinsic- and extrinsic-initiated apoptotic pathway in association with cell cycle arresting, telomerase inhibiting, autophagy, and oxidative stress is involved in the antiproliferative and apoptosis inductive activities of *Ganoderma* and its extracts. The inhibition of tumor cell adhesion, invasion, and migration by *Ganoderma* and its extracts involves molecular mechanisms such as AP-1, NF-κB, MMP, cadherin, β-integrin, c-Met, FAK, EMT, and so on. Targeting the major pro-angiogenic stimulus, VEGF, and its receptor contributes to the inhibition of tumor angiogenesis by *Ganoderma* and its extracts. Inhibition against the ATP-dependent transmembrane drug transporter such as P-glycoprotein (P-gp) on the surface of resistant tumor cells to prevent reduction of the intracellular accumulation of anticancer drugs by pumping out the drugs plays an important role in the activities of *Ganoderma* and its extracts to reverse tumor cell multidrug resistance.

Keywords *Ganoderma* · Lingzhi · Tumor · Cytotoxicity · Apoptosis · Invasion · Migration · Angiogenesis · Multidrug resistance

Natural products contain plenty of compounds with a broad spectrum of therapeutic indication suggesting that functional moieties act as a core pharmacophore. *Ganoderma* (Lingzhi), a species of the *Basidiomycetes* class, is a mushroom with a long history as a remedy used in some Asian countries to promote health and longevity. *Ganoderma* has considerably become an attractive natural product for researchers and has been attracting international attention because of its paramount and multiple pharmaceutical effects. Modern research methods gradually reveal its chemical constituents as well as their respective functions.

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The anticancer potential of the extracts derived from *Ganoderma* has been widely demonstrated. It was suggested that polysaccharide or aqueous extract (essentially contains polysaccharide) derived from *Ganoderma lucidum* (*G. lucidum*) have no direct cytotoxic or apoptotic effects on tumor cells in vitro, according to researches on some cancer cell lines, such as sarcoma-180 (S-180), human lung cancer (PG), and human leukemia (HL-60), without cytotoxicity and apoptosis found after direct addition of aqueous extract or the polysaccharide of *G. lucidum* into the culture medium, although the inhibitory effects against the tumors could be found in mice in vivo [1–6]. Controversially, there are some researches with different results showing direct inhibitory effects against tumor cells in vitro. Yue GG et al. (2006) compared the effects of the aqueous extracts derived from *G. lucidum, Ganoderma sinense, and Ganoderma tsugae,* on two human breast cancer cell lines, MCF-7 and MDA-MB-231, showing direct proliferation inhibitory effects on the cancer cells with invalid effects on human normal mammary epithelial cells [7]. Shen J et al. (2014) reported that *G. lucidum* polysaccharides (GLPs) can inhibit human liver carcinoma HepG2 cells directly through the regulation of hepatocarcinoma genes [8].

For triterpenoid or ethanol extract (essentially contains triterpenoid), varieties of researches revealed their direct inhibitory effects on the cancer cells in vitro. Amen YM et al. (2016) reported that a new oxygenated lanostane-type triterpene, named lucidumol C, isolated from the chloroform extract of the fruiting bodies of *Ganoderma* lingzhi showed cytotoxic activities against human colorectal carcinoma (HCT-116, Caco-2), human liver carcinoma (HepG2), and human cervical carcinoma (HeLa) cell lines [9]. Smina TP et al. (2016) found high cytotoxicity of *G. lucidum* total triterpenes against Dalton's lymphoma ascites (DLA) and Ehrlich's ascites carcinoma (EAC) cell lines with IC_{50} values 5 ± 0.32 and 3.9 ± 0.2 μg/ml, respectively, together with the induction of apoptosis in both cell lines which is evidenced from the DNA fragmentation assay, in addition to the anticancer activity of the total triterpenes in DLA-induced solid and EAC-induced ascites tumor models in Swiss albino mice [10]. Satria D et al. (2018) reported that, lucidumol D, a new lanostane-type triterpenoid isolated from the fruiting bodies of *Ganoderma* lingzhi showed selective antiproliferative and cytotoxic effects against MCF-7, HepG2, HeLa, Caco-2, and HCT-116 cancer cell lines [11]. Du GH et al. (2017) investigated that among 26 triterpenoids isolated from *G. lucidum* used in molecular docking by LibDock module of Discovery Studio 2016 software with 11 target proteins, the ganoderic acid Y and 7-oxo-ganoderic acid Z2 showed certain inhibitory activity on lung cancer cell H460 (IC_{50} 22.4 and 43.1 μmol/L, respectively), in addition to the other triterpenoids that had no antitumor activity in the detected tumor cell lines, demonstrated in MTT experiments [12].

The extracts derived from *Ganoderma* exhibit a broad spectrum of antitumor properties, including antiproliferative and apoptosis inductive, antimetastatic, anti-angiogenic, and multidrug resistance reversional activities, involving a variety of molecular mechanisms besides antitumor immunology.

3.1 The Antiproliferative and Apoptosis Inductive Activities and Their Molecular Mechanisms

Cancer, a multifactorial disease, causes alterations in behaviors and metabolisms and leads to uncontrolled cell proliferation and weakened immune system. The balance between cell proliferation and cell death is so crucial that it determines whether a cell progresses into survival or apoptosis. Accordingly, antiproliferation and pro-apoptosis in cancer cells are crucial to cancer therapy. Extracts of *Ganoderma* inhibit tumor cell proliferation and collaterally induce apoptosis involving some signaling pathways.

Xie JT et al. (2006) reported that among the two different fractions of *G. lucidum* extract, a fraction containing mainly polysaccharides (GLE-1) and a triterpenoid fraction (GLE-2), both with significant inhibitory effects against the proliferation of human colon cancer SW 480 cells, GLE-2 showed much stronger inhibitory effect than that of GLE-1, and the GLE-1 showed effects to inhibit DNA synthesis in the cells and reduce the formation of DPPH radicals [13].

Gao JJ et al. (2002) revealed that, among the three lanostane-type triterpene aldehydes, named lucialdehydes A, B, and C, isolated from the fruiting bodies of *G. lucidum*, lucialdehydes B and C showed cytotoxic effects against Lewis lung carcinoma (LLC), T-47D, sarcoma-180, and Meth-A tumor cell lines in vitro (with ED_{50} values of 10.7, 4.7, 3.1, and 3.8 μg/ml, respectively, for lucialdehyde C which exhibited the strongest cytotoxicity) [14]. Min BS et al. (2000) demonstrated the cytotoxicity of six new highly oxygenated lanostane-type triterpenes isolated from the *G. lucidum* spores against Meth-A and LLC tumor cell lines in vitro [15].

Ruan W et al. (2015) isolated and identified six triterpenoids that were ganolucidic acid E, lucidumol A, ganodermanontriol, 7-oxo-ganoderic acid Z, 15-hydroxy-ganoderic acid S, and ganoderic acid DM from an extract of the mushroom *G. lucidum*, and all compounds showed inhibitory activities against cell growth in three human carcinoma cells (Caco-2, HepG2, and HeLa cells) in a dose-dependent manner with LC_{50} from 20.87 to 84.36 μM, along with the induction of apoptosis in HeLa cells besides Caco-2 cells in which four of the compounds caused apoptosis, but none of the compounds induced apoptosis in HepG2 cells [16].

Zhang J et al. (2016) reported that both submerge-cultured intracellular *G. lucidum* polysaccharides (GLP) and fruiting body GLP inhibited the growth of p53 functional human carcinoma cell lines including HCT-116 $p53^{+/+}$, HepG2, A549 (human lung carcinoma cell), U2OS (human osteosarcoma cell), OCI-AML2 (human acute myeloid leukemia cell), MKN-45 (human gastric cell), and MCF-7, but they failed or were resisted in p53 dysfunctional human carcinoma cell lines, such as HCT-116 $p53^{-/-}$, Saos-2, H1299, HL-60, and MDA-MB-157, indicating that the inhibitory activity of GLP against cancer cells was p53 guided [17]. Furthermore, Jiang D et al. (2017) demonstrated that GLPs, alone or together with 5-fluorouracil (5-FU), reactivated mutant p53 in colorectal cancer HT-29 ($p53^{R273H}$) and SW480 ($p53^{R273H\&P309S}$) cells and further induced cell growth inhibition and apoptosis [18].

Dai J et al. (2017) found, using a fibroblast cell-quiescence model, that two natural compounds purified from *G. lucidum*, ergosterol peroxide and ganodermanondiol, exhibited cytotoxicity not only against fast proliferating cells but also against quiescent, slow-cycling cells due to apoptosis induction, and association with a shallower quiescent state in compound-treated cells, resultant from the increased basal activity of an Rb-E2F bistable switch that controls quiescence exit. The two compounds preferentially killed quiescent breast cancer cells (MCF7), compared to its non-transformed counterpart (MCF10A), presumably due to their already less stable quiescent state, suggesting the potential of the two compounds in future antitumor development against the slow-cycling cancer cell subpopulations including cancer stem and progenitor cells [19].

Chung WT et al. (2001) researched two types of purified samples, water-soluble (sample A) and water-insoluble (sample C) samples obtained from the culture broth of *G. lucidum* mycelium, showing that both samples much effectively inhibited the growth of human cancer cell lines, Hep3B, AGS, and A549 (with 93–85% growth inhibition) with the least cytotoxicity on the normal human lung cell line, WRL68. Compared with the sample A, the sample C showed greater inhibition on cancer cell growth and had higher antimutagenicity on mutagens 4NQO or MMNG according to Chinese hamster ovary cell line (CHO test) or *Salmonella typhimurium* (Ames test). The sample C accelerated apoptosis of human carcinoma cells (human lung cancer cell line, A549) up to 70% while the control less than 50%. After 4 days of cultivation, the sample C increased the differentiation ratio of HL-60 cells up to 58% while in the case of no sample supplementation 18% [20] Table 3.1.

The antiproliferative and proapoptotic activities of *Ganoderma* extracts in cancer cells are executed through mechanisms like intrinsic- and extrinsic-initiated apoptosis, cell cycle arresting, telomerase inhibition, autophagy, and oxidative stress, involving some signaling pathways (Fig. 3.1).

3.1.1 Intrinsic- and Extrinsic-Initiated Proapoptotic Activities Involving Some Signaling Pathways

Apoptosis, a programmed cell death, consists of two major pathways in mammalian cells: mitochondria-initiated intrinsic pathway and death receptor-stimulated extrinsic pathway [21–24]. In mitochondria-initiated intrinsic pathway, proapoptotic signals trigger a release from mitochondrial intermembranous space into cytosol of cytochrome c, which forms apoptosome, a complex with Apaf-1 and dATP, and provokes caspase-9 activation. The subsequent activation of executioner caspases, such as caspase-3, caspase-6, and caspase-7, resulted from the activation of caspase-9 that in turn causes a series of apoptotic events, which eventually result in cell death [25, 26]. The extrinsic pathway starts when Fas ligand binds to Fas death receptor, and with the recruitment of an adaptor molecule to the receptor, caspase-8 is combined and proteolytically activated. After activation, caspase-8 cleaves

Table 3.1 Growth inhibition against human cancer cell lines by partially purified culture broth of *G. lucidum*

	AGS				A549			
	Sample A		Sample C		Sample A		Sample C	
Concentration (mg/L)	Inhibition ratio (%)	Selectivity	Inhibition ratio (%)	Selectivity	Inhibition ratio (%)	Selectivity	Inhibition ratio (%)	Selectivity
0.2	24 ± 0.04[a]	0.41	24 ± 0.04[a]	1.11	21 ± 0.03[a]	0.76	28 ± 0.01[a]	0.98
0.4	29 ± 0.03[a]	0.36	29 ± 0.02[b]	0.92	31 ± 0.03[b]	0.84	25 ± 0.01[c]	1.31
0.6	36 ± 0.03[a]	0.93	55 ± 0.02[b]	1.04	48 ± 0.03[a]	0.88	59 ± 0.04[c]	1.32
0.8	48 ± 0.04[c]	1.10	71 ± 0.03[b]	1.10	62 ± 0.02[a]	0.84	78 ± 0.01[a]	1.14
1.0	68 ± 0.01[a]	1.11	85 ± 0.02[b]	1.32	24 ± 0.04[b]	1.10	24 ± 0.04[a]	1.58

Significantly different from the control: [a]$p < 0.001$; [b]$p < 0.05$; [c]$p < 0.01$

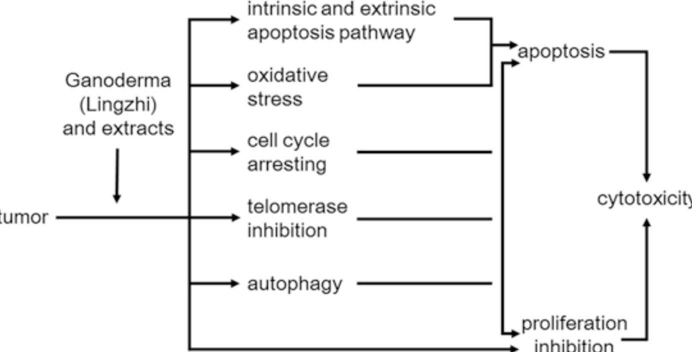

Fig. 3.1 The antiproliferative and apoptosis inductive activities of *Ganoderma* against tumor. *Ganoderma* and its extracts have antitumor activities with inhibition on proliferation and induction on apoptosis involving intrinsic and extrinsic apoptosis pathways in association with cell cycle arresting, telomerase inhibiting, autophagy, and oxidative stress

effector caspase-3, caspase-6, and caspase-7, causing apoptotic cell death [27]. It is well known that Bax, Bcl-2, and caspase-3 are mitochondrial apoptosis pathway-related factors. Upregulating the expression of Bax gene and downregulating the expression of Bcl-2 gene can induce the apoptosis [28]. Besides the abovementioned pathways, endoplasmic reticulum (ER), which normally regulates protein synthesis and intracellular calcium (Ca^{2+}) homeostasis, can also induce apoptosis [29]. Excessive ER stress triggers apoptosis via many mechanisms such as alteration in Ca^{2+} level, redox imbalance, and activation of Bcl-2 family proteins [30]. Because ER stress can also activate caspase-8 and caspase-9, there is cross talk with the two well-characterized apoptotic pathways [31–33]. Extracts of *Ganoderma*, besides inhibiting proliferation involving some signaling pathways, induce intrinsic and extrinsic apoptosis in tumor cells.

Li CH et al. (2005) found that among the methanol extracts of *Ganoderma amboinense* (*G. amboinense*) with the bioactivities to inhibit the growth of variety of cancer cell lines including hepatoma HuH-7 cells, colorectal carcinoma HCT-116, Burkitt's lymphoma Raji cells, and acute promyelocytic leukemia HL-60 cell (some of the methanol extracts inhibited the activities of topoisomerases I and II alpha) in vitro, one ganoderic acid X (GAX) of the most potent triterpene was identified (3 alpha-hydroxy-15 alpha-acetoxy-lanosta-7, 9(11), 24-trien-26-oic acid) with immediate inhibition of DNA synthesis as well as activation of ERK and JNK mitogen-activated protein kinases, and cell apoptosis in HuH-7 cells elucidated with molecular events of apoptosis including degradation of chromosomal DNA, decrease of Bcl-xL level, the disruption of mitochondrial membrane, cytosolic release of cytochrome c, and activation of caspase-3 [34].

Liang Z et al. (2014) found that *G. lucidum* polysaccharides (GLPs) time and dose dependently reduced cell viability on human colorectal cancer cells HCT-116 with cell apoptosis determined by morphological changes, DNA fragmentation, mitochondrial membrane potential decrease, S phase population increase, and

caspase-3 and caspase-9 activation. The upregulated expression of Bax (vs Bcl-2), caspase-3, and poly (ADP-ribose) polymerase (PARP) by GLP and dramatic decrease of the GLP-induced apoptosis by inhibition of c-Jun N-terminal kinase (JNK) using SP600125 demonstrated that apoptosis stimulated by GLP in human colorectal cancer cells was associated with activation of mitochondrial and mitogen-activated protein kinase (MAPK) pathways [35].

Smina TP et al. (2017) evaluated the cytotoxicity and proapoptotic effect of *G. lucidum* total triterpenes in human breast adenocarcinoma (MCF-7) cell line using MTT assay and DNA fragmentation analysis, showing that the total triterpenes induced apoptosis in MCF-7 cells by downregulation of the levels of cyclin DB, Bcl-2, and Bcl-xL and also by upregulation of the levels of Bax and caspase-9. Using skin papilloma and mammary adenocarcinoma in Swiss albino mice and Wistar rats, respectively, inducing with dimethylbenz [a] anthracene (DMBA), it was shown that topical application of 5, 10, and 20 mg total triterpenes reduced the incidence of skin papilloma by 62.5%, 33.5%, and 12.5%, respectively, as well as the incidence of the mammary tumor by 33.33%, 66.67%, and 16.67% in 10, 50, and 100 mg/kg b.wt. total triterpenes treated animals, respectively. The average number of tumors per animal was reduced, and the tumor latency period was extended by the total triterpenes in both models [36].

Wu K et al. (2018) demonstrated that polysaccharides were extracted from the powder of sporoderm-broken spores of *G. lucidum* (GLP) significantly inhibited cell viability in a time- and dose-dependent manner along with the induction of late apoptosis accompanying poly (ADP-ribose) polymerase 1 (PARP) cleavage and inhibition of pro-caspase-3, pro-caspase-6, and pro-caspase-9 protein expression, in PC-3 cells. Meanwhile, the GLP upregulated the expression levels of NAG-1, as well as its transcriptional factor early growth response-1, in a time- and dose-dependent manner. With a luciferase assay, GLP demonstrated its effect on induction of the NAG-1 promoter activity, indicating the transcriptional regulatory effect of GLP on NAG-1. GLP-induced apoptosis was significantly, but not completely, prevented by inhibition of NAG-1 expression using small interfering RNA, and the effects of GLP on PARP and pro-caspase expression were reversed. Furthermore, the phosphorylation of protein kinase B and mitogen-activated protein kinase/extracellular signal-regulated kinase signaling were inhibited by GLP in PC-3 cells. These results indicate that the apoptosis induced by GLP in PCa cells may partially be mediated through NAG-1 induction [37].

Zheng L et al. (2018) purified a steroid from *G. lucidum* on a submerged culture and investigated its antitumor mechanisms on A375 human malignant melanoma cells by MTT, flow cytometry, and Western blotting, demonstrating that apoptotic mechanisms of the steroid were caspase-dependent and mediated via the mitochondrial pathway. It significantly caused downregulation of induced myeloid leukemia cell differentiation protein Mcl-1 (Mcl-1) in melanoma cells, but not significant changes in the expression levels of Bcl-2 family proteins, such as Bcl-2-like protein 11, p53 upregulated modulator of apoptosis, Bcl-2-associated X protein, BH3 interacting-domain death agonist, Bcl-2-associated death promoter, and Bcl-2, suggesting that Mcl-1 is crucial in regulating apoptosis of melanoma cells induced by the steroid [38].

Calviño E et al. (2010) found that, derived from *G. lucidum* fruiting body, two aqueous extracts slightly reduced cell viability and induced DNA fragmentation in NB4 cells while methanol-extracted semipurified fraction significantly reduced the viability of these leukemia cells (treated for 19 h) along with the induction of DNA fragmentation and induction of apoptosis with a reduction of p53 levels, of the Bcl-2/Bax relationship as well as reduced levels of both unphosphorylated and phosphorylated Akt (protein kinase Akt, protein kinase B) and Erk (Erk1 and Erk2) [39].

Jang KJ et al. (2010) investigated the significant viability decreasing effects of ethanol extracts of *G. lucidum* (EGL) on the AGS human gastric carcinoma cells in a dose- and time-dependent manner which attributed to apoptotic cell death, with observed chromatin condensation and an accumulation of apoptotic fraction, caused by induction of the expression of death receptor-related proteins such as death receptor 5 and tumor necrosis factor-related apoptosis-inducing ligand, which further triggered the activation of caspase-8 and the cleavage of Bid, besides activation of caspase-9 and caspase-3, downregulation of IAP family proteins such as XIAP and survivin, and concomitant degradation of poly (ADP-ribose) polymerase; meanwhile the activity of Akt was downregulated in EGL-treated cells, and the phosphatidylinositol-3 kinase/Akt inhibitor LY294002 sensitized the cells to EGL-induced apoptosis [40]. Thereby, it could be indicated that the apoptosis of AGS cells induced by EGL involves both signaling cascade of death receptor-mediated extrinsic and mitochondria-mediated intrinsic, caspase pathways associating with inactivation of the Akt signal pathway (Fig. 3.2).

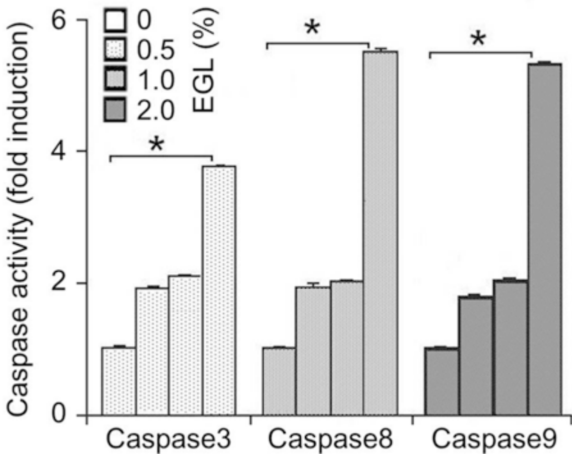

Fig. 3.2 Activation of caspases by ethanol extracts of *G. lucidum* (EGL). The AGS human gastric carcinoma cells were incubated with EGL for 72 h and then were collected and lysed. Aliquots were incubated with Asp-Glu-Val-Asp-p-nitroaniline, Ile-Glu-Thr-Asp-p-nitroaniline, and Leu-Glu-His-Asp-p-nitroaniline for caspase-3, caspase-8, and caspase-9, individually, at 37 °C for 1 h. The released fluorescent products were measured. Three independent experiments were performed. Asterisks indicate Student's *t*-test *p* values <0.05. (Adapted from Ref. [40])

3.1.2 Cell Cycle-Arresting Activities and Their Molecular Mechanisms

Cell cycle is a continuous process for cell proliferation, with the duplication of chromosomes and their distribution to a pair of genetically identical daughter cells, known as cell division (mitosis). Cell cycle consists of four distinct cell cycle phases: G1 phase (gap 1, interphase 1), S phase (synthesis), G2 phase (gap 2, interphase 2), and M phase (mitosis or meiosis). The progression from one phase to another is regulated by a set of conserved serine-threonine cyclin-dependent kinases (CDKs) in complex with temporarily expressed special adaptor proteins, cyclins. Classically, cyclin D1 bind CDK4 and CDK6 drive the entry into G1 phase, with the phosphorylation of retinoblastoma protein (Rb) and release of E2F (E2 factor) transcription factor. E2F, in turn, triggers the expression of cyclins E and A. The transition from G1 to S phase is driven by association of CDK2 with cyclin E, and the replacement of cyclin E by cyclin A in the complex initiates S phase. CDK1 in complex with cyclins A and B drives the S to G2 transition and M phase, respectively. At each phase, a set of specific inhibitors such as p15, p21, p27, or WEE1 controls the activity of each CDK complexes [41–44]. Cell cycle arrest results in inhibition of cell proliferation and induction of apoptosis in cancer cells. Extracts of *Ganoderma* were reported to have the cell cycle-arresting activity.

Müller CI et al. (2006) screened *G. lucidum* extract for its antiproliferative activity using a panel of 26 human cancer cell lines and revealed the facts that HL-60 (ED_{50} 26 μg/ml), U937 (63 μg/ml), K562 (50 μg/ml), Blin-1 (38 μg/ml), Nalm-6 (30 μg/ml), and RPMI8226 (40 μg/ml) were the six most sensitive hematologic cell lines, that HL-60 cells demonstrated the most prominent cell cycle arrest (at G2/M), and that four hematopoietic cell lines (HL-60, Blin-1, U937, RPMI8226) showed apoptosis ranged between 21% and 92% [45].

Zhu HS et al. (2000) investigated two alcohol extracts from sporoderm-broken spores of *G. lucidum* (termed extract I and extract III) both with strong inhibitory effects on the growth of HeLa cells (extract III was more effective than extract I), and extract III showed capability to block cell cycle at the transition from G1 to S phase and induce a marked decrease of intracellular calcium level, which might influence cellular signal transduction by altering the calcium transport system [46] (Table 3.2).

Lin SB et al. (2003) reported a triterpene-enriched fraction, WEES-G6, prepared from mycelia of *G. lucidum* and found that 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 resulted in a prolonged G2 cell cycle phase and strong growth inhibition, on human hepatoma Huh-7 cells, but not the normal liver cells [47].

Hu H et al. (2002) found that alcohol extract of *G. lucidum* caused nearly a 70% inhibition of cell growth in MCF-7 cells compared to the control (after 48 h of treatment at 500 μg/ml), and induced cell cycle arrest at G1 phase (12~48 h after treatment) with increased level of p21 (12 h after treatment remaining elevation up to 48 h) in contrast to the decreased levels of cyclin D1, cdk4 and E2F, meanwhile induced apoptosis with the increased level of Bax protein (after 12 h of treatment

Table 3.2 Cell cycle distribution of Hela cells after treatment with extract III from *G. lucidum* spores

	0 h			24 h			48 h		
Concentration (mg/ml)	G1	S	G2 + M	G1	S	G2 + M	G1	S	G2 + M
0	55 ± 4	36 ± 4	9 ± 2	60 ± 6	30 ± 2	10 ± 2	66 ± 7	25 ± 5	9 ± 2
0.2				67 ± 7	22 ± 5	11 ± 3	69 ± 7	22 ± 3	9 ± 2
0.4				72 ± 8	18 ± 3	10 ± 2	71 ± 2	20 ± 1	9 ± 1

Values are the mean ± SD of three determinations

remaining elevation up to 48 h) and unchanged expression of Bcl-2, suggesting mediation of the inhibition of cell proliferation and cell cycle arrest via upregulation of p21/Waf1 and downregulation of cyclin D1, as well as mediation of the apoptosis via upregulation of a proapoptotic Bax protein [48].

Chang UM et al. (2006) found that Ganoderiol F (GolF), a tetracyclic triterpene isolated from *G. amboinense*, induced growth arrest of cancer cell lines HepG2, Huh-7, and K562, with much less effect in hepatoma Hep3B cells and normal lung fibroblast MRC-5 cells, and no effect on peripheral blood mononuclear cells resulted in prompt inhibition of DNA synthesis and arrest of cell progression cycle in G1 phase in the cancer cells except Hep3B, with the inhibitory activity on topoisomerases which may contribute to the inhibition of cellular DNA synthesis, and for HepG2 cells, after 18 days of continuous treatment with 30 μM GolF, over 50% of cells were enlarged and flattened, with beta-galactosidase positive phenotypes of senescent cells. It is presumed that the activation of the mitogen-activated protein kinase EKR and upregulation of cyclin-dependent kinase inhibitor p16 in early stages of GolF treatment lead to cell cycle arrest and induce premature senescence of HepG2 cells [49].

Kuo HP et al. (2013) investigated a quality assured extract of *Ganoderma tsugae* (*G. tsugae*, GTE) which exhibited activities of inhibition on the growth of HER2-overexpressing cancer cells in vitro and in vivo and enhancement of the growth inhibitory effect of antitumor drugs (e.g., taxol and cisplatin) in these cells, accompanying cell cycle arrest by interfering with the HER2/PI3K/Akt signaling pathway, besides the curtailed expression of the HER2 protein by modulating the transcriptional activity of the HER2 gene and the stability/degradation of the HER2 protein [50].

Sun Z et al. (2014) prepared a sulfated polysaccharide (SCGLP1) from the fruiting bodies of *G. lucidum* and demonstrated that treatment with SCGLP1 in human osteosarcoma MG63 cells dose and time dependently inhibited cell proliferation and cell viability, induced apoptotic death with an increase in G0/G1 phase arrest, but had minor cytotoxic effect on human normal osteoblast (NHOst) cells. SCGLP1 increased Bax and Bad, decreased Bcl-2 and Bcl-XL, suppressed the loss of mitochondrial membrane potential (Δψm) and the release of mitochondrial cytochrome c to cytosol, and inhibited cleavage of caspase-9, caspase-3, and poly (ADP-ribose) polymerase (PARP). In addition, pan-caspase inhibitor (z-VAD-fmk) antagonized the SCGLP1-induced apoptosis in MG63 cells. The results indicate that the apoptosis induced by SCGLP1 is primarily associated with caspase-3- and caspase-9-dependent apoptotic pathway [51].

Wang T et al. (2015) found that *G. lucidum* triterpenoids (GLTs) dose dependently inhibited prostate cancer cell growth through induction of apoptosis by activation of caspases-9 and caspases-3 to trigger downstream apoptotic events and cell cycle arrest at G1 phase by upregulation of p21 expression at the early time and downregulation of cyclin-dependent kinase 4 (CDK4) and E2F1 expression at the late time [52].

Shao Y et al. (2016) found that the anticancer effects of triterpenes from *G. lucidum* might be associated with histone acetylation and interphase of mitotic cell cycle through modulating general control non-derepressible 5 (GCN5) and cyclin-dependent kinase-2 (CDK2), respectively [53].

Na K et al. (2017) showed that the water extract from sporoderm-broken spores of *G. lucidum* (BSGLWE) time and dose dependently inhibited colorectal cancer HCT-116 cell viability with the disruption of cell cycle progression at G2/M phase through decreasing cyclin B1 and cyclin A2 and increasing P21 at mRNA levels. Besides, BSGLWE induced apoptosis via downregulation of Bcl-2 and survivin at mRNA levels and decreasing Bcl-2, PARP, pro-caspase-3, and pro-caspase-9 at protein levels. In addition, BSGLWE inhibited tumor growth in vivo because of regulation on the expression of genes and proteins related to cell cycle and apoptosis, such as reduction of Ki67, PCNA, and Bcl-2 expression. Furthermore, BSGLWE treatment significantly upregulated NSAID activated gene-1 (NAG-1), a proapoptotic gene, in vivo and in vitro at both mRNA and protein levels, with the upregulated relative amounts of secreted NAG-1 in cell culture medium or serum of nude mice, suggesting a role of NAG-1 in BSGLWE-induced anticolorectal cancer activity [54].

Cui ML et al. (2017) reported that transformed soybean isoflavones with the homogenized slurry of *G. lucidum* (TSI) dramatically inhibited the viability of HTL-9 cells and MCF-7 cells but no detectable cytotoxicity found in GES-1 normal cells at the TSI concentration lower than 100 μg/ml. HTL-9 cells were arrested in the G1 phase by 100 μg/ml of TSI, which also primarily induced late apoptosis, accompanied with partial early apoptosis. The expressions of Bax, caspase-3, caspase-8, and cytochrome C were upregulated by TSI (100 μg/ml) in HTL-9 cells, according to Western blot and RT-PCR analysis, indicating the main mediation of TSI-induced cell apoptosis via mitochondrial pathway. Besides, the expression of p53 was elevated, and the expression of survivin and nuclear factor κB (NF-κB) was inhibited. Cell cycle was arrested in the G1 phase by TSI which induced primarily early apoptosis in MCF-7 cells as well [55].

Gill BS et al. (2018) described that among the constituents of *G. lucidum*, the major terpenoid, i.e., ganoderic acid, was found to interact with membrane receptors, mainly receptor tyrosine kinase (RTKs). It performed anticancer activities by interaction and modulation of the signaling network in IR, IGFR-1, IGFR-2, VEGFR-1, VEFGR-2, and EGFR in cancer signaling pathways, primarily acting on NF-κB, RAS-MAPK, PI3K/Akt/mTOR, and cell cycle resulting in apoptosis [56].

Lin TY et al. (2017) demonstrated that a recombinant medicinal mushroom *G. lucidum* protein, recombinant LZ-8 (rLZ-8), downregulated the expression of both wild-type and mutated EGFR; inhibited EGFR downstream effectors, AKT and ERK1/2; and therefore induced cell cycle arrest and apoptosis in lung cancer cells. In mouse model, the lung cancer progression, as well as EGFR expression, was also effectively inhibited by rLZ-8 in lung tumor lesions. The rLZ-8 altered EGFR localization that enhanced the EGF-induced degradation of EGFR, therefore reducing the amount of EGFR in cell membranes. Binding rLZ-8 to EGFR caused EGFR autophosphorylation at tyrosine 1045 with ubiquitination by the formation of EGFR/Cbl complexes, leading to the degradation of EGFR, but the EGFR degradation was abolished by Cbl-shRNA. Accordingly, it was shown that rLZ-8 inhibited growth and induced apoptosis of lung cancer cells by promoting EGFR degradation [57].

3.1.3 Telomerase Inhibiting Activities and Their Molecular Mechanisms

Telomere and telomerase have become attractive targets to develop anticancer therapies because of their important roles in cancer cell immortality [58]. Telomere is a specialized chromatin terminal structure with the function to protect chromosome end from dangerous processing events. Telomere exerts a finite limit on cell growth because of its shortening after each round of cell division. Telomere length is influenced by a complex balance between "shortening" and "elongation" signals. Telomerase is the only enzyme with the function to maintain telomere length by elongating a telomeric DNA chain and telomeric repeat-containing RNA (TERRA). In most of mammal cancer cells, maintenance of telomere length by telomerase to bypass the growth limitation plays an important role in cellular immortalization and oncogenesis [59]. *Ganoderma* and its extracts have the inhibitory activity on telomerase with anticancer effects.

Gonul O et al. (2015) demonstrated that, among the *G. lucidum* extracts, the ether extract with the highest cytotoxic potency (IC_{50} 100 μg/mL) after 72 h of incubation displayed the inhibitory effects on the telomerase activity in MCF-7 cells, showing that the telomerase activity was 32.2% lower in the ether extract-treated MCF-7 cells (100 μg/mL in DMSO) than that of the 1% DMSO-treated MCF-7 cells as control. Among the predicted miRNAs targeting the telomerase reverse transcriptase (TERT, which is the catalytic subunit of the telomerase), two of them, miR-3687 and miR-1207-5p, were upregulated by at least twofold, indicating involvement of the two miRNAs in the inhibitory effect on telomerase activity in MCF-7 breast cancer cells treated with *G. lucidum* ether extract [60].

Liao CH et al. (2006) expressed and purified the recombinant fungal immunomodulatory protein (reFIP-gts, which is originally isolated from *G. tsugae*) in E. coli and found that reFIP-gts significantly and selectively inhibited the growth of A549 cancer cells without affecting the growth of normal MRC-5 fibroblasts. The reFIP-gts suppressed telomerase activity in a concentration-dependent manner, with the downregulation of the telomerase catalytic subunit (hTERT). It was also found at the mRNA level. With the transient transfections of A549 cells with pGL3-Basic plasmid constructs containing the functional hTERT promoter and its E-box-deleted sequences cloned upstream of a luciferase reporter gene, determined by electrophoretic mobility shift assays and Western blotting, it was demonstrated that reFIP-gts inhibited binding of c-myc transcriptional factor to the E-box sequence on the hTERT promoter, indicating that reFIP-gts suppresses telomerase activity and inhibits transcriptional regulation of hTERT via a c-myc-responsive element-dependent mechanism [61].

Telomeres have G-quadruplex (G4) structures which are formed by telomeric DNA containing repetitive DNA sequence (TTAGGG)n, and the G4 inhibits telomerase activity [62]. Therefore, binding to and stabilizing G4 complex structures have the ability to inhibit the telomerase. Using the docking tool AutoDock4,

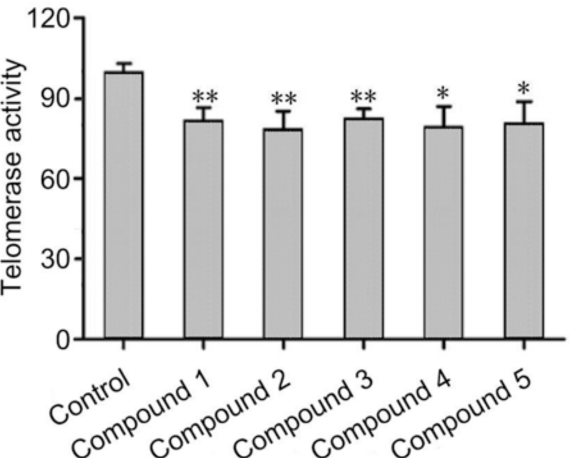

Fig. 3.3 Relative inhibition of telomerase by the triterpenoids from *G. lucidum*. Asterisks indicate p values <0.05 and double asterisks indicate p values <0.01 compared with the control group ($n = 3$). (Adapted from Ref. [64])

Sillapapongwarakorn S et al. (2017) screened 208 GLTs (triterpenoids isolated from *G. lucidum*) for ligands with high binding affinity and selectively to stabilize the pG4DNA, showing that ganoderic acid A and ganoderic acid Df exhibit high binding affinity and selectively bind to the lateral groove of pG4DNA, suggesting the triterpenoid might be a new class of G-quadruplex groove-binding ligands with telomerase inhibiting activity and thus act as potential anticancer agents [63].

Zheng DS et al. (2017) identified five triterpenoids, including ganoderic acid A (compound 1), ganoderic acid B (compound 2), ganoderol B (compound 3), ganodermanontriol (compound 4), and ganodermanondiol (compound 5), on the basis of spectroscopic analysis, and found that the triterpenoids exhibited significant inhibitory effects on telomerase in human nasopharyngeal carcinoma 5–8 F cells (the relative inhibition ratio ranged from 78.62 ± 6.41 to 82.79 ± 3.12% without notable difference in the inhibitory effects among these compounds) detected via the TRAP-PCR-ELISA method, with the inhibitory activities on the viability of NPC 5–8 F cells [64] (Fig. 3.3).

3.1.4 Activities on Autophagy and Their Molecular Mechanism

Autophagy is a highly conserved cellular process in which cytoplasmic materials such as intracellular proteins and organelles are captured and degraded in lysosomes and the degraded materials are subsequently released from lysosomes into metabolic and biosynthetic pathways for recycling. Basal autophagy eliminates damaged cellular components to maintain protein and organelle health and homeostasis. Starvation-induced autophagy plays a vital role in the survival of yeast and mammals via intracellular nutrient scavenging during starvation. Autophagy restrains oxidative stress, chronic tissue damage, and oncogenic signaling by prevention

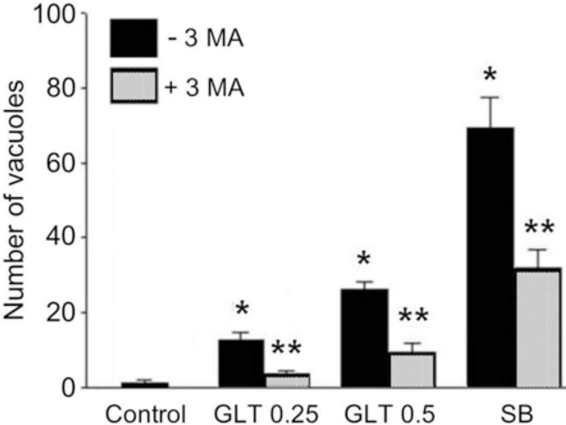

Fig. 3.4 *G. lucidum* triterpene extract (GLT)-induced autophagy with the formation of autophagic vacuoles in HT-29 cells. The autophagic vacuoles were determined under the phase microscope after 6 h treatment of the HT-29 cells with 0.25 mg/ml GLT, 0.5 mg/ml GLT, and SB202190 in the absence or presence of 10 mM 3-methyladenine (3MA). The number of vacuoles was compared with the control; asterisks indicate p values <0.001 ($n = 3$). The number of vacuoles in the presence vs absence of 3-MA was also compared; double asterisks indicate p values <0.005. (Adapted from Ref. [71])

from toxic accumulation of damaged protein and organelles (particularly mitochondria), therefore inhibiting cancer initiation [65–67]. Moreover, autophagic cell death, another mechanism of programmed cell death differing from apoptosis and programmed necrosis, is often observed in tumor cells [68]. *Ganoderma* and its extracts have the activity on autophagy with anticancer effects.

Thyagarajan A et al. (2010) demonstrated that *G. lucidum* triterpene extract (GLT) induced autophagy with the formation of autophagic vacuoles and expressional elevation of two autophagic proteins, Beclin-1 (1.3-fold increase) and LC-3 (3.3-fold increase), which is associated with the induction of autophagy in mammalian [69, 70], in colon cancer cells HT-29, and in tumors in a xenograft model (Beclin-1, 3.9-fold increase; LC-3, 1.9-fold increase), along with the inhibitory effects on proliferation of human colon cancer cells with cell cycle arrest at G0/G1 in vitro as well as on tumor growth in a xenograft model of colon cancer in vivo. Considering the effect of p38 mitogen-activated protein kinase (p38 MAPK) inhibitor, SB202190, on induction of autophagy with the elevation of Beclin-1 (1.2-fold increase) and LC-3 (3.4-fold increase) expression, markedly decreasing phosphorylation of p38 by GLT in a dose- and time-dependent manner in HT-29 cells indicates that autophagy is mediated through the inhibition of p38 MAPK [71] (Fig. 3.4).

Hahne JC et al. (2014) determined the effect of *Cordyceps* extract and a mixture of *G. lucidum/Agaricus Blazi Murill* extract on three endometrial cancer cell lines, Ishikawa, Hec-1A, and AN3-CA (derived from endometrial cancers grade I, II, and III, respectively), showing that all fungi extracts had an inhibitory effect on cell viability and proliferation, but z-VAD-fmk, a broad caspase-inhibitor, or necrostatin-1, a necroptosis inhibitor, did not markedly abolish the inhibitory effect after

72 h incubation, concluding that neither necrosis nor the conventional programmed cell death (apoptosis) played a predominant role in the fungi extracts mediated cell death in the endometrial cancer cells. It was found that incubation with fungi extracts increased the autophagy in the endometrial cancer cells in comparison to the untreated control cells and the solvent controls according to acridine orange staining and FACS assay, while addition of 3′-methyladenine, a well-established autophagy inhibitor, completely abolished the autophagic effects of the extracts. The autophagic effects of the extracts were verified by the results that incubation with fungi extracts led to an increased amount of LC3B-II in AN3-CA cells and Ishikawa cells (not Hec-1A cells because it is difficult to detect an increase in LC3B-II due to the already increased amount of autophagic cells in the untreated cells), because the conversion of LC3B-I to the faster-migrating form LC3B-II is a well-established indicator of autophagy [72].

Oliveira M et al. (2014) investigated the bioactive properties of the methanolic extract from *G. lucidum* fruiting body which showed most potent inhibitory activity on the growth of a gastric cancer cell line (AGS) among various extracts of *G. lucidum* from both the fruiting body and the spores. Besides cell cycle arrest, treatment with the methanolic extract of fruiting body induced more autophagy determined by increased autophagosomes and autophagy marker LC3-II in AGS cells [73].

Reis FS et al. (2015) confirmed that, together with the increase of autophagosomes formation and the increase of cellular levels of LC3-II and the decrease of cellular levels of p62 (an autophagy substrate that is degraded by autophagy and can be used to monitor the autophagic flux), the *G. lucidum* methanolic extract affects cellular autophagy in AGS cells. Together with lysosomal protease inhibitors (E-64d/pepstatin, known later-stage autophagy inhibitors), the extract caused a further increase in the cellular LC3-II levels with an increase in p62 levels, confirming that the methanolic extract of *G. lucidum* induces autophagy rather than inhibits autophagic flux [74].

Hseu Y C et al. (2019) reported that treatment with ethanol extract isolated from *G. tsugae* (*G*T) 200~400 μg/mL significantly reduced cell viability and caused G2/M arrest in K562 cells. In addition, GT induced mitochondrial and death receptor-mediated apoptosis and correlated with DNA fragmentation, followed by cytochrome c release; caspase-3, caspase-8, and caspase-9 activation; PARP cleavage; Fas activation; Bid cleavage; and Bax/Bcl-2 dysregulation. Cytoprotective autophagy was found to be induced by GT, as was revealed by increased LC3-II accumulation, Beclin-1/Bcl-2 dysregulation, acidic vesicular organelle formation, and p62/SQSTM1 activation. Notably, pretreatment of cells with the autophagy inhibitors 3-MA and CQ enhanced GT-induced apoptosis. Interestingly, reactive oxygen species production in cells was not triggered by GT administration; equally, the antioxidant N-acetylcysteine was found to be incapable of preventing apoptosis and autophagy induced by GT treatment. It also discovered that cytoprotective autophagy induced by GT was associated with EGFR and PI3K/AKT/mTOR signaling cascade suppression [75].

Hsin IL et al. (2011) reported that recombinant fungal immunomodulatory protein, GMI, was cloned from *Ganoderma microsporum* (*G. microsporum*) and purified. This research demonstrated that GMI induces lung cancer cell death by

activating autophagy, but does not induce apoptotic cell death. On Western blot, GMI increased LC3 conversion and decreased p53 expression in a time- and concentration-dependent manner. Cytoplasmic calcium chelator BAPTA-AM was used to prove that GMI promotes autophagy via a calcium-mediated signaling pathway. 3-methyladenine (3-MA), an autophagy inhibitor, enhanced the cytotoxicity of GMI on cell viability assay. Using VZV-G pseudotyped lentivirus-shRNA system for autophagy-related genes silencing, the capabilities of GMI to reduce cell viability and colony formation were abolished in autophagy-defective cells. Furthermore, GMI did not stimulate apoptosis after blocking of autophagy by 3-MA or shRNA knockdown system. In xenograft studies, oral administration of GMI inhibited the tumor growth and induced autophagy significantly in nude mice that had received a subcutaneous injection of A549 cells [76].

Chiu LY et al. (2015) proved that a fungal protein from *G. microsporum* (GMI) elevated the intracellular calcium level and reduces the growth of MDR subline via autophagy and apoptosis, regardless of p-glycoprotein (P-gp) overexpression, in mice xenograft tumors. Further, GMI treatment inhibited the phosphorylation of Akt/S473 and p70S6K/T389. However, the phosphorylation of ERK was not associated with GMI-induced autophagy. These results suggest that autophagy plays a pro-death role in acquired MDR and upregulation of autophagy by GMI via Akt/mTOR inhibition provides a potential strategy for overcoming MDR in the treatment of lung cancers [77].

Autophagy has opposing, context-dependent roles in cancer. Cancers are more autophagy-dependent than normal tissues because autophagy-mediated recycling for maintenance of mitochondrial function and energy homeostasis may meet the elevated demand of metabolism for cell growth and proliferation. Thus, autophagy inhibition may be used in cancer therapy depending on the context [66].

Dan X et al. (2016) reported that, besides its potent inhibitory activities against proliferation and colony formation on HT-29 and HCT-116 colorectal cancer cells with cell cycle arrest in G1 phase via the expressional regulation of cyclin D1 and P53, a ribonuclease isolated from wild *G. lucidum* (GLR) suppressed cell autophagy in both HT-29 cells and HCT-116 cells according to its accumulation of P62, upregulation of LC3-I and downregulation of LC3-II, and greater abundance of LC3 protein in GLR-treated HCT-116 cells and a portion of GLR-treated HT-29 cells [78].

3.1.5 Activities on Oxidative Stress and Their Molecular Mechanisms

Oxidative stress, important for physiology and pathology of various diseases, occurs when formation of reactive oxygen species (ROS) exceeds elimination to a critical level which disrupts the balance. Accumulation of excess ROS often causes multifarious impairment to cells, including reduction of ATP level in cells, increase of cytosolic Ca^{2+}, DNA damage, dysfunction of biological function in lipid bilayer, and so on. The major endogenous factors for ROS formation are cytochrome P450

and peroxisomes in the mitochondria. To keep redox homeostasis, some protective molecules named “antioxidant defenses” play their roles in maintaining the balance between formation and elimination of ROS. There are two groups of cellular antioxidant systems currently known as enzymatic and nonenzymatic groups. The enzymatic group includes catalase, superoxide dismutase (SOD), glutathione peroxidase (Gpx), and glutathione-S-transferase (GST). The nonenzymatic group comprises molecules such as vitamins C and E, lipoic acid, carotenoids, flavonoids, and others [79–81]. It is reported that oxidative stress induces cancer cell death by ROS production in signaling via the mitochondrial pathway [82].

Liu RM et al. (2015) investigated that 5~40 μM of four structurally related GAs (ganoderic acids produced by *G. lucidum*), i.e., GA-T, GA-Mk, and two deacetylated derivatives of GA-T (GA-T1 and GA-T2), induced apoptotic cell death in cervical cancer cells HeLa, with their activities to intensify the generation of intracellular ROS and weaken antioxidant defense system by reducing glutathione (GSH) level, superoxide dismutase (SOD), and glutathione peroxidase (GPX) activities, but were obviously antagonized by the exogenous antioxidants, i.e., N-acetylcysteine, catalase, and diphenyleneiodonium chloride, besides their activities to decrease mitochondrial membrane potential and activate caspase-9 and caspase-3. The data indicate the mediation of GAs induced mitochondria-dependent cell apoptosis in HeLa cells through enhancing oxidative stress and depressing antioxidant defense [83] (Fig. 3.5).

Kim TH et al. (2015) found that, along with its activities on reduction of mitochondrial membrane potential levels; disruption of the mitochondrial membrane potential; elevation of calcium concentration; increase of caspase-3, PARP, caspase-

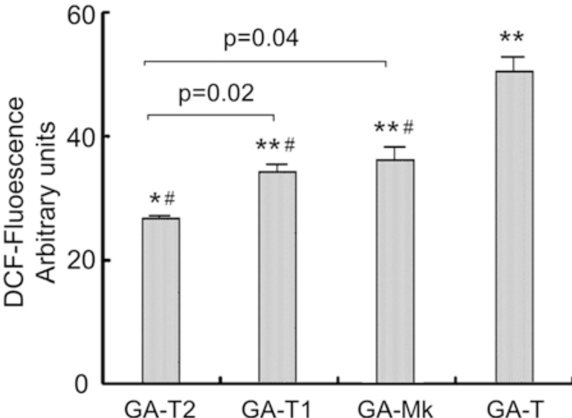

Fig. 3.5 Induction of ROS generation by *G. lucidum* produced ganoderic acids (GAs) in Hela cells. Flow cytometry analysis with DCF staining was used to determine the intracellular ROS in Hela cells after treatment of GAs for 24 h. Data represents the mean ± SD ($n = 3$). Asterisks indicate p values <0.05; double asterisks p values <0.01, compared with the control group. Octothorpes indicate p values <0.05 compared with the GA-T group. (Adapted from Ref. [83])

7, and caspase-9 levels; and decrease of Bcl-2 protein levels, Khz (a fusion mycelium of *G. lucidum* and *Polyporus umbellatus* mycelia, isolated from *G. lucidum* and *P. umbellatus*) promoted reactive oxygen species generation in HCT-116 cells, with cell viability inhibition and percentage elevation of the apoptotic cells determined by their sub-G1 phase of the cell cycle [84].

Kim TH et al. (2012) showed that Khz-induced apoptosis preferentially in HepG2 cells and transformed cells (1198 and 1170-I cell lines transformed from BEAS-2B cells exposed in vivo to beeswax pellets containing cigarette smoke condensate) in contrast to non-transformed cells (BEAS-2B, an immortalized normal human bronchial epithelial cell line, and 1799, a non-transformed cell line derived from BEAS-2B cells exposed to beeswax alone) for which only minimal effects were found. Khz increased cytoplasmic ROS levels in HepG2 cells but prevented by DPI (a flavoprotein inhibitor) and apocynin (a $p47^{phox}$ inhibitor) as well as silencing the expression of Nox2 and Nox4 using specific siRNAs. Khz activated NADPH oxidase according to the translocation of the cytosolic subunits of NADPH oxidase $p47^{phox}$ and $p67^{phox}$ to the cell membrane. MitoSOX Red staining showed mitochondrial ROS generation by Khz, but it was ceased by pretreatment with DPI or apocynin suggesting the importance of NADPH oxidase in mitochondrial ROS production. Suppression of mitochondrial cytochrome c release and apoptosis by pretreatment of cells with DPI or apocynin in Khz-treated cells indicate that ROS generation through NADPH oxidase was necessary for the induction of apoptosis. Both EGTA and BAPTA-AM, which are extracellular and intracellular Ca^{2+} chelators, respectively, prevented NADPH oxidase activation, inhibited ROS generation, and blocked Khz-induced mitochondrial cytochrome c release and apoptosis, indicating that Khz triggers a rapid and sustained increase in intracellular Ca^{2+} concentration that, in turn, activates NADPH oxidase to induce ROS generation and, finally, apoptosis. JNK was activated by Khz treatment but a chemical inhibitor of JNK (SP600125) blocked activation of NADPH oxidase as well as mitochondrial cytochrome c release and apoptosis induced by Khz treatment. Khz-induced ROS production was suppressed by inhibition of JNK using siRNA transfection or pretreatment with chemical inhibitors. Collectively, it was strongly indicated that JNK is activated by Khz via an increase in the intracellular Ca^{2+} concentration thereby triggering ROS generation by NADPH oxidase and the induction of apoptosis [85].

Kim TH et al. (2014) reported that Khz-cp, a crude polysaccharide extract that is obtained after nuclear fusion in *G. lucidum* and *P. umbellatus* mycelia (Khz), induced apoptosis preferentially in human gastric cancer cell line SNU-1 and transformed cells (1198 and 1170-I cell lines) in contrast to non-transformed cells (BEAS-2B and 1799 cell line) for which only minimal effects were found, by increasing the intracellular Ca^{2+} concentration and activating P38 to generate reactive oxygen species (ROS) via NADPH oxidase and via mitochondria (mitochondrial ROS production was required for Khz-cp-induced apoptosis secondary to critical ROS generation by NADPH oxidase). Khz-cp-induced apoptosis was caspase-dependent and occurred via a mitochondrial pathway. P38 played a key role in the activation of NADPH oxidase according to the abrogation of membrane translocation of the $p47^{phox}$ and $p67^{phox}$ subunits and ROS generation by inhibition of P38 expression or activity [86].

Harhaji Trajković LM et al. (2009) demonstrated that both two extracts prepared from *G. lucidum* – a methanol extract containing total terpenoids (GLme) and a purified methanol extract containing mainly acidic terpenoids (GLpme) – reduced viability of B16 mouse melanoma cells in vitro and inhibited tumor growth of B16 cells inoculated subcutaneously into syngeneic C57BL/6 mice in vivo, whereby GLme exhibited stronger effect, with the inhibition of cell proliferation and induction of caspase-dependent apoptotic cell death mediated by upregulated p53 and inhibited Bcl-2 expression. The intensified production of ROS and partial recovery of cell viability resulted from their neutralization by the antioxidant, N-acetyl cysteine, indicate the involvement of intensified ROS generation in the antitumor effect of the GLme [87].

Yue QX et al. (2008) found that, in addition to its synergism to doxorubicin (DOX) for the cytotoxicity in HeLa cells, *Ganoderma* triterpenes (GTS) caused sensitization of cells to chemotherapeutics through enhancement of oxidative stress, DNA damage, and apoptosis in HeLa cells. According to proteomic study, GTS treatment led to alteration of 14 proteins' expression, which was important for cell proliferation, cell cycle, apoptosis, and oxidative stress. GTS significantly increased the intracellular levels of ROS in HeLa cells, and the use of the ROS scavenger, *N*-acetyl cysteine, particularly caused a strong decrease in the cytotoxicity of GTS, indicating the involvement of oxidative stress in cytotoxicity of GTS in HeLa cells [88].

3.2 The Inhibition of Tumor Cell Adhesion, Invasion, Migration, and Their Molecular Mechanism

Metastasis, for which cell adhesion, migration, and invasion are critical steps, is important for cancer, and many molecular mechanisms are involved in, such as AP-1, NF-κB, MMP, cadherin, β-integrin, c-Met, FAK, EMT, and so on. Acting at these molecules may contribute to suppression of cancer metastasis. It was reported by researches that *Ganoderma* and its extracts have bioactivities in these aspects (Fig. 3.6).

Sliva D et al. (2002) demonstrated that both spores or dried fruiting body of *G. lucidum* significantly suppressed constitutive migration of the highly invasive estrogen receptor-negative breast cancer cells MDA-MB-231 as well as the highly invasive androgen receptor-negative prostate cancer cells PC-3 in a dose-dependent manner, along with their activities to inhibit constitutively active transcription factors AP-1 and NF-κB; secretion of uPA; expression of uPA and uPAR in breast MDA-MB-231 and prostate PC-3 cancer cells, considering that cell migration of highly invasive and chemotherapy-resistant human breast cancer cells MDA-MB-231 is dependent on constitutively active AP-1 and NF-κB; and secretion of uPA. In contrast to unaffected constitutive DNA-binding activity of AP-1 when pretreated with spores and fruiting body of *G. lucidum*, the constitutive DNA-binding activity of NF-κB was markedly reduced, whereas constitutive AP-1 activity was suppressed at the transactivation level without changing AP-1 DNA-binding, suggesting that *Ganoderma* inhibited both DNA-binding and transactivation of NF-κB, whereas the inhibition of AP-1 occurred only at the transactivation level [89].

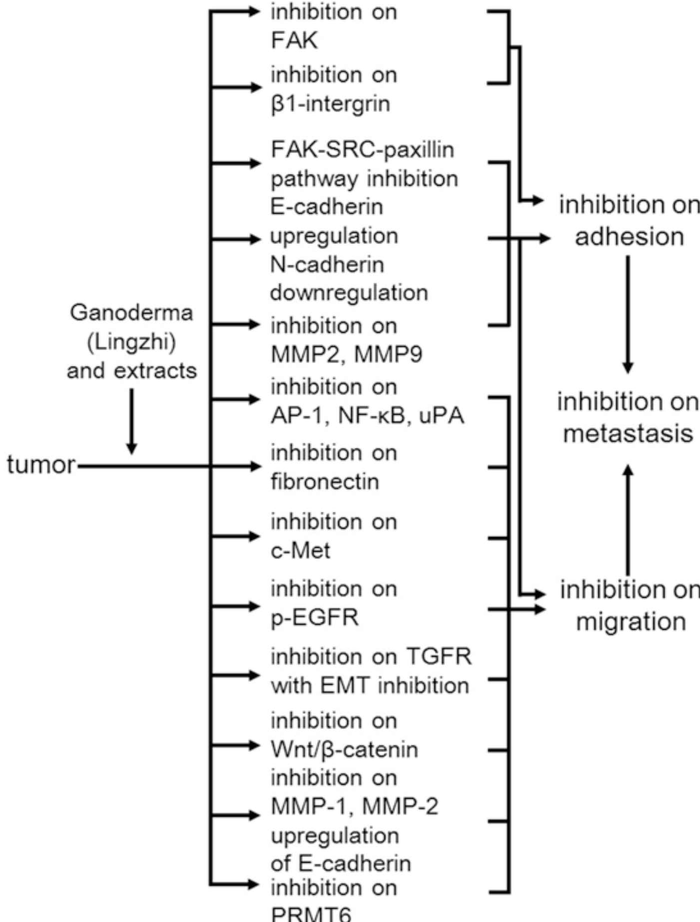

Fig. 3.6 The inhibition of tumor cell adhesion, invasion, migration, and their molecular mechanism of *Ganoderma* against tumor. *Ganoderma* and its extracts have activities to inhibit tumor cell adhesion, invasion, and migration involving molecular mechanisms such as AP-1, NF-κB, MMP, cadherin, β-integrin, c-Met, FAK, EMT, and so on

Sliva D et al. (2003) exhibited that some of the samples of *G. lucidum*, such as samples containing mushroom powder (sample E and sample D), broken spores (sample B), and whole spores (sample A), demonstrated strong inhibiting effect on cancer cell migration in highly invasive estrogen receptor-negative breast cancer cells MDA-MB-231 (86.5%, 54.3%, 71.9%, 89.3%, respectively) and androgen receptor-negative prostate cancer cells PC-3 (93.6%, 39.3%, 95.9%, 83.8%, respectively), which is directly linked to the inhibition of constitutively active transcription factor NF-κB. It was comparable to the effects of some samples such as sample A, sample B, sample E, and mushroom powdered extract with spores (sample F) on the inhibition of constitutively active NF-κB in both MDA-MB-231 cells and PC-3 cells, respectively [90].

Cao QZ et al. (2007) found that *G. lucidum* polysaccharide peptide (GlPP) significantly inhibited cell motility of PG human lung carcinoma cells in vitro without direct inhibition on proliferation. Adhesion of PG cells was also inhibited. The activity of MMP-9 was dose dependently inhibited (the inhibition ratio was 41.53% at a dose of 100 mg/L GlPP) accompanied with the inhibition on MMP-9 mRNA expression, suggesting that inhibition of activity and expression of MMP-9 might be the target of GlPP to mediate anti-invasion activity [91].

Zhao SF et al. (2010) reported that, besides its inhibitory activities on proliferation of A2780CP (cisplatin-resistant) and A2780CP (cisplatin-sensitive) human ovarian cancer cells, the water-soluble extract of *G. lucidum* spores significantly inhibited the adhesion, migration, invasion, as well as multicell spheroid and colony formation in human ovarian cancer SKOV3 cells, accompanied with dose-dependent upregulation of E-cadherin and downregulation of N-cadherin and vimentin, suggesting the roles of these regulations in the mechanism of the extract to inhibit invasion and metastasis in human ovarian cancer cells [92].

Thyagarajan A et al. (2006) showed that *G. lucidum* extract (containing 13.5% polysaccharides and 6% triterpenes) inhibited oxidative stress-induced migration in poorly invasive breast cancer cells MCF-7 in a dose-response manner. *G. lucidum* extract markedly suppressed oxidative stress-induced phosphorylation of Erk1/2, oxidative stress-induced expression of c-Fos and the constitutive expression of c-Jun, two subunits of AP-1 whose activity is induced by Erk1/2, in MCF-7 cells. Oxidative stress-induced AP-1 activity and DNA-binding of AP-1 were significantly inhibited by *G. lucidum* extract. The oxidative stress-induced activity of NF-κB (which is linked to oxidative stress) and its DNA-binding were markedly suppressed by *G. lucidum* extract. Oxidative stress-induced secretion of IL-8 (whose expression is controlled by AP-1 and NF-κB) from MCF-7 cells was inhibited by *G. lucidum* extract as well. It was suggested that *G. lucidum* extract inhibited the oxidative stress-induced invasive behavior of breast cancer cells by modulating Erk1/2 signaling pathway involving the suppression of interleukin-8 secretion [93].

Wu GS et al. (2013) showed that GAEE, a *G. lucidum* triterpenoid extract mainly containing GA, dihydrogenated GA, and GA isomer, inhibited cell migration and adhesion with slight decrease of invasion in MDA-MB-231 cells without alteration of the expression levels of integrin β1 and integrin β4 but does dependently decreased FAK and p-FAK expression. GAEE inhibited autophosporylation of FAK at Y397 and subsequent phosphorylation at Y925, without alteration of the expression of SRC and p-SRC (Y416 and Y527), but decreased the formation of FAK/SRC complex, whereby attenuated FAK full activation (p-FAK at Y925) and might impair downstream signaling. The expression of active form of paxillin (p-paxillin at Y118, which plays a pivotal role in completely activating FAK/paxillin complex and subsequently inducing cell migration) was decreased, whereas that of the total paxillin expression was not changed after GAEE treatment. GAEE attenuated the binding affinity between FAK and paxillin, which is regulated by p-paxillin at Y118 and might affect cell migration and adhesion. GAEE decreased protein levels of Rac1, RhoA, and Cdc42, suggesting that GAEE might inhibit cell migration and adhesion

through disruption of cell polarity, actin assembly, and focal adhesion formation, according to the critical role of RhoA, Rac1, and Cdc42 in modulation of the cell cytoskeleton reorganization and actin-associated adhesion during cell migration, and influence of cell polarity, microtubule dynamics, and membrane transport pathways. GAEE did not alter the expression of N-WASP, a key regulator of reorganization of actin cytoskeleton and an essential component of invadopodia, but suppressed the interaction between N-WASP and Cdc42 and β-actin which might cause decreased actin polymerization and disrupt the process of focal adhesion turnover during migration. It was conclusively indicated that *G. lucidum* triterpenoids could suppress cell migration and adhesion through FAK-SRC-paxillin signaling pathway [94].

Wu QP et al. (2006) exhibited different degrees of cell adhesion inhibitory effects in malignant human breast carcinoma cells (MT-1) caused by different preparations of *G. lucidum* spores, which were sporoderm-broken spores (broken by an enzymatic method) > sporoderm-broken spores (broken by a physical method) > intact spores>buffer control. Similarly, polysaccharides from different *G. lucidum* sources showed different degrees of their effects, and the greatest inhibitory activity on cell adhesion was seen in the polysaccharides isolated from *G. lucidum* fruiting bodies grown on logs of wood, in a dose-dependent manner. The increase of cell adhesion by inoculation of the cells on polysaccharide-coated Petri plates indicated the interaction of the polysaccharide with cell surface proteins (the cancer cells used do not attach very well to the Petri plates originally, and if the cells bound to the polysaccharides, it would be able to detect cell attachment after incubation). Considering that β1-integrin is associated with cell adhesion, it was further demonstrated by Western blot analysis that β1-integrin expression was greatly reduced, suggesting the specific role of β1-integrin in inhibition of cell adhesion by *G. lucidum* spore products [95].

Loganathan J et al. (2014) showed significant inhibition of breast-to-lung cancer metastases caused by GLE (a well-characterized extract from *G. lucidum*) from 33.9 ± 15.2% in control to 10.2 ± 5.4% in GLE-treated animal models (nude mice, which were implanted with MDA-MB-231 human breast cancer cells into the mammary fat pads) with statistically nonsignificant inhibition of tumor growth without observable changes in the tumor volumes. Oligo GEArray Human Tumor Metastasis Microarray revealed the downregulatory effects of GLE on the expression of *HRAS*, *VIL2*, *S100A4*, *MCAM*, *I2PP2A*, and *FN1* genes by more than 20%, which were further confirmed by qRT-PCR. The indeed responsibilities of these genes targeted by GLE for the migration of MDA-MB-231 cells were evaluated by gen silencing with siRNA. Gene silencing of *HRAS and S100A4* by siRNA suppressed, and silencing of *VIL2* slightly suppressed, the migration of MDA-MB-231 cells, but silencing of *MCAM* did not affect the migration of MDA-MB-231 cells. Gene silencing of *I2PP2A* and *FN1* resulted in the downregulation of SET and FN1 protein expression, respectively, and also inhibited the migration of MDA-MB-231 cells. Transfection of pooled siRNAs (mixture of siRNAs for *HRAS*, *VIL2*, *S100A4*, *MCAM*, *I2PP2A*, and *FN1*) suppressed migration of MDA-MB-231 cells according to wound healing assay and cell migration assay in Boyden chambers, with the downregulation of the expression of ezrin, S100A4, MCAM, SET, and fibronectin,

except for HRAS. Furthermore, combination of pooled siRNA and GLE treatment showed the strongest inhibition of fibronectin expression, suggesting that fibronectin was the major target for inhibiting cell invasiveness [96].

Wu JR et al. (2015) found that LZ8, a medicinal peptide purified from the herb Lingzhi, markedly suppressed the constitutive cell migration of both c-Met-positive (HCC372) and c-Met-negative (HCC329) hepatocellular carcinoma, in addition to its inhibitory effects on the cell growth. LZ8 markedly reduced primary HCC329 tumor growth on the middle liver lobe and intra-metastasis toward the right and left liver lobes in the SCID mice, compared with those in the DMEM- and JNJ (the c-Met specific antagonists, JNJ-38877605, at IC_{50}: 26.5 nM)-treated mice. The LZ8 exerted different effects on the signal transduction in HCC372 and in HCC329 cells in several aspects. Treatment with LZ8 decreased the phosphorylation of JNK, ERK, and AKT in HCC329 cells, whereas greatly suppressed both the expression of c-Met and phosphorylation of c-Met (Tyr1234), and abolished p-ERK and p-AKT on the downstream level, without reduction of p-JNK, in HCC372 cells. Using a receptor array, which can simultaneously detect 49 essential RTKs in phosphorylated forms, it was demonstrated that p-EGFR was markedly reduced in HCC329 treated with LZ8, compared with that in untreated HCC329, while inhibitors of EGFR, AG1748, and SU5416 suppressed the cell migration (using wound healing method), and the level of p-JNK was reduced by treating HCC329 with AG1748, indicating that EGFR-JNK signaling was required for mediating cell migration of HCC329, which could be effectively suppressed by LZ8. In HepG2, LZ8 suppressed the HGF-induced c-Met signaling including p-c-Met, p-JNK, p-ERK, and p-paxillin (Ser 178) by 85% to 90% and suppressed the HGF-induced cell migration (assayed by wound healing method) more efficiently than did JNJ. These data demonstrated the anti-HCC activities of LZ8 by blocking the c-Met-dependent or the c-Met-independent pathway [97].

Liang ZE et al. (2015) demonstrated that *G. lucidum* polysaccharides (GLPs) dose dependently suppressed cell migration in LoVo human colon cancer cells, besides the GLP-mediated cytotoxicity with apoptosis mediated via activation of the Fas/caspase-dependent pathway according to the increased activity of caspases-3, caspases-8, and caspases-9 and the expression of Fas and caspase-3 proteins and the reduced expression of cleaved poly (ADP-ribose) polymerase (PARP), in a dose-dependent manner [98].

Lin TY et al. (2016) demonstrated that rLZ-8, a *G. lucidum* recombinant protein, suppressed tumor metastasis and increased the survival rate in Lewis lung carcinoma cell-bearing mice. The epithelial to mesenchymal transition (EMT) process, which is regarded as the critical event in tumor metastasis, was effectively suppressed by rLZ-8 via interfering with cell adhesion and focal adhesion kinase (FAK) functions in lung cancer cells (such as A549 and CL1-5 human NSCLC adenocarcinoma cell lines and LLC1 Lewis lung carcinoma cell line). The rLZ-8-induced FAK inactivation downregulated Slug, a transcription factor that suppresses E-cadherin transcription which is known as a critical event in EMT and tumor metastasis, resulted in enhancement of E-cadherin expression and suppression of cancer cell mobility. The rLZ-8 promoted the degradation of Slug through ubiquiti-

nation proteasome pathway (UPP) in CL1-5 cells and mechanistically increased the interaction between MDM2 and Slug, leading to Slug degradation, whereas MDM2-shRNA abolished the rLZ-8-promoted Slug degradation. It could be concluded that rLZ-8 performed antimetastatic activity by suppression of EMT and cell mobility via the negative modulation of FAK, which resulted in the ubiquitination and degradation of Slug [99].

Li X et al. (2016) showed that synthetic ergosterol peroxide (5α, 8α-epidioxiergosta-6, 22-dien-3β-ol), which is originally purified from *G. lucidum*, inhibited cell migration, along with its induction of cell death, and inhibition of cell cycle progression and colony growth in human hepatocellular carcinoma HepG2 cells. The expression of Foxo3 mRNA and protein was enhanced with the reduction of its upstream signal proteins pAKT and c-Myc, which represses Foxo3 functions, and the levels of proapoptotic proteins, Puma and Bax, were effectively increased as well. These results suggest the role of Foxo3 activity promotion by pAKT and c-Myc inhibition and proapoptotic protein Puma and Bax activation in anticancer activities of the ergosterol peroxide against hepatocellular carcinoma [100].

Li L et al. (2016) demonstrated that a supercritical-CO_2 extract of *G. lucidum* spores (GLE), which was obtained from completely sporoderm-broken germinating *G. lucidum* spores by supercritical fluid carbon dioxide (SCF-CO_2) extraction, inhibited TGF-β1-induced migration of cholangiocarcinoma cells TFK-1, with the suppression of TGF-β1-induced EMT which was identified by suppression of TGF-β1-induced morphological changes and the changes in cadherin expression and inhibition of the formation of F-actin stress fibers, evidencing the mechanism of inhibition of TGF-β1-induced EMT in the suppression of cholangiocarcinoma migration in vitro by GLE [101].

Chen Y et al. (2016) showed that ethanol/ethanol extract (E/E-SBGS), but not ethanol/aqueous extract (E/A-SBGS), which were both prepared from the sporoderm-broken spores of *G. lucidum* (SBGS), inhibited the migration of human lung cancer cells H441 and survival of human lung cancer cell lines A549, H441, and H661 in a dose-dependent manner. In A549 cells, colony formation was markedly inhibited, cell cycle progression was arrested in the G2/M phase, and apoptosis was triggered by suppressing the expression and activity of cell cycle regulators, cyclin B1 and cdc2, as well as antiapoptotic proteins, Bcl-2 and Bcl-xl, with the mechanism that E/E-SBGS dose dependently suppressed the activation of Akt, the mammalian target of rapamycin (mTOR), and their downstream molecules S6 kinase and 4E-BP1. In vivo experiments showed significant tumor volume and weight suppression by both E/E-SBGS and SBGS in A549 human lung cancer xenograft mouse model [102].

Tsao SM et al. (2016) demonstrated the migration inhibitory activity of a fucose-containing fraction of Ling-Zhi (FFLZ) in higher spontaneous metastasis breast cancer cells 4 T1, with the inhibition of EMT phenotype in vitro, and suppression of tumor growth and metastasis in 4 T1-bearing mice in vivo. It was indicated that the effects of FFLZ to inhibit EMT and prevent metastasis were mediated by promotion of ubiquitination-dependent TGFR degradation and abolishment of TGFR signaling pathways, according to the activities of FFLZ to downregulate TGFR and

its downstream signaling pathways, including the phosphorylation of Smad2/3 and the expression of Smad4, and enhance the Smurf2-dependent ubiquitination of TGFR by disrupting the balance of the lipid rafts, promote the "re-localization" of the TGFR to the caveolae, and facilitate the degradation of TGFR [103].

Barbieri A et al. (2017) found that *G. lucidum* extracts, which were prepared using common organic solvents, significantly decreased cell migration with the reduction of cell viability in both MDA-MB 231 (triple-negative breast cancer) and B16-F10 (melanoma) cell lines, besides the significant inhibitory effects, under pro-inflammatory conditions (incubation with lipopolysaccharide, LPS), on the release of IL-8, IL-6, MMP-2, and MMP-9. The migration inhibitory effects of the *G. lucidum* extracts were correlated with a lower release of matrix metalloproteases [104].

Zhang Y (2017) demonstrated the migration inhibitory activities of *G. lucidum* extract in MDA-MB-231 breast cancer cells which have migration ability, along with its inhibition against Wnt-induced hyper-proliferation in both MDA-MB-231 and 4 T1 breast cancer cells. The *G. lucidum* extract significantly inhibited the phosphorylation of LRP6 and the expression of Axin2, the Wnt3a-activated Wnt target gene, indicating that the *G. lucidum* extract suppressed breast cancer cell growth and migration by blocking Wnt/β-catenin signaling through inhibiting the phosphorylation of Wnt co-receptor LRP6 [105].

Li K et al. (2017) revealed that ethanol extracts of sporoderm-broken spores of *G. lucidum* (BSGLEE), which mainly contain triterpenoids, significantly suppressed cell migration in HCT-116 colorectal cancer cells with the decrease of MMP-1 and MMP-2 and increase of E-cadherin expression at mRNA levels, in addition to the activities of proliferation inhibition, apoptosis induction, and promotion of cell cycle arrest at G0/G1 phase which was correlated to the inhibition of the expression of some apoptosis and cell cycle cascades regulating crucial genes and proteins, including p21, p16, cyclin D1, Bcl-2, Bax, NAG-1, PARP, and caspase-3. HCT-116 xenograft tumor growth in nude mice was significantly inhibited by BSGLEE as well, with the decreased Ki-67 staining as detected by immunochemistry [106].

Zhao X et al. (2018) demonstrated that *G. lucidum* polysaccharides (GLPs) significantly inhibited cell migration in LNCaP human prostate cancer cells, besides the effects to inhibit cell growth and induce cell cycle arrest. Transfection with the protein arginine methyltransferase 6 (PRMT6) overexpression plasmid increased cell migration in LNCaP cells accompanied by enhanced expression of PRMT6, cyclin-dependent kinase-2 (CDK2), FAK, and steroid receptor coactivator (SRC) and reduced p21 expression, whereas GLP treatment antagonized the promotion of cell migration, as well as enhancement of PRMT6, CDK2, FAK, and SRC expression, and reduction of p21 expression. PRMT6 knockdown by siRNA inhibited cell migration; reduced PRMT6, CDK2, FAK, and SRC expression; and enhanced p21 expression, while the effects of GLP on cell migration were abolished. It was indicated that the inhibitory effect of GLP on cell migration may exert via the PRMT6 signaling pathway [107].

Qu L et al. (2017) demonstrated that high dose (2 mg/mL) of triterpenes from *G. lucidum* (GLT) significantly inhibited cell migration and invasion in DU-145 human prostate cancer cells with the significantly decreased expression of

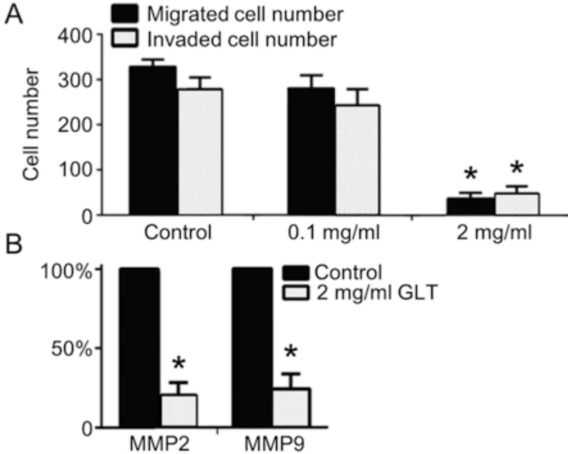

Fig. 3.7 Inhibition of cell migration and invasion as well as inhibition of MMP-2 and MMP-9 expression in DU-145 cells by *G. lucidum* triterpene (GLT). The cell migration and invasion were determined by migration chamber and invasion chamber (**a**), while the expression of MMP-2 and MMP-9 were determined by Western blot (**b**) after 24 h treatment with GLT in DU-145 cells. Asterisks indicate p values <0.05 compared with the control group and 0.1 mg/ml GLT group (**a**) or compared with the control group (**b**). (Adapted from Ref. [108])

migration−/invasion-associated genes, MMP-2 and MMP-9, besides its actions on cell viability inhibition and apoptosis induction, indicating the inhibitory effect of GLT on cell migration and invasion may occur via the suppression of MMPs [108] (Fig. 3.7).

3.3 The Inhibition of Tumor Angiogenesis and Its Molecular Mechanism

Angiogenesis is a process of the growth of new blood vessels from an existing vasculature and is critical in tumor growth and progression for supplying oxygen and nutrients, besides its key role in other aspects of tumor pathology such as metabolic alteration and tumor dissemination/metastasis, and is hence considered an essential pathologic feature of cancer [109]. Therapies against angiogenesis in cancer have focused on targeting the major pro-angiogenic stimulus, vascular endothelial growth factor (VEGF) [110]. The activities of *Ganoderma* and its extracts against angiogenesis and its stimulus such as VEGF in cancer have been reported (Fig. 3.8).

Cao QZ et al. (2004) investigated the antitumor and antiangiogenic effects of *G. lucidum* polysaccharide peptide (GLPP). Results showed that GLPP (50, 100, and 200 mg/kg) inhibited growth of sarcoma-180 in BALB/c nude mice markedly by 35.2%, 45.2%, and 61.9%, respectively, in vivo. GLPP was directly added to the cultured medium which did not inhibit PG cell proliferation in vitro, but GLPP 50,

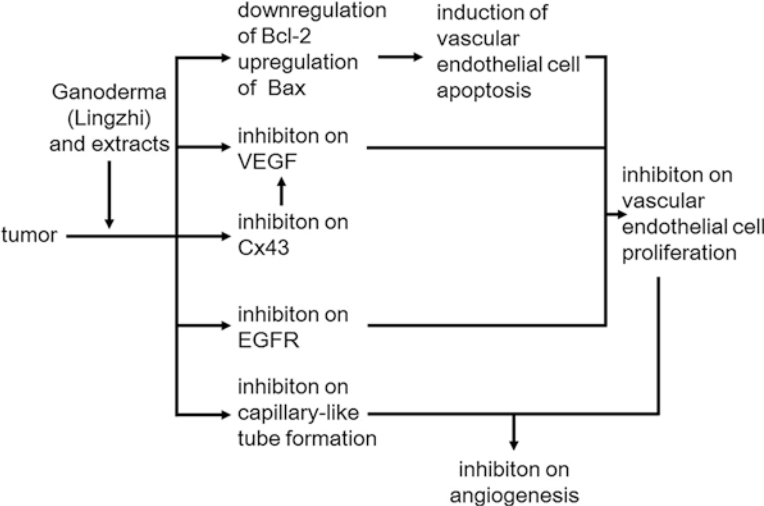

Fig. 3.8 The inhibition of tumor angiogenesis by *Ganoderma* and the molecular mechanisms. *Ganoderma* and its extracts inhibit tumor angiogenesis by targeting the major pro-angiogenic stimulus, VEGF, and its receptor, along with induction of vascular endothelial cell apoptosis and inhibition of vascular endothelial cell proliferation

100, and 200 mg/kg-treated serum potently inhibited human lung carcinoma (PG) cell proliferation by 22.5%, 26.8%, and 30.3%, respectively, in vitro and reduced the xenograft in BALB/c nude mice greatly in vivo by 55.5%, 46.0%, and 46.8%, respectively. Lymphocytes proliferation of nude mice could be stimulated by LPS 5 mg/L, but not by ConA 2.5 mg/L, indicating that GLPP could not promote the T lymphocyte proliferation and neutral red phagocytosis of peritoneal macrophages of nude mice. The chick chorioallantoic membrane assay showed that GLPP- and GLPP-treated serum had antiangiogenic effect. In addition, GLPP (1, 10, and 100 mg/L) inhibited human umbilical cord vascular endothelial cell (HUVEC) proliferation in vitro with the inhibitory rate of 9.4%, 15.6%, and 40.4%, respectively. It was suggested that GLPP fulfills its antitumor effect via antiangiogenic activity by HUVEC proliferation inhibition [5].

Cao QZ et al. (2006) indicated that *G. lucidum* polysaccharide peptide (GLPP) dose dependently inhibited proliferation of human umbilical cord vascular endothelial cells (HUVECs) with the direct induction of cell apoptosis (determined by flow cytometry with Annexin V-FITC/PI staining) without cytotoxicity (determined by the lactate dehydrogenase (LDH) assay). The reduced secretion of VEGF (determined by ELISA assay) by high dose of GLPP in human lung carcinoma cells PG in hypoxia, together with the downregulation of Bcl-2 antiapoptotic protein expression and upregulation of Bax proapoptotic protein expression in HUVECs (determined by Western blot assay), revealed that the mechanism of the antiangiogenic potential of GLPP was direct inhibition of proliferation of vascular endothelial cell by induction of the cell apoptosis via downregulation of Bcl-2 and upregulation of Bax and indirect reduction of growth factor expression in tumor cells [111] (Fig. 3.9).

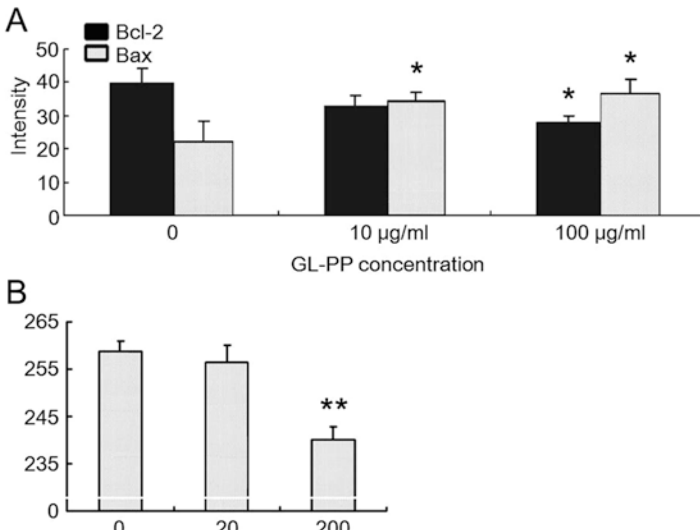

Fig. 3.9 Protein expression of Bcl-2 and Bax in HUVEC and VEGF expression in culture supernatants of PG cell in hypoxia. (**a**) Protein expression of Bcl-2 and Bax in HUVEC determined by Western blot after treatment with *G. lucidum* polysaccharide peptide (*Gl*PP) for 48 h. (**b**) VEGF expression in culture supernatants of PG cells determined by ELISA after treatment with *Gl*PP in hypoxia (95% N_2/5% CO_2) for 18 h. Data represent the mean ± SD (n = 3). Asterisks indicate p values <0.05, and double asterisks indicate p values <0.01 compared with the control group

Kimura Y et al. (2002) demonstrated that triterpenoid fraction (100 and 200 mg/kg) of the fruit bodies of *G. lucidum* inhibited angiogenesis induced by Matrigel, a soluble basement membrane extract of the Engelbreth-Holm-Swarm (EHS) tumor, in combination with VEGF and heparin in an in vivo model, suggesting that the inhibition of tumor-induced angiogenesis might contribute to the antitumor and antimetastasis activities of the triterpenoid fraction, in which compound I was identified (as ganoderic acid F) [112].

Song YS et al. (2004) showed that the ethanol extract of fresh fruit bodies of *G. lucidum* (GL, 1.25, 2.5, 5, or 10 µg per egg) significantly inhibited angiogenesis (the percentage inhibition of angiogenesis were 43.1, 53.6, 64.7, or 63.1%, respectively) detected using a chick embryo chorioallantoic membrane assay, besides its inhibition against LPS-induced NO production in RAW 264.7 macrophages [113].

Zhang XC et al. (2005) reported that *G. lucidum* polysaccharides (GLPs) significantly suppressed the angiogenesis in chicken chorioallantoic membrane angiogenesis model (CAM) at the dosages of 0.2~5 µg/CAM, with the remarkable inhibition on adhesion of human prostate cancer cells PC-3 M-1E8 at a concentration of 0.33~33 g/L, in a dose-dependent manner [114].

Wang XJ et al. (2006) reported that oral administration of *G. lucidum* spore significantly inhibited microvessel density (MVD) and expression of VEGF in HepG2 xenograft tumor in nude mice besides inhibition of the tumor growth with more necrosis and less degree of atypia, suggesting that *G. lucidum* spore fulfills antian-

giogenetic and growth inhibitory effects in hepatocellular carcinoma in vivo by inhibition of VEGF expression [115].

Hsu SC et al. (2009) indicated that, besides the reduction of cell viability, *G. tsugae* methanol extract (GTME) inhibited epidermal growth factor receptor (EGFR) expression as well as VEGF secretion and expression in EGFR-overexpressing human epidermoid carcinoma A-431 cells, with dramatic downregulation of phospho-PI3K, phospho-Akt, and phospho-mTOR, but the GTME-induced downregulation in phospho-EGFR and phospho-Akt and the suppression of VEGF were restored by EGF, a member of ligands that interact with and activate EGFR. GTME directly inhibited capillary-like tube formation, an important step in angiogenesis, in HUVECs. The EGFR and VEGF expression in tumor cells in A-431 tumor-bearing athymic nude mice were dramatically downregulated by GTME as well. These findings suggest that the GTME significantly inhibits EGFR and VEGF expression in vitro and in vivo, which are important for tumor angiogenesis and growth [116].

Guo PR et al. (2014) reported that *G. lucidum* polysaccharide (GLP) in combination with cisplatin significantly reduced MVD and expression of VEGF and bFGF (basic fibroblast growth factor) in mRNA and protein levels, besides its growth inhibitory effects, in T24 (bladder cancer)-bearing nude mouse model, suggesting the mechanism related to the downregulation of VEGF and bFGF expressions [117].

Dai S et al. (2014) reported that a standard extract of *G lucidum*, containing 6% triterpenes and 13.5% polysaccharides, effectively inhibited VEGF mRNA and protein expression in human ovarian cancer cells HO 8910 (HOCC), with its promotive effects on Cx43 mRNA and protein expression (Cx43 was suggested to be the downstream of VEGF according to the experiment showing that knockdown of Cx43 abrogated the inhibitory effect of the *G. lucidum* extract on HOCC proliferation without alteration on the attenuation of VEGF induced by the *G. lucidum* extract) [118].

3.4 The Reversion of Tumor Cell Multidrug Resistance and Its Molecular Mechanism

As a major obstruction in conventional chemotherapy, multidrug resistance (MDR) usually results in the failure of cancer treatment. It is well-known that the overexpression of ATP-dependent transmembrane drug transporter such as P-glycoprotein (P-gp) on the surface of resistant tumor cells plays an important role in pumping out a variety of anticancer drugs as the substrates of P-gp, leading to reduction of the intracellular drug accumulation followed by the ineffectiveness of drugs in clinical therapy. Therefore, reversion of tumor cell multidrug resistance by inhibition of the P-gp activity is very valuable to effective chemotherapy in cancer. Because the synthetic P-gp inhibitors are serious toxic, it is underway to search for more potent and less toxic inhibitors of natural origin [119]. The activities of *Ganoderma* and its extracts on reversion of tumor cell multidrug resistance and its molecular mechanism have been reported (Fig. 3.10).

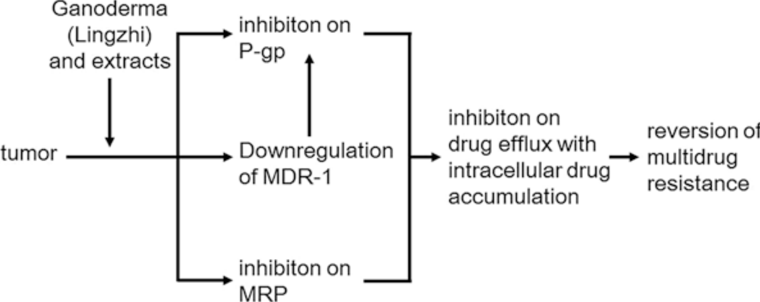

Fig. 3.10 The reversion of tumor cell multidrug resistance by *Ganoderma* and the molecular mechanisms. *Ganoderma* and its extracts reverse tumor cell multidrug resistance by inhibition against the ATP-dependent transmembrane drug transporter such as P-gp (or MRP) on the surface of resistant tumor cells to prevent reduction of the intracellular accumulation of anticancer drugs by pumping out the drugs

Li WD et al. (2008) demonstrated that *G. lucidum* polysaccharides (*Gl*-PS) obviously reversed the resistance to adriamycin (ADM) in the ADM-resistant leukemic cell line K562/ADM cells and the RF (reversing factor), which was calculated according to the formula: RF=IC_{50} (control group)/IC_{50} (drug-treated group), were 2.96, 6.46, 6.80, and 3.35 in the 5, 10, 20, and 40 mg/L *Gl*-PS-treated groups, respectively. Confocal laser scanning microscopy and flow cytometric analysis showed that the less intracellular ADM accumulation in the K562/ADM cells than that in the K562 cells (ADM-sensitive) became almost equal or higher after treatment with *Gl*-PS. The expression rate of P-glycoprotein (P-gp), as well as the levels of MDR-1 and MRP1 mRNA, was reduced distinctly, which may explain the antagonistic effect of *Gl*-PS against the resistance to ADM in the K562/ADM cells because MDR has been associated with the overexpression of P-gp or MDR-associated protein (MRP), 2 transmembrane transporters that act as pumps to remove toxic drugs from tumor cells, and the MDR modulators may either block the induction of MDR-1 gene, which encodes MDR transporter P-gp-170 in human, or MRP1 gene expression or downregulate P-gp expression [120] (Fig. 3.11).

Sadava D et al. (2009) demonstrated that preincubation with water extract of *G. lucidum* in multidrug-resistant (VPA) human small-cell lung cancer (SCLC) cells significantly reduced the IC_{50} for two chemotherapeutic drugs, etoposide (15.9-fold) and doxorubicin (5.1-fold), besides its reduction of the IC_{50} in drug-sensitive (H69) human SCLC cells (10.2-fold for etoposide and 5.6-fold for doxorubicin), indicating the activity of the water extract of *G. lucidum* to reverse multidrug resistance to etoposide and doxorubicin in multidrug-resistant (VPA) SCLC cells and sensitize drug-sensitive (H69) human SCLC cells to etoposide and doxorubicin. Enhancement of DNA fragmentation within cells as measured by ELISA, enhancement of TUNEL staining for DNA breaks, and enhancement of specific activities of caspases-3 and caspases-9, but not caspase-8 by colorimetric assays, indicated proapoptotic activities of water extracts of several *Ganoderma* species via endogenous pathway, with pattern changes similar to SCLC cells treated with chemotherapeutic drugs in the

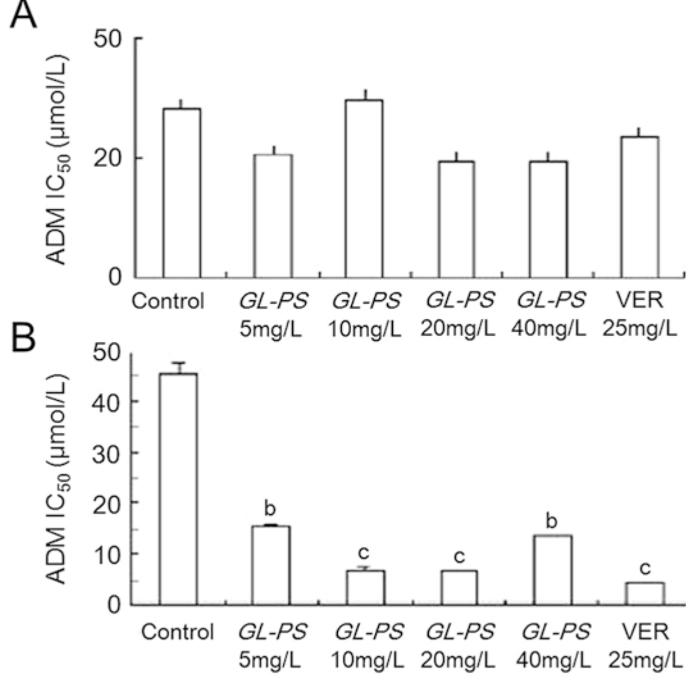

Fig. 3.11 Effects of *Gl*-PS on sensitivities of K562 cells (**a**) and K562/ADM cells (**b**) to ADM. Cells were incubated with *Gl*-PS in presence of ADM for 44 h. Verapamil (VER) was used as positive control. The effects were determined by MTT assay. (Adapted from Ref. [120])

expressions of 9 genes involved in the cell cycle/apoptosis, as measured by RT-PCR and capillary electrophoresis [121].

Tang W (2013) reported that ganoderic acid T (GA-T), which possessed cytotoxicity and proapoptotic activity on multidrug-resistant KB-A-1/Dox cell and sensitive KB-A-1 cell at high concentrations (the IC_{50} was both 50 μg/mL), reduced the IC_{50} of doxorubicin on multidrug-resistant KB-A-1/Dox cell from 2.294 μg/mL to 0.103 μg/mL (22.3-fold) after 5 μg/mL of GA-T treatment without significant change of IC_{50} in sensitive KB-A-1 cell. The content of doxorubicin and rhodamine 123, which can be transported outside from the cells by P-gp therefore intracellular change of rhodamine-123 content may indicate the function of P-gp, in multidrug-resistant KB-A-1/Dox cell after GA-T treatment increased 40% and 20%, respectively, indicating that GA-T sensitizes the multidrug-resistant KB-A-1/Dox cell to doxorubicin by improving intake of the drug into the cells or inhibiting transportation of the drug outside from the cells by P-gp [122].

Tang Y et al. (2013) reported that ethyl acetate extract and n-butyl alcohol extract of *G. lucidum* after ethanol extraction dose dependently reversed the multidrug resistance to adriamycin in multidrug-resistant gastric carcinoma cell line SGC7901/ADR, improved accumulation of adriamycin in the cells, reduced transcription of *MDR1* gen, and decreased expression of P-gp [123].

Suárez-Arroyo IJ et al. (2016) showed that *G. lucidum* extract (GLE) overcame erlotinib resistance in intrinsic erlotinib-resistant MDA-MB-231 cells and a successfully developed erlotinib-resistant cell line, rSUM-149 (inflammatory breast cancer), with its synergistic effect with erlotinib for sensitization in SUM-149 cells, besides its inhibitory effects on viability, proliferation, migration, and invasion in SUM-149 cells involving inactivation of AKT and ERK signaling pathways [124].

Li P et al. (2017) revealed that ethyl lucidenates A (Ela), a triterpenoid isolated from *G. lucidum,* (2.5~10 μM) concentration dependently decreased the IC_{50} of vincristine (VCR) against K562/A02 cells (a cell line with about 116-fold resistance to VCR in comparison with the parental K562 cells determined in this study) along with the effective increase of the intracellular accumulation of Rh123 in a dose-dependent manner without promotion of P-gp protein expression, indicating that the multidrug resistance reversal effect of Ela is probably due to inhibition of P-gp drug transporter function, instead of P-gp protein expression, thereby elevating accumulation of VCR in K562/A02 cells to enhance VCR-induced cytotoxicity toward K562/A02 cells [125].

Liu DL et al. (2015) demonstrated that ganoderenic acid B (GAB), a lanostane-type triterpene isolated from *G. lucidum*, remarkably reversed the resistance of HepG2/ADM cells (a drug-resistant cell line) to doxorubicin (DOX), vincristine (VCR) and paclitaxel and significantly reversed the resistance of ABCB1 (a 170-kDa transmembrane glycoprotein encoded by the *MDR1* gene, has been most extensively identified in MDR cancer cells)-overexpressing MCF-7/ADR cells (a DOX-induced drug-resistant cell line) to DOX. Failure in sensitization of both HepG2/ADM and HepG2 cells to cisplatin, a water-soluble chemotherapeutic drug insensitive to HepG2/ADM and HepG2 cells and impossible to be transported by ABCB1, together with the increased intracellular content of Rhm-123, a substrate transported out of the cells by ABCB1 conventionally, in HepG2/ADM cells suggests that GAB may affect the drug transport of ABCB1, resulting in the increase of the intracellular drug accumulation and inhibition of drug efflux. Attenuation of reversal effect to VCR by ABCB1 siRNA-transfection, and unaltered protein expression level of ABCB1, in HepG2/ADM cells after GAB treatment without inhibition of ATPase and CYP3A4 activity suggests that GAB reverses ABCB1-mediated MDR by effective inhibition of the transport function of ABCB1 and enhancement of the intracellular drug accumulation in MDR cells without influence on ABCB1 expression or ATPase in cells [126].

Chiu LY et al. (2015) demonstrated that a fungal protein from *G. microsporum*, GMI, increased the intracellular level of calcium in two MDR sublines (both sublines are cross-resistant to DOC and VCR), A549/D16 subline which expresses high P-gp and mediates MDR by ABC transporters (typical MDR) and A549/V16 which mediates MDR by non-ABC transporter-associated factors (atypical MDR), with inhibition on the cell growth in vitro, as well as inhibition on the growth of xenograft tumors in mice in vivo, involving apoptosis and autophagy with Akt/mTOR inhibition [77].

Wu QP et al. (2012) demonstrated that *Ganoderma* oil significantly overcame the resistance of the human breast carcinoma cell line MDA-MB-231 (stably transfected with miR-378, which contribute to multiple drug resistance) to epirubicin,

besides its induction of cancer cell death more effectively in miR-378-expressing MDA-MB-231 cells than that in control cells (GFP-transfected cells), and the effective ergosterol peroxide to induce cancer cell death was isolated and identified from the *Ganoderma* oil [127].

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Chapter 4
Protective Effect of *Ganoderma* (Lingzhi) on Radiation and Chemotherapy

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Abstract Radiation and chemotherapy are common and mainstay treatments for cancer patients. But they are also usually associated with some toxicity and side effects in most of the patients. *Ganoderma* (Lingzhi) is considered as a major kind of complementary/alternative medicine and used to prevent the adverse effects caused by radiation and chemotherapy. This chapter reviewed the protective effects of *Ganoderma* (Lingzhi) on radiation and chemotherapy, including the preventive effects on myelosuppression, intestinal injury, nephrotoxicity, cardiovascular toxicity, and other side effects. Both basic researches and clinical studies of *Ganoderma* (Lingzhi) in preventing side effects induced by radiation and chemotherapy were reviewed.

Keywords *Ganoderma* · Lingzhi · Polysaccharides · Polysaccharide peptides · Myelosuppression · Intestinal mucosal immune

4.1 Introduction

Chemotherapy, radiotherapy, and surgery are three major modern anticancer treatments. In advanced stages of the disease, the therapy most often employed is chemotherapy and/or radiation. However, chemotherapy and radiation are highly cytotoxic, inducing a number of severe adverse effects [1]. Cancer patients not only bear the biological effects of cancer but also suffer from the high cost, toxicity, drug resistance, and regulatory limitations of the therapy [2–4].Therefore, it is crucial to develop safe, tolerable, and effective chemo- and radioprotective compounds for

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human applications in the cancer treatment by complementary and alternative medicine options, which include vitamins, traditional Chinese medicine (TCM), or other biological products [5].

Dietary components, especially mushrooms, can serve as excellent pharmaceutical agents because of their low toxicity profile and ease of administration. Medicinal mushrooms are abundant sources of a wide range of useful native products and new compounds with interesting biological activities [6–8].

Ganoderma lucidum (Fr.) Karst (Polyporaceae), as one of the most famous TCM, is used as a drug in the Ancient China for more than 2000 years. Studies have proved the profound antioxidant, anti-inflammatory, antitumor, antinociceptive, antimutagenic, anticarcinogenic, cardioprotective, radioprotective, liver-protective, and nephroprotective effects of various extracts of *Ganoderma* [9, 10].

This article mainly summarized the protective effect of *Ganoderma* on radiation and chemotherapy.

4.2 The Basic Researches of *Ganoderma* (Lingzhi) in Preventing Side Effects Induced by Radiation and Chemotherapy

Radiation and chemotherapy are common and mainstay treatments for cancer patients. However, these treatments are usually associated with some toxicity and side effects in most of the patients. These include hematological toxicity (such as leukopenia and neutropenia), gastrointestinal toxicity (such as mucositis, diarrhea, and constipation), alopecia, fatigue, and organ toxicity (such as cardiotoxicity and nephrotoxicity) [11].

Complementary/alternative medicine has been considered as other medicine that complements mainstream medicine and provides diagnostic, therapeutic, and preventive methods that mainstream medicine cannot achieve [12]. It refers to a variety of health care, treatment systems, or methods independent of Western medicine [12]. Richardson MA et al. (2000) assessed the prevalence of complementary/alternative medicine which has been used in a comprehensive cancer center. The results showed that in 453 participants, the complementary/alternative medicine, including spiritual practices, movement and physical therapies, vitamins, and herbs, was used at least in one approach in 83.3% cancer patients [13].

One kind of such complementary/alternative medicine is *Ganoderma*, also called Lingzhi, which has been used for more than 2000 years in China and other Asian countries. It has been used to prevent and cure various human diseases such as tumorigenic diseases, hepatitis, bronchitis, hypertension, and other immunological disorders [14]. TCM theory suggests that *Ganoderma* can enhance body resistance and strengthen the constitution of patients, which has been called “Fuzheng Guben,” a major principle in TCM. The main *Ganoderma* species include *G. lucidum*, *G. tsugae*, *G. sinensis*, *G. atrum*, and *G. applanatum*. *Pharmacopoeia of the People’s*

Republic of China 2000, 2005, 2010, and 2015 editions included *G. lucidum* and *G. sinensis*. *G. lucidum* is also recorded in USA.

Pharmacopeia/National Formulary USP40-NF35 [15].

4.2.1 Effects of Ganoderma on Myelosuppression Induced by Radiation and Chemotherapy

ZB Lin et al. (1980) firstly found that before and after radiation each 20 days, *G. lucidum* solution [10 g(equal crude material)/kg] administrated by gavage once a day could significantly reduce the mortality in lethal dose of ^{60}Co γ-ray-radiated mice. However, after lethal dose of ^{60}Co γ-ray irradiation, intraperitoneal injection of *G. lucidum* solution only can significantly increase the average survival time in mice [16].

Cyclophosphamide is a kind of broad-spectrum anticancer drug and has been used extensively in the therapy of malignant lymphoma, multiple myeloma, breast cancer, ovarian cancer, soft tissue sarcoma, acute leukemia, chronic lymphoblastic leukemia, etc. [17]. Its major toxicity is myelosuppression. In the myelosuppressed mice model induced by cyclophosphamide, treatment with 2.5 mg/kg *G. lucidum* polysaccharides (Gl-PS) intraperitoneally once daily could promote myelopoiesis. Yet Gl-PS did not directly stimulate hematopoietic progenitor proliferation and inhibition of apoptosis in bone marrow cells. The research showed that Gl-PS selectively bound to mice bone marrow stromal cells promoted the colony formation of colony-forming unit-mixed colony (CFU-Mix), colony-forming unit-granulocyte macrophage (CFU-GM), colony-forming unit-erythrocyte (CFU-E), and colony-forming unit-fibroblast (CFU-F) and increased the production of hematopoietic growth factors interleukin-6 (IL-6), IL-1, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and stem cell factor (SCF)) (Table 4.1, Figs. 4.1 and 4.2). The findings suggest that Gl-PS can be used as a possible alternative therapeutic strategy to lessen chemotherapy-induced myelosuppression by means of myelopoiesis potentiation [18].

Ionizing radiations can induce cellular DNA and membrane damages to inflict deleterious effects on living cells; therefore, radiotherapy is one major effective tool for cancer treatment. Unfortunately, the normal tissues are also damaged by radiation, which limit the therapeutic gains, and we need to develop an effective radioprotector for human. *G. lucidum* is considered as a potential radioprotector due to its high phytochemical concentration.

TP Smina et al. (2008) investigated the radiation protective effect of total triterpenes isolated from *G. lucidum* [19]. The human blood was collected with finger prick method and added with total triterpenes before 2 Gy radiation ^{60}Co γ radiation. The DNA damage in human blood lymphocytes was analyzed by methods of comet assay or single-cell gel electrophoresis. The effects of total triterpenes on membrane

Table 4.1 Levels of hematopoietic growth factors in Gl-PS-SCM and Gl-PS-BMSCM. Ref [18]

Concentration of *Gl*-PS (μg/ml)	G-CSF	GM-CSF	IL-1β	IL-6	SCF
Gl-PS-SCM					
0	0.0 ± 0.0	5.9 ± 2.3	12.7 ± 4.8	80.3 ± 6.4	0.0 ± 0.0
25	472.6 ± 53.7[a]	15.0 ± 3.4[a]	99.5 ± 12.6[a]	289.2 ± 22.4[a]	0.0 ± 0.0
50	634.8 ± 82.1[a]	26.7 ± 4.9[a]	169.0 ± 14.7[a]	439.8 ± 41.0[a]	0.0 ± 0.0
100	801.3 ± 99.2[a]	38.7 ± 4.6[a]	281.4 ± 22.5[a]	635.7 ± 77.0[a]	0.0 ± 0.0
200	1070.2 ± 123.0[a]	50.2 ± 6.3[a]	479.5 ± 40.7[a]	857.3 ± 104.6[a]	0.0 ± 0.0
Gl-PS-BMSCM					
0	8.0 ± 3.1	3.2 ± 1.5	5.3 ± 3.1	38.2 ± 5.3	20.3 ± 4.1
25	20.8 ± 4.8[a]	11.5 ± 2.4[a]	9.5 ± 3.6	155.7 ± 6.9	43.2 ± 7.5
50	38.8 ± 7.3[a]	18.7 ± 4.8[a]	7.8 ± 3.1	298.8 ± 15.2[a]	76.0 ± 10.7[a]
100	71.2 ± 11.2[a]	49.2 ± 10.3[a]	8.2 ± 5.2	797.0 ± 33.3[a]	92.5 ± 13.6[a]
200	101.7 ± 20.5[a]	50.7 ± 17.0[a]	9.0 ± 4.9	922.2 ± 72.2[a]	155.6 ± 19.5[a]

[a]$P < 0.05$ versus control (concentration of Gl-PS: 0μg/ml)
Splenocytes (1×10^7) or bone marrow stromal cells (1×10^6) were cultured with Gl-PS (0~200 μg/ml) for 48 h. Gl-PS-splenocyte-conditioned medium (Gl-PS-SCM) and conditioned medium of bone marrow stromal cells (BMSCM) induced by Gl-PS were collected to measure cytokines by ELISA. $P < 0.05$ vs. control (concentration of Gl-PS: 0 μg/ml) [18]

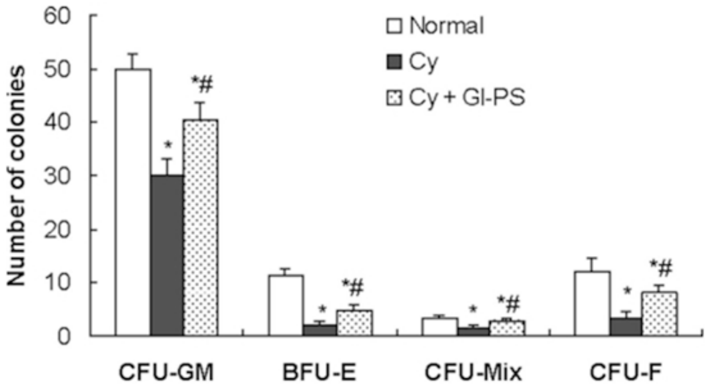

Fig. 4.1 Effects of *Gl*-PS on the clonogenic activity of various hematopoietic progenitor cells and stromal cells in the bone marrow in vivo. On day 11 after Cy treatment, BMC (5×10^4) were plated on CFU-GM, BFU-E, and CFU-Mix culture system. $*P < 0.05$ versus the normal control; $^{\#}P < 0.05$ versus the vehicle-administered Cy group. Ref [18]

damage and plasmid pBR 322 DNA were also assessed in vitro. The results showed that radiation could significantly induce the membrane damage, appearing as a rise in TBARS (thiobarbituric acid reactive substances) and LOOH (lipid hydroperoxides) levels. Total triterpenes could dose-dependently reduce the levels of TBARS and LOOH. Total triterpenes (100 μg/ml) were able to adjust TBARS and LOOH

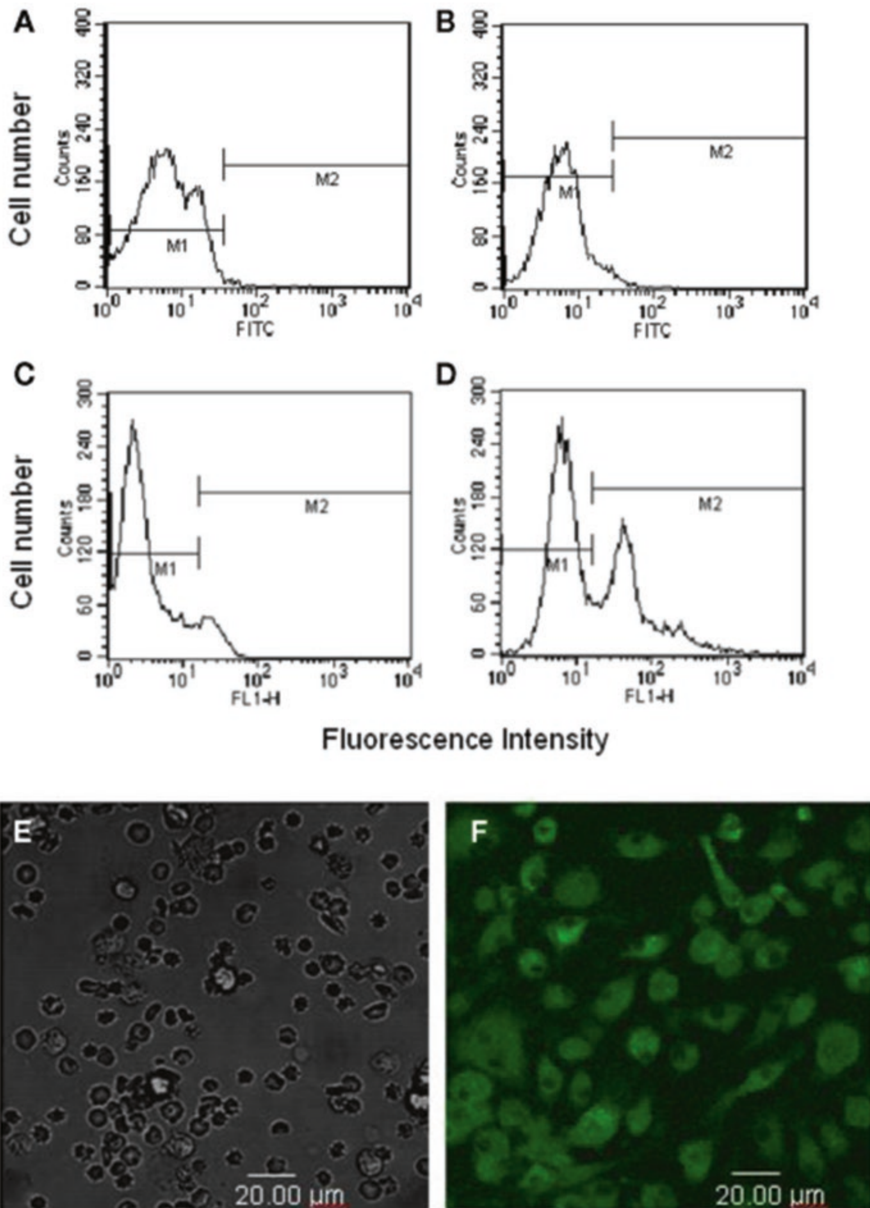

Fig. 4.2 Selective binding of FITC–*Gl*-PS to BMSC. Nonadherent hematopoietic cells (**a**, **b**) and adherent BMSC (**c**, **d**) were stained with FITC-dextran [negative control (**a**, **c**)] or FITC-*Gl*-PS (**b**, **d**) for flow cytometric analysis. Hematopoietic cells (**e**) and BMSC (**f**) stained with FITC-*Gl*-PS were also observed under a confocal laser-scanning microscope. Ref [18]

levels to normal. Exposure to radiation could result in the DNA undergoing strand breaks, and total triterpenes could reverse these effects. 25 Gy radiation reduced pBR DNA to 23.35% in supercoiled form, while 50 μg of total triterpenes was able to raise supercoiled form to 98.87%. In human blood lymphocytes, compared to normal control, 2 Gy radiation could significantly increase DNA in tail, tail length, tail moment, and olive tail moment from 3.78 ± 0.52, 7.70 ± 0.72, 0.61 ± 0.27, and 0.74 ± 0.15 to 19.27 ± 1.00, 25.27 ± 0.31, 5.63 ± 0.22, and 5.46 ± 0.31. Total triterpenes (100 μg/ml) could effectively reduce the damage, representing as reducing % DNA in tail, tail length, tail moment, and olive tail moment to 5.64 ± 1.12, 11.78 ± 1.32, 0.89 ± 0.36, and 1.39 ± 0.42. Total triterpenes were also able to reduce the micronuclei formation in mice bone marrow cells increased by radiation. These results indicate the significant effectiveness of total triterpenes from *G. lucidum* in protecting the DNA and membrane damages induced by radiation and suggest *Ganoderma* triterpenes as a potential radioprotector.

T.P. Smina et al. (2011) further reported the radioprotective effect of *G. lucidum* total triterpenes on splenic lymphocytes in vitro [20]. The splenic lymphocytes were isolated from male Swiss albino mice and pretreated with different concentrations of total triterpenes before exposed to radiation. Result of MTT [3-(4, 5-dimethyl -2-thiozolyl)-2-5-diphenyl-2-H-tetrazolium bromide] assay showed that *G. lucidum* total triterpenes did not inhibit cell proliferation. DNA ladder assay showed that 2 Gy radiation induced apoptosis in splenic lymphocytes, resulting in the laddering of DNA. However, all the tested concentrations of total triterpenes could significantly inhibit DNA fragmentation and apoptosis induced by radiation. The quantified result of DNA damage by the comet assay showed that irradiation caused obvious DNA damage, representing as elevated levels of tail length, tail DNA, tail moment, and olive tail moment. Total triterpenes (100 μg/ml) was able to reduce the tail DNA from 9.30 ± 2.68 to 3.65 ± 0.23 ($P < 0.001$ vs. radiation alone group) and tail length from 15.16 ± 3.70 to 8.31 ± 2.50 ($P < 0.01$ vs. radiation alone group). The tail moment and olive tail moment were also significantly decreased by total triterpenes. The quantification of apoptosis was assessed by flow cytometry, and the results showed that 50.84 ± 3.4% of cells in radiation group were apoptotic cells. Treatment with 100 μg/ml of total triterpenes could reduce the apoptotic cells percentage to 25.80 ± 5.1%. Furthermore, total triterpenes effectively decreased the formation of intracellular reactive oxygen species and increased endogenous antioxidant enzyme activity [SOD, GPx, and GR (glutathione reductase)] in splenic lymphocytes exposure to irradiation.

The polysaccharides isolated from *G. lucidum* producing in southern parts of India were reported as a novel radioprotective agent by Pillai TG et al. (2008) [21]. The whole bodies of Swiss albino mice were exposed to 10Gy ^{60}Co γ radiation in the presence or absence of *G. lucidum* polysaccharides. A clinically used radioprotective drug, amifostine, was used as positive control. TBARS, an indicator of lipid peroxidation status in mice liver microsomal membrane, were increased by gamma radiation in a dose-dependent manner. All the tested polysaccharide fractions from *G. lucidum* could effectively reduce the elevated levels of TBARS induced by 350 Gy r-rays. Neutral polysaccharide was the most effective fraction in protecting

plasmid pBR 322 DNA against radiation. The survival studies showed that the survival animals in group of neutral polysaccharides were the same as amifostine (more than 60%) on the 30th day, while in the radiation alone group, no animals survived after 25 days. In addition, the increased micronuclei in polychromatic erythrocytes by 4 Gy exposure were reduced from 28.160 ± 3.049 to 6.300 ± 2.422 and 16.024 ± 2.074 by neutral polysaccharides at doses of 500 and 250 μg/kg body wt, respectively. Amifostine with a dosage of 300 mg/kg body wt could reduce to 10.400 ± 2.581. These in vitro and in vivo results indicated that the *Ganoderma* polysaccharides could effectively protect radiation-induced damages and suggested the *Ganoderma* polysaccharides can be a potential radioprotective agent.

TG Pillai and PU Devi (2013) investigated the radioprotection of beta-glucan, a polysaccharide from *G. lucidum* [22]. The survival test result showed that no mice survived on 30th day after irradiation, while 66% of the mice administration with beta-glucan (500 μg/kg body weight) past irradiation survived at 30th day. The hemoglobin level and total leukocyte count were significantly decreased by radiation. The level of GSH was reduced, while MDA was increased significantly by radiation. Beta-glucan administration could dose-dependently inhibit these changes. In addition, beta-glucan markedly decreased the number of aberrant cells induced by radiation. These results suggested beta-glucan isolated from *G. lucidum* may be potential candidate for radioprotection.

Pillai TG et al. (2014) reported the radioprotective properties of beta-glucan isolated from *G. lucidum* on human lymphocytes exposed to gamma radiation [23]. The results showed that the marked radioprotection ability of beta-glucan was related with its antioxidant activity and DNA-repairing ability.

Zhao W et al. (2012) reported that *G. lucidum* polysaccharides (GLP) had no effects on peripheral white blood in mice exposed to ^{60}Co gamma-irradiation, but it could significantly increase the nucleated cell in bone marrow in a dose-dependent manner. In addition, DNA damage, micronuclei formation, and lipid peroxidation induced by radiation were all reduced by GLP. These results indicated GPL-possessed radioprotective effects [24].

Liu GJ et al. (2017) reported that *G. lucidum* spore oil emulsion exerted reduction effects of toxicity to mice undergoing chemotherapy [25]. The tumor-bearing model of rats was intraperitoneally injected with cyclophosphamide combined with or without oral administration with *G. lucidum* spore oil emulsion for 21 days. The results showed that the white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), reticulocyte (RET), and the number of bone marrow nucleated cells (BMNC) were all significantly decreased by cyclophosphamide. Administration with *G. lucidum* spore oil emulsion could inhibit these changes. In addition, *G. lucidum* spore oil emulsion was able to reduce the liver damage and attenuate the decreased spleen coefficient, thymus index, and number of splenic nodules induced by cyclophosphamide.

Chen CH et al. (2008) reported the efficacy-enhancing effect of compound Lingzhi spore oil on S180 tumor-bearing mice after chemotherapy [26]. S180-transplanted tumor mice were treated with 5-fluorouracil alone or in combination with compound Lingzhi spore oil. Then the change in peripheral blood cells and indexes of the spleen

and thymus was detected. The results showed that Lingzhi spore oil could markedly increase the white cell count and the number of platelet in S180 tumor-bearing mice treated with 5-fluorouracil. The dropped spleen and thymus indexes induced by 5-fluorouracil were also enhanced by compound Lingzhi spore oil.

Yu SQ et al. (1997) reported that Lingzhi spore was able to inhibit the reduction of white blood cells in 870 rad ^{60}Co γ-ray-treated mice [27].

4.2.2 Effects of Ganoderma Intestinal Injury Induced by Radiation and Chemotherapy

Naoki Kashimoto et al. (2010) [28] investigated the effects of a water-soluble extract from the culture media of *G. lucidum* mycelia (MAK) on the damaged small intestine caused by anticancer drugs, including 5-fluorouracil (5-FU), tegafur with uracil (UFT), cisplatin, cyclophosphamide, and gefitinib. MAK contains different kinds of substances, such as water-soluble lignin, triterpenes, proteins, and polysaccharides. Six-week-old male B6C3F1/Crlj mice were fed with different dosages (1.25%, 2.5%, and 5.0%) of MAK orally for 7 days prior to administration with 5-FU intravenously (i.v.) or intraperitoneally (i.p.). The results showed that both treatment with 5-FU i.v. and i.p. could significantly reduce the number of regenerative crypts compared to normal control. These decrease could be significantly attenuated by administration with 2.5% and 5% MAK, but 1.25% MAK group showed no such effect. For other anticancer drug-induced small intestinal injury, MAK could significantly increase the number of regenerative crypts. These results suggest that MAK can effectively ameliorate the small intestinal damage caused by chemotherapy.

Naoki Kashimoto research team (2005) also reported the radioprotective effect of MAK [29]. MAK previously has been reported to stimulate the acquired immune system or the natural immune system; therefore, MAK was hypothesized to afford radioprotection effects. The commercial diet with a 1.25, 2.5, and 5% supplement of MAK was given to B6C3F1 mice, respectively, for a week before irradiation and kept for 3.5 days after irradiation. Then the mice were sacrificed and the crypts survival was determined. The results showed that irradiation could dose-dependently decrease the number of crypt (0 Gy: 116.53 ± 13.39, 8 Gy: 84.54 ± 11.74, 10 Gy: 43.74 ± 8.42, 12 Gy: 24.76 ± 5.62). A 5% MAK could significantly increase the surviving crypts in every dose of X-irradiation group (8 Gy: 117.00 ± 12.47, 10 Gy: 68.06 ± 9.63, 12 Gy: 43.77 ± 7.64).

GMI, one fungal immunomodulatory protein isolated from *Ganoderma microsporum*, was found to play a protective role in 5-FU-induced oral and intestinal mucositis while strengthening its anticancer effects in oral cancer [30]. BALB/C mice were treated with 5-FU intraperitoneally for two cycles to induce oral and intestinal mucositis. GMI (36 mg/day) were pretreated for 3 days before the first 5-FU injection and continued for 10days. In vitro, human tongue squamous cell carcinoma cell line SCC9 and human oral squamous cell carcinoma cell line SAS

were administrated with different concentrations of 5-FU and GMI. The results showed that GMI had no effects for 5-FU-induced leukopenia. The histology of jejunum samples showed that 5-FU caused intestinal mucositis, including inflammatory cell infiltration, flattened epithelial layer, and shortened villi. GMI administration reduced the structural damage of the mucosal layer and increased the intestinal villi length in 5-FU-treated mice. The apoptosis of mice jejunum tissues was analyzed by Western blot and assessed the expressions of cleaved caspase 7 and Bcl-2. The results showed that there were no significant differences among normal control, 5-FU and 5-FU+GMI groups, indicating that GMI did not protect intestine by attenuating apoptosis of enterocytes. 5-FU also caused the oral mucositis, representing as thinner tongue mucosa and reduction in the total number of filiform papilla. GMI administration could recover the thickness of tongue mucosa to a degree similar to the control and raised the numbers of filiform papilla. 5-FU caused cell death of SCC9 cells and SAS cells in a dose-dependent and time-dependent manner in vitro. Co-treatment with GMI could enhance cytotoxicity of 5-FU but had no side effect on normal cells. Apoptosis-related proteins including caspase 7 and PARP were detected by Western blot. The number of apoptotic cells was analyzed with flow cytometry with annexin-V and propidium iodide staining. The results showed that GMI dose-dependently enhanced apoptosis induced by 5-FU in both two oral cancer cells. The above data suggest GMI may be a candidate against chemotherapy-induced mucositis in oral and intestine.

In 2011, we reported the protective effects of *G. lucidum* polysaccharides (Gl-PS) on damaged intestine induced by chemotherapy [31]. In vivo, BALB/C mice were intraperitoneally injected with methotrexate (MTX) to induce small intestinal damage. Different concentrations of Gl-PS (50, 100, and 200 mg/kg) were administrated by gavage to MTX-treated mice. In vitro, normal rat intestinal cell IEC-6 was treated with MTX and Gl-PS. The results showed that MTX treatment induced severe mucosal damage, significantly elevated small intestine MDA levels, and reduced SOD and serum immunoglobulin A (IgA) levels in the mice. Administration of 100 and 200 mg/kg Gl-PS could significantly reverse the MTX effects. In IEC-6 cells, MTT assay showed that Gl-PS (0.1, 1, and 10 μg/mL) could stimulate the cell proliferation in a dose-dependent manner (Figs. 4.3 and 4.4). In addition, Gl-PS was able to stimulate the cell migration and increase the expression of ODC and c-Myc mRNAs in vitro. These results indicated that Gl-PS could protect the small intestine against MTX-induced injury through induction of epithelial cell proliferation and migration.

4.2.3 *Effects of Ganoderma on Nephrotoxicity Induced by Chemotherapy*

Cisplatin has been used extensively in clinic for a variety of cancers, particularly of the testis, ovary, bladder, head, and neck. Unfortunately, accompanied by curative effects in controlling and management of oncological disorders, cisplatin often

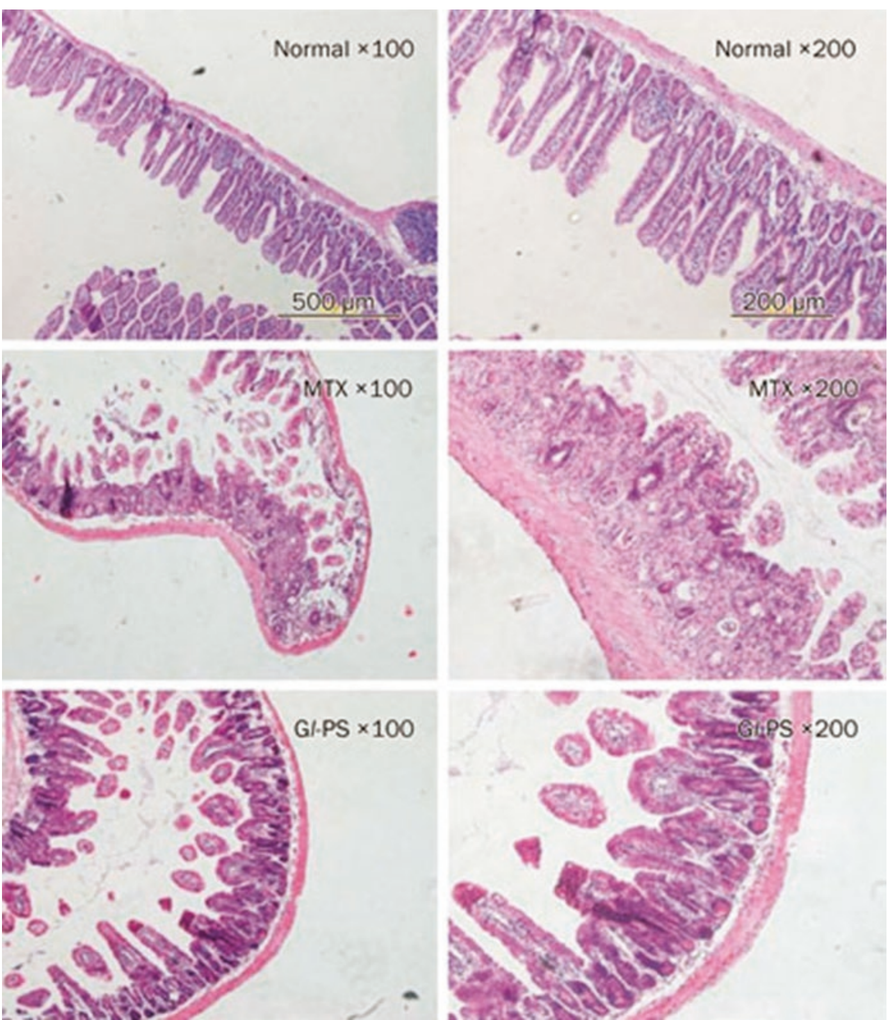

Fig. 4.3 Morphology of the murine jejunum with administration of Gl-PS in MTX-treated mice under magnification of 100 and 200. Normal, group of normal control; MTX, group of MTX model; G*l*-PS, group of 100 mg/kg G*l*-PS under MTX stress. These photographs are representative examples of a group of nine mice. Ref [31]

induces nephrotoxicity and neurotoxicity. The nephrotoxicity caused by cisplatin is considered to be associated with free radicals, and the damage is the consequence of enhanced lipid peroxidation with decreased renal antioxidant enzyme activity [32]. Sheena et al. (2003) reported the prevention of *G. lucidum* for cisplatin-induced nephrotoxicity by its antioxidant activity [33]. The 6- to 8-week-old male Swiss albino mice were given orally 250 or 500 mg/kg body weight of methanolic extract of *G. lucidum* 1 h before cisplatin injection. 72 h later, the blood and kidneys were collected. The results showed that cisplatin injection induced elevated serum

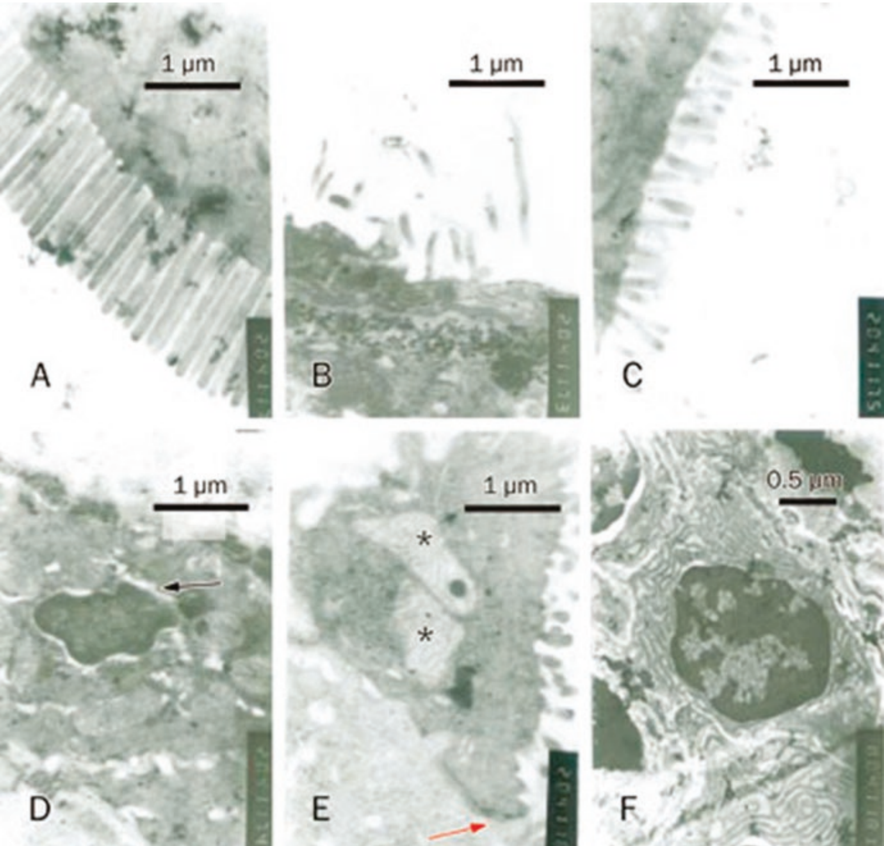

Fig. 4.4 Changes of intestinal ultrastructure with administration of Gl-PS in MTX-treated mice. (**a**) Normal control group. (**b**, **d**, **e**) MTX model groups. (**c**, **f**) Group of 100 mg/kg G*l*-PS under MTX stress. The arrow (**d**) indicates swelling of nuclear membrane. The arrow (**d**, **e**) indicates tight junction. ∗ indicates swelling of mitochondria. Ref [31]

creatinine and urea levels while decreasing antioxidant status markers such as catalase (CAT), superoxide dismutase (SOD), concentration of reduced glutathione (GSH), and glutathione peroxidase (GPx) in mice. Methanolic extract of *G. lucidum* could significantly lower the elevated serum creatinine and urea concentrations. Furthermore, administration of methanolic extract of *G. lucidum* in cisplatin-treated animals could dose-dependently restore the renal SOD activity, GSH, CAT, and GPx levels to normal.

The other component of *G. lucidum*, terpenes, also showed prevention of cisplatin-induced nephrotoxicity. The male Swiss albino mice were administered orally with terpene (50 and 100 mg/kg body wt, respectively) 1 h before cisplatin (16 mg/kg body wt) injection. The results showed that 72 h after cisplatin injection, compared to the normal control group, cisplatin induced significantly elevated the serum urea, creatinine, alkaline phosphate levels, and the renal malondialdehyde

(MDA) activities. On the contrary, the renal catalase, SOD, and GPx activities were markedly reduced by the administration of cisplatin. The kidney histopathological observation showed that cisplatin alone-treated animals were induced nephrotoxicity, including renal cell necrosis and more plasma ultrafiltrate accumulation found in the proximal convoluted tubule. Administration of terpene could dose-dependently reverse these changes induced by cisplatin. The results indicated that in cisplatin-treated mice, combined therapy with *G. lucidum* terpenes showed good antioxidant ability, which might be associated with its nephroprotection [34].

Lingzhilactones from *Ganoderma* Lingzhi were found to be able to protect against renal injuries induced by Adriamycin [35]. BALB/C mice were intravenously injected with Adriamycin at a dose of 10 mg/kg body weight for only one time to induce nephropathy. Lingzhilactones were administered intraperitoneally at dose of 25 mg/kg body weight daily for 3 weeks. The results showed that lingzhilactone B could reduce the markedly elevated urinary albumin levels and fibrotic area induced by Adriamycin injection. Several fibrogenic genes in kidney were evaluated by immunofluorescence staining and Western blot analysis. The results showed that in Adriamycin-treated mice, the expressions of fibronectin and α-SMA were significantly upregulated. Treatment with lingzhilactone B almost abolished induction of these genes. The mRNA expression of collagen I in Adriamycin nephropathy was suppressed more than 80% by lingzhilactone B. In addition, Western blot and immunostaining analysis results showed that lingzhilactone B nearly completely inhibited the phosphorylation of Smad3 induced by Adriamycin. Furthermore, lingzhilactone B was confirmed safely in rodent experiments. These results indicated that lingzhilactone B displayed against nephropathy induced by Adriamycin. The mechanisms were associated with disruption of Smad3.

4.2.4 Effects of Ganoderma on Cardiovascular Toxicity Induced by Anticancer Agents

Doxorubicin (DOX) is a kind of anthracycline antibiotic and used widely for treating cancer, including hematological cancers, carcinomas, and sarcomas. Yet its utility in clinic is compromised by developing cardiac complications. Dox-induced cardiotoxicity was linked to increased oxidative stress in cardiac and myofibrillar deterioration, intracellular calcium dysregulation, apoptosis in cardiomyocytes and endothelial cells, disturbances of myocardial adrenergic signaling, etc. [36]. *G. lucidum* was previously reported by Sudheesh NP et al. (2013) to be able to protect cardiomyocytes from mitochondrial oxidative stress and have significant protective effects against mitochondrial damage in the course of myocardial infarction [37]. This research team further investigated the ability of an aqueous ethanol extract of *G. lucidum* (GLE) against DOX-induced cardiotoxicity in rats [38]. 250 and 500 mg/kg GLE were given to Wistar rats 5 days before 6 mg/kg DOX intraperitoneal administration for 3 days. Administration of GLE continued for 3 more days along with DOX injection. The results showed that DOX could significantly increase the levels of creatine kinase

(CK, from 88.59 ± 7.89 to 570.9 ± 55.46 units/L) and lactate dehydrogenase (LDH, from 288.0 ± 22.67 to 1763.3 ± 138.82 units/L). GLE was able to reduce their levels dose-dependently. The decreased levels of cardiac antioxidant enzymes such as GSH, SOD, GPx, and CAT by DOX administration were significantly increased by treatment with 500 mg/kg GLE. The oxidant status markers such as malondialdehyde (MDA), advanced oxidation protein products (AOPPs), and protein carbonyls (PCOs) were notably declined by GLE administration in DOX-treated group. Hematology profiles, histopathological observations, and electrocardiography parameters all supported the effects of GLE preventing DOX-induced cardiotoxicity. High-performance thin-layer chromatography (HPTLC) analysis revealed that GLE contained 42.27% polysaccharides and 21.6% protein. These experimental results suggested the potential therapeutic effects of GLE to ameliorate DOX-induced cardiotoxicity.

G. lucidum polysaccharides (GLPS) are the most important bioactive substances of *G. lucidum*. Fan Xu et al. (2017) assessed the effects of GLPS against DOX-induced cardiotoxicity and investigated the associated mechanisms in vitro and in vivo [39]. For in vitro experiment, rat cardiomyocytes H9c2 were pretreated with GLPS 8 h before DOX administration and continued for 16 h. Sprague-Dawley (SD) rats received daily oral gavage with GLPS for 15 days prior to DOX and continued for 6 days. In vitro results showed that DOX treatment decreased the cell viability of H9c2 cells and increased the level of lactate dehydrogenase (LDH). GLPS pretreatment didn't affect the cell viability but could significantly prevent DOX-induced cardiomyocyte death and decrease LDH in a dose-dependent manner. In vivo, DOX treatment led to cardiotoxicity, representing as decreased heart weight index and elevated serum levels of cardiac enzymes (creatine kinase, aspartate aminotransferase, and LDH). GLPS pretreatment could significantly reverse these changes. Histopathological examination of heart tissues also confirmed the cardioprotection effects of GLPS. In addition, DOX-induced cardiomyocyte apoptosis was markedly suppressed by GLPS pretreatment through mitochondrion-dependent apoptotic pathway. DOX potentiated cardiac oxidative stress, such as increased MDA level and reduced levels of SOD, CAT, and GSH in vivo and in vitro. These effects were dose-dependently attenuated by GLPS pretreatment. At the end of experiment period, both mRNA and protein levels of pro-inflammatory cytokines such as TNF-α and IL-6, anti-inflammatory cytokine IL-10 in H9c2 cells, and heart tissues of rats were detected. The results showed that DOX caused elevated pro-inflammatory cytokines and reduced anti-inflammatory cytokine. GLPS pretreatment reversed these changes. The transcription factor NF-E2-related factor 2 (Nrf2) is regarded as an important transcription factor to regulate oxidative stress, and Keap1 (kelch ECH-associating protein 1)-Nrf2-ARE (antioxidant response elements) signaling plays a major role in cell survival responses to endogenous and exogenous stresses [40]. Therefore, the expressions of Nrf2 and its related molecules were detected to elucidate the potential mechanisms. GLPS pretreatment dose-dependently increased the expression of Nrf2 by suppressing Clu3 expression, resulting in elevated levels of MDM2 and HO-1 and decreased expression of P53 and p-P65, which was associated with the effects of GLPS in preventing DOX-induced cardiotoxicity through attenuating apoptosis, oxidative stress, and inflammation.

The cardioprotective effect of *G. lucidum* ethanol extract (GDE) against Adriamycin-induced toxicity was reported by M. Rajasekaran and C. Kalaimagal (2012) [41]. Adriamycin was given to Wistar rats for 3 weeks in the presence or absence of GDE. The results showed that compared to control, Adriamycin administration induced elevated serum levels of marker enzymes including alanine aminotransferase (ALT) from 131.51 ± 2.76 to 217.72 ± 1.80 UL-1, AST from 109.52 ± 2.52 to 233.34 ± 1.40 UL-1, LDH from 192.0 ± 1.36 to 286.15 ± 2.06 UL-1, and CK from 93.17 ± 1.17 to 182.51 ± 1.97 UL-1. The lipid peroxidation (LPO) in the heart of rats was also increased from 34.83 ± 1.13 to 99.12 ± 2.12 nmol/mg protein. But the antioxidant enzymes were significantly reduced by Adriamycin compared to control, such as GSH (78.83 ± 1.49 vs. 30.33 ± 1.82 nmol/g tissue), glutathione peroxidase (GPx, 33.83 ± 1.62 vs. 10.50 ± 0.76 μmol GSH oxidized/min/mg protein), glutathione-S-transferase (GST, 26.83 ± 1.01 vs. 12.21 ± 1.56 U/min/mg protein), SOD (21.50 ± 1.84 vs. 7.67 ± 0.55 U/g protein), and CAT (16.67 ± 1.66 vs. 6.67 ± 0.42 μmol/min/mg protein). However, GDE administration could ameliorate these changes induced by Adriamycin to be nearer to the normal control. This study showed the effect of GDE on antioxidant property and illustrated its cardioprotective potential against Adriamycin-induced toxicity.

4.2.5 Other Protective Effects of Ganoderma on Radiation and Chemotherapy

Chemotherapy-related fatigue occurs in 80–96% non-small cell lung cancer patients who receive cisplatin therapy, one most common and active anticancer agent [42, 43]. Fatigue seriously affects the quality of life of the cancer patients receiving chemotherapy and even results in interruption treatment [44, 45]. Therefore, the strategies without side effects are urgently needed for chemotherapy-related fatigue treatment. Since *G. lucidum* polysaccharides (GLPs) have been widely reported to be able to improve the nutritional status of individuals and the quality of life [46, 47], its effect on fatigue caused by cisplatin was investigated by Ouyang Mingzi et al. (2016) [48]. In a chemotherapy-related fatigue mouse model, 100 mgl/kg GLPs (GLP-M) and 200 mg/kg GLPs (GLP-H) could significantly increase the weight-loaded swimming time, which was reduced in the tumor control group (TCG) and the cisplatin control group (PCG). The fatigue caused by cisplatin was more severe and occurred earlier than that induced by the tumor (Fig. 4.5). The upregulated serum levels of TNF-α and IL-6 were also reduced by GLP-M and

Fig. 4.5 (continued) mean ± SE. Compared with the TCG, $*P < 0.05$, $**P < 0.01$. Compared with the PCG, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$. (**b**) Effects of GLPs on the weight-loaded swimming test of the A549 cell-inoculated mice. Each value represents the mean ± SE. Compared with the TCG, $*P < 0.05$, $**P < 0.01$. (**c**) Effects of GLPs on the weight-loaded swimming test of the A549 cell-inoculated and cisplatin-treated mice. Each value represents the mean ± SE. Compared with the PCG, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$. Ref [48]

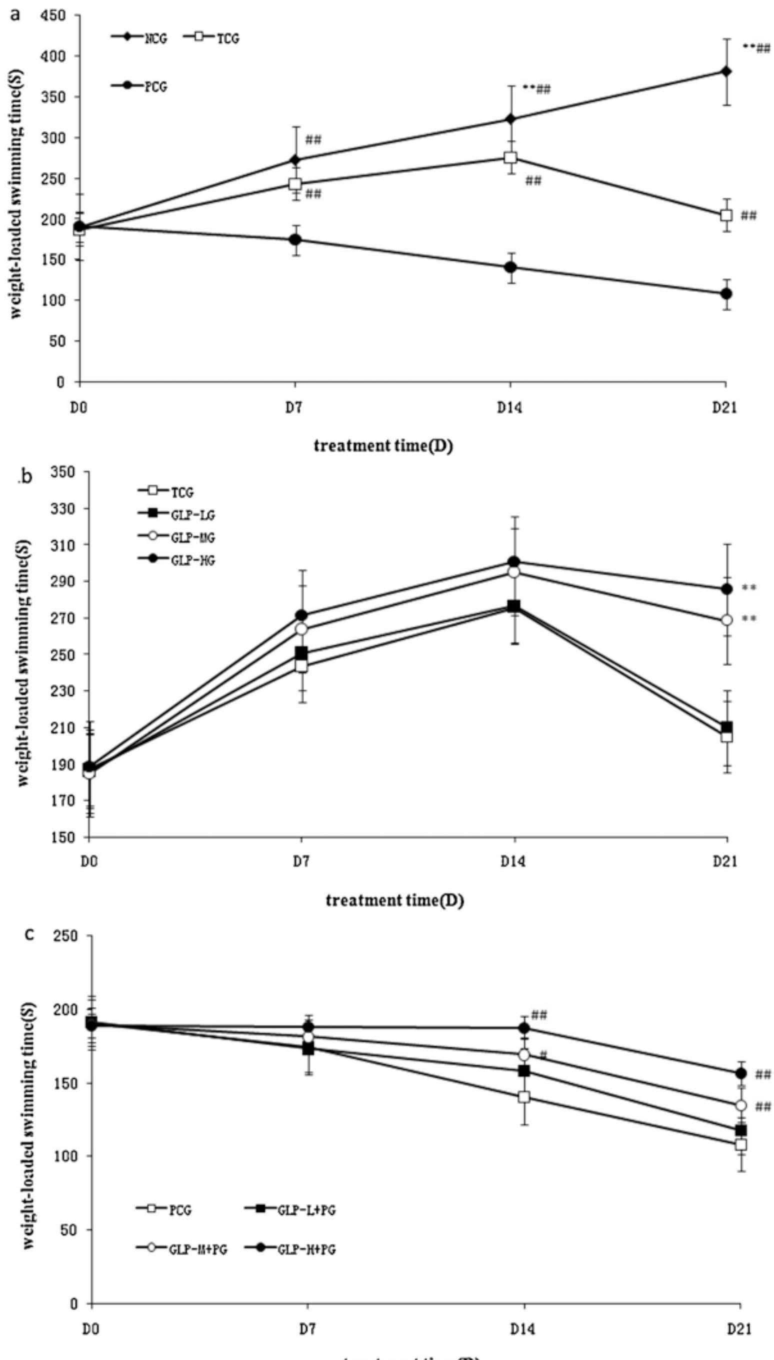

Fig. 4.5 The effects of GLPs on weight-loaded swimming test. (**a**) Effects of A549 cell inoculation and cisplatin treatment on the weight-loaded swimming test. Each value represents the

GLP-H. In addition, GLP-H could reduce the levels of MDA and increase the SOD activity in the muscle compared to TCG and PCG. The survival time test showed that the mice treated with GLP-M and GLP-H could live longer than those in TCG and PCG. Cisplatin increased the levels of serum blood urea nitrogen and creatinine, while GLP-M and GLP-H could restore these changes. These results indicated that GLPs could improve chemotherapy-related fatigue through regulating inflammatory responses and oxidative stress and reducing nephrotoxicity.

Nausea and vomiting are two most common side effects of chemotherapy and radiation therapy [42]. 50–80% of patients undergoing radiotherapy are affected by radiation-induced nausea and vomiting [49]. The incidence of chemotherapy-induced nausea and vomiting (CINV) is reported to range from 30% to 90% [50]. The severity of these adverse effects even leads to patients giving up the subsequent cycles of chemotherapy and also increases significantly the presence of anticipatory emesis. Therefore, complementary and alternative medicine is often used to prevent the side effects of chemotherapy for cancer patients, and its current usage rates are increased to 87% in the past two decades [51]. C Z Wang et al. (2005) investigated the effects of *G. lucidum* extract (GLE) on CINV in a rat model [52]. The male Wistar rats were injected intraperitoneally with cisplatin to induce pica in the presence or absence of different concentrations of GLE. Kaolin intake test reflects the nausea and vomiting action induced by cisplatin. The results showed that after cisplatin injection, the kaolin intake by rats was significantly increased, while the food intake was significantly reduced. GLE administration could dose-dependently decrease the kaolin intake ($P < 0.01$) while increasing the food intake ($P < 0.01$). Administration of GLE in a dosage of 1, 3, and 10 mg/kg reduced the area under the curves for kaolin intake to 81.6%, 51.6%, and 27.1%, respectively. At 24 h, the cisplatin injection decreased the food intake from 22.5 g to 11.0 g ($P < 0.01$). 10 mg/kg GLE administration increased the food intake close to 20 g ($P < 0.01$). These results suggested that GLE played a supportive effect on general body condition.

For normal cells damaged by chemotherapy and radiotherapy, *G. lucidum* also has protective effect. Wang DH and Wang XC (2006) reported the protective effects of extracts of *G. lucidum* on damaged HL-7702 cells caused by chemotherapy and radiotherapy [53]. HL-7702 is a kind of normal human's liver cell. Different kinds of GLE (*G. lucidum*, the extract of ethyl acetate, the extract of chloroform, and the remains after two-time extraction) were pretreated or posttreated to HL-7702, which were damaged by 2.5 μg/mL cisplatin or ^{60}Co γ-ray irradiation. The results showed that posttreatment with all the four tested extracts did not restore the damaged effects of HL-7702 induced by cisplatin, while pretreatment with the extract of chloroform in a dosage of 0.25 and 0.5 μg/mL could reduce the damaged degree significantly. Pretreatment of all the four kinds of extracts was able to reduce the damaged degree by ^{60}Co γ-ray radiation for HL-7702 cells. However, only the extract of chloroform showed restoring effect to some degree to the HL-7702 cells damaged by ^{60}Co γ-ray irradiation. These results indicated the protective effects of chloroform extract of *G. lucidum* on the damaged normal cells induced by chemotherapy and radiotherapy.

4.3 The Clinical Application of *Ganoderma* (Lingzhi) in Preventing the Side Effects Induced by Radiation and Chemotherapy

Clinical study discovered that when *G. lucidum* is combined with radio- and chemotherapy for treating esophageal cancer, gastric cancer, colorectal cancer, lung cancer, liver cancer, prostate cancer, cervical cancer, leukemia, etc., it can not only reduce the chemo- and/or radiation-induced side effects, such as bone marrow suppression, leukopenia and thrombocytopenia, stomach intestinal injury, and liver and kidney injury but also can enhance the antitumor immunity and improve cancer patients' quality of life, which indicates that *G. lucidum* enhance curative effects and reduce toxicity and side effects caused by chemotherapy or radiotherapy drugs [54].

Liang J et al. (2002) found that the radiotherapy combined with LingZhi-912 might increase the effect on treating esophageal cancer and decrease the side effect. After 122 patients received radiotherapy combined with a medicine LingZhi-912 capsu1e (MRT) (0.4 g/time, 3 times/day, receiving orally for 30 days) and 76 patients received radiotherapy alone (RT), the curative effect in the MRT group and in the RT group reached 90. 2% (110/122) and 72. 4% (55/76), respective1y. The count of leukocyte and platelet and the activity of phagocyte were increased in the MRT group significant1y. The symptoms for chest pain and vomit were relaxed. The medium surviva1 time was 27.5 mo in the MRT group and 14.7 mo in the RT group. The 1- and 3-year surviva1 rates were 78.1% and 40.5% in the MRT and 53.2% and 26.0% in the RT, respective1y [55].

Zhuang SR et al. (2012) reported that a total of 58 breast cancer patients who received chemotherapy or radiotherapy were enrolled. Immune cell levels in patient serum were determined before and 6 weeks after cancer treatment for patients receiving either nine capsules of RG-CMH, which represents a mixture of rose geranium and extracts of *Ganoderma tsugae*, *Codonopsis pilosula*, and *Angelica sinensis*, or a placebo each day, and patients received medicinal herb treatment and chemotherapy concomitantly. Administration of RG-CMH was associated with a significant reduction in levels of leukocytes from 31.5% for the placebo group to 13.4% for the RG-CMH group. Similarly, levels of neutrophils significantly decreased from 35.6% for the placebo group to 11.0% for the RG-CMH group. RG-CMH intervention was also associated with a decrease in levels of T cells, helper T cells, cytotoxic T cells, and natural killer cells compared with the placebo group. These results revealed that administration of RG-CMH to patients receiving chemotherapy/radiotherapy may have the capacity to delay, or ease, the reduction in levels of leukocytes and neutrophils that are experienced by patients during cancer treatment [56].

Zhao FY et al. (2015) reported that 59 patients with (non-small cell lung carcinoma) NSCLC were divided into the observation group (31 cases) and the control group (28 cases). Both groups were treated with paclitaxel + cisplatin for chemotherapy. *G. lucidum* spore capsules (4 pills/time, 3 times/day, with 21 days as one course of treatment) were taken orally, and placebo was added to the control group

on the basis of chemotherapy. After six treatment courses, the clinical efficacy and the improvement of immune function before and after treatment were compared between the two groups. Results showed that after chemotherapy combined with *G. lucidum* spore capsule treatment, $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ levels were higher than the control group, and $CD8^+$ levels were lower than the control group. The rates of bone marrow suppression and gastrointestinal dysfunction in the observation group were lower than those in the control group, suggesting that *G. lucidum* spore capsule combined with paclitaxel + cisplatin in the treatment NSCLC can improve clinical efficacy, reduce the impact on immune function, and at the same time reduce bone marrow toxicity and gastrointestinal reaction [57].

Wang J et al. (2016) studied 134 patients with NSCLC, which were randomly divided into the observation group and the control group, and the control group received conventional chemotherapy, observation group received *G. lucidum* spore capsule orally (4 pills/time, 3 times/day, with 21 days as one course of treatment) for four courses, and the clinical efficacy and immune function of the two groups were observed. The treatment efficiency and total effective rate in the observation group were significantly higher than those in the control group, while the incidence of myelosuppression and gastrointestinal reaction was significantly lower than that of the control group. In the observation group after treatment, $CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ were significantly improved, and the improvement was significantly better than that of control group, indicating that it can effectively improve clinical efficacy, alleviate adverse reactions caused by chemotherapy, and significantly improve patients' immune function [58].

Yan BK et al. (1998) investigated the efficacy of Lingzhi oral liquid combined with chemotherapy for the treatment of advanced non-small cell lung cancer, and patients were randomly divided into the treatment group and the control group. The effective rates were 65.71% and 42.85%, respectively. The results showed that Lingzhi oral liquid (20 ml/time, 3 times/day, 1 month regarded as one course of treatment) could alleviate the toxic and side effects of chemotherapy and improve the effect of chemotherapy and the quality of life of tumor patients and can reduce the inhibition of hematopoietic function of bone marrow by chemotherapy while improving the suppressed cellular immune function and T cells in tumor patients after two-course treatment [59].

Yu Y (2001) reported that after 94 tumor patients received internal medicine therapeutics or operation combined with chemo- and radiotherapy, peripheral blood cells and state of illness have been reduced and stabilized after receiving the spore powder of *Ganoderma* (0.9~1.5 g/time, 3 time/day, 24 days for as one course of treatment) for 2~4 courses. The doctors followed up for 6 to 10 months to the patients who received Lingzhi voluntarily. The results showed that among the 94 patients, 88 patients' peripheral blood cell numbers were increased, and the effective rate reached 93.61% [60].

Sun DJ et al. (1999) studied 140 common malignant tumor patients diagnosed with esophageal cancer, gastric cancer, colon cancer, liver cancer, lung cancer, breast cancer, malignant lymphoma, and ovarian cancer and divided them into experimental group (100 cases) and control group (40 cases). Experimental group

received *Ganoderma* Fuzheng capsule (15 g/time, 2 times a day) which composed of *Ganoderma* and ginseng, orally, three times a day, seven granules for each time after chemotherapy. In the experimental group, seven cases (7%) had nausea and vomiting. The control group had 17 cases of nausea and vomiting, accounting for 42.5%. In the whole chemotherapy process, the experimental group had significantly less gastrointestinal reaction than the control group and 6 patients with leukocyte count lower than 3.0×10^9 /L during chemotherapy, accounting for 6% in experimental group, in control group, 7 cases with leukocyte count number was less than 3.0×10^9 /L, accounting for 17.5%, suggesting that the *G. lucidum* Fuzheng capsule was used to protect bone marrow function and leukocyte decline, it was significantly better than the control group [61].

Qi YF et al. (1999) studied the *Ganoderma* spore powder capsule as an adjuvant chemotherapy in 200 tumor patients and reported that it has a good therapeutic effect on gastric cancer, esophageal cancer, liver cancer, colorectal cancer, and so on. The results showed that the efficacy rate of *Ganoderma* spore powder capsule (1 g/time, 3 times/day, orally) reached 43%, significantly better than the control in short-term objective efficacy. There were significant differences in quality of life and body weight between the two groups. The two groups had similar effects in reducing hematological toxicity and stimulating bone marrow hematopoiesis, but the experimental group was better than the control group in increasing immune function, especially cellular immunity. It shows that *Ganoderma* spore powder capsule is an effective agent for reducing toxicity and enhancing efficacy of chemotherapy [62].

Kuang JM (2007) studied 56 patients and randomly divided them into the treatment group and the control group. Patients in the observation group were given broken *Ganoderma* spore powder by oral administration for one course of treatment, 0.9 g/time, three times a day combined with chemotherapy, and control group only received chemotherapy. Results showed that efficacy rate reached 92.9% in observation group and 57.1% in control group after chemotherapy. Weakened immunity was found in 2 cases in observation group and 10 cases in control group. Bone marrow and gastrointestinal dysfunction were found in 4 cases in the observation group and 20 cases in control group, indicating that *Ganoderma* broken spore powder combined with chemotherapy for malignant tumor has the effect of reducing toxicity and enhancing efficacy [63].

Wang YH et al. (2014) investigated 64 patients with advanced colorectal cancer and divided them into two groups, and the treatment group received broken *Ganoderma* spore powder(10 g/time, 3 times/day, 4 weeks as a one course of treatment) combined with oxaliplatin. The control group was treated with XELOX chemotherapy alone in three courses of treatment, and in the short-term efficacy, adverse reactions and quality of life were evaluated before and after chemotherapy. The effective rate was 46.9% in the treatment group and 37.5% in the control group. There was no difference in statistical significance. However, the disease control rate was 84.4% in the treatment group and 56.3% in the control group with statistically significant difference. The incidence of leukopenia, nausea, and vomiting in the treatment group was lower than that in the control group, and the quality of life in the treatment group was significantly higher than the control group [64].

Lin ND (2004) studied 114 patients diagnosed with gastric cancer, esophageal cancer, lung cancer, liver cancer, cervical cancer, and colon and bladder cancer, which were randomly divided into the chemotherapy + *Ganoderma lucidum* group (66 cases) (4 pills each time, 4 times a day, 40 days for 1 treatment course) and chemotherapy-alone group (48 cases). Results showed that before and after treatment with chemotherapy + Lingzhi, the activity of NK cells was 51.24 ± 7.90% and 48.10 ± 7.90 ($P > 0.05$), respectively. The chemotherapy group was 51.40 ± 6.62% and 44.43 ± 7.19% ($P < 0.05$). Before and after treatment in the chemotherapy + *G. lucidum* group, there was no significant change in CD3, CD4, and CD8 cell subtypes (%), but after treatment of chemotherapy alone, the percentage of CD3, CD4, and CD8 cell subsets was significantly decreased ($P < 0.05$), indicating that *G. lucidum* extract capsule combined with chemotherapy can significantly improve cellular immune function in cancer patients [65].

4.4 Conclusion

In this chapter, it was reviewed that the protective effect of *Ganoderma* (Lingzhi) on radiation and chemotherapy included improving the conditions of neutropenia and leukopenia, ameliorating the small intestinal damage; reducing nephrotoxicity and cardiovascular toxicity, preventing chemotherapy-related fatigue, nausea, and vomiting, etc. Basically, the action mechanisms of *Ganoderma* (Lingzhi) on radiation and chemotherapy were focused on reducing cellular DNA and membrane damages and alleviating oxidative stress conditions. The water extract, ethanol extract, and chloroform extract of *Ganoderma* (Lingzhi) are all investigated by researchers. Most components of *Ganoderma*, such as polysaccharides, beta-glucan, lingzhilactones, terpenes, and GMI (one fungal immunomodulatory protein isolated from *Ganoderma microsporum*), were effective in preventing the side effects of radiation and chemotherapy. In clinical studies, the researchers associated the protective effects of *Ganoderma* (Lingzhi) on radiation and chemotherapy with its immunological mechanisms. Primary clinical studies also confirmed the protective effects of *Ganoderma* (Lingzhi) on radiation and chemotherapy. However, the large-scale, randomized clinical researches are absent in this field.

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Chapter 5
Neuropharmacological Effect and Clinical Applications of *Ganoderma* (Lingzhi)

Xiangyu Cui and Yonghe Zhang

Abstract *Ganoderma lucidum* (*G. lucidum*, Lingzhi) is a kind of medical mushroom with various pharmacological compounds. It has been used for clinical applications for thousands of years as a highly nutritious and significantly effective medicinal herb. Compared with its immunomodulatory effect, there are a few studies on the neuropharmacological actions of *Ganoderma*, and the mechanism has not been fully elucidated. As far as we know, *Ganoderma* regulate the central nervous system (CNS) at least in part through its immunomodulatory activity. The neuropharmacological effects of *G. lucidum* mainly include but not limited to sedative and hypnotic, neuroprotective, antinociceptive and analgesic, antiepileptic, and antidepressant effects. Clinical trials of *G. lucidum* in the patients with these disorders are still rare. To date, there are no *Ganoderma*-related drugs approved by the US Food and Drug Administration (FDA). In this chapter, we will summarize and elucidate recent progress of such effects of *Ganoderma* and its ingredients from both the preclinical and clinical points of view.

Keywords *Ganoderma* · Sedative and hypnotic effects · Neuroprotective effects · Antinociceptive and analgesic effects · Antiepileptic effects · Antidepressant effects

5.1 Introduction

G. lucidum have been used for centuries as medical mushrooms to treat various diseases in the world, especially in China. Among these diseases, therapeutic effects of *Ganoderma* on the central nervous system (CNS) disorders have been well documented. The mechanisms of action are largely unknown due to the lag of the mechanistic research on these neurological diseases. Currently, it was considered that

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neuroprotective and anti-inflammatory effects of *Ganoderma* may play an important role. In the neuropharmacological effects of *G. lucidum*, we mainly focused on the sedative and hypnotic, neuroprotective, antinociceptive and analgesic, antiepileptic, and antidepressant effects and describe them in the following sections. Note that some of the articles were written in Chinese and may be not retrieved in the PubMed website.

5.2 Sedative and Hypnotic Effects

In China, *G. lucidum* has been used as a tranquilizing agent to treat insomnia for thousands of years. Its "An-Shen" effect reflects tranquilizing activity in the treatment of restlessness, palpitation, and insomnia [1]. Early studies found that sedation effects were observed 1–2 min after administration of *G. lucidum* tincture (5 g/kg, i.p.), *G. lucidum* fermentation concentrate (10 ml/kg, i.p.), or mycelium solution (5 g/kg, i.p.). Meanwhile, the locomotor activity and muscle tension were significantly decreased. Injection of *G. lucidum* solution (20 g/kg) significantly enhances the anesthetic effect of pentobarbital sodium and decreases the effective dose of pentobarbital sodium that makes mice anesthesia. The median effective dose (ED_{50}) of pentobarbital sodium was 25.4 mg/kg in the *G. lucidum* solution group, while that in the control group was 35.0 mg/kg. There was a significant difference between the two groups [2, 3]. Administration of *G. lucidum* leachate obtained under constant temperature (i.p.) or *G. lucidum* concentrate (i.g. or i.p.) also decreased locomotor activity in mice, and this effect will last for 3–6 days. *G. lucidum* leachate obtained under constant temperature significantly prolongs the duration of anesthesia induced by hexenal sodium. Injection of the fermentation broth of *Ganoderma* sp. reduced locomotor activity of mice and enhanced sedative effect induced by chlorpromazine and reserpine, while it antagonizes excitatory effect induced by phenylalanine [4]. Other formulations of *Ganoderma*, such as *G. lucidum* spore powder [5], *G. lucidum* extract [6], and *G. lucidum* granules [7], also decrease locomotor activity and sleep latency induced by pentobarbital sodium with prolonged sleep time. *G. lucidum* granules can protect mice from convulsion onset induced by strychnine [8].

Chu QP et al. (2007) suggested that the sedative and hypnotic effects of *G. lucidum* extract involve benzodiazepine receptor, the target of traditional sedative and hypnotic drugs [9]. In pentobarbital-treated (45 mg/kg) mice, administration of *G. lucidum* extract (GLE) not only decreased sleep latency but also prolonged the duration of sleep significantly at doses of 80 and 120 mg/kg. By sleep electroencephalogram (EEG) analysis, GLE (80 mg/kg) decreased sleep latency and increased both total sleep and non-rapid eye movement (NREM) sleep but revealed no significant influence on REM sleep. GLE has minimal effects on sleep architecture at dose up to 120 mg/kg. Flumazenil (a benzodiazepine antagonist) at a dose that caused no

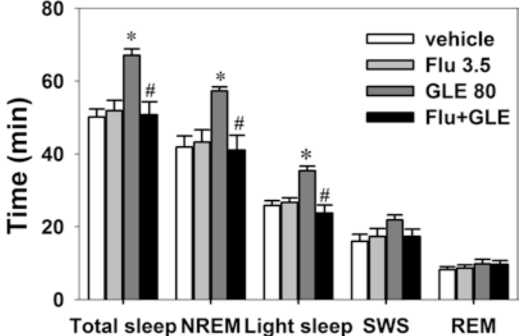

Fig. 5.1 Effects of flumazenil on the changes in sleep parameters induced by GLE in pentobarbital-treated rats. Total sleep [$F(3, 15) = 9.00$, $P = 0.001$], REM [$F(3, 15) = 0.57$, $P = 0.646$], NREM [$F(3, 15) = 6.18$, $P=0.006$], SWS [$F(3, 15) = 1.85$, $P = 0.182$], and light sleep [$F(3, 15) = 10.94$, $P = 0.000$]] were assessed. Data are presented as mean ± S.E.M (n = 4~5). $*P < 0.05$ and $**P < 0.01$ vs. vehicle and $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. group treated with GLE alone. Rats were administered GLE (80 mg/kg, p.o.) consecutively for 3 days, and flumazenil (3.5 mg/kg, i.p.) was administered 20 min prior to the last administration of GLE. 60 min after GLE administration, rats were treated with pentobarbital (35 mg/kg, i.p.), and the EEG and EMG were recorded immediately until rats were awake. (Reproduced with permission from Ref. [9])

obvious effect when used alone showed a significant antagonistic effect on the decrease in sleep latency, increase in sleeping time, non-REM sleep time or light sleep time, as well as suppression of locomotor activity in rats induced by GLE, as shown in Fig. 5.1. GLE increased delta activity during NREM sleep, which is often regarded as an indicator of sleep intensity, while flumazenil showed no significant antagonistic effect on that (Table 5.1). Studies have shown that GLE may inhibit GABA reuptake by inhibiting GABA transporter, which may be related to its hypnotic effect [10]. Cui XY et al. (2012) reported that the immunoregulatory mechanism of *G. lucidum* may be involved in its regulatory effect on sleep [11]. TNFα is posited to be a key cytokine involved in sleep regulation [12–14]. EEG delta activity during NREM sleep is positively correlated with plasma and brain levels of TNFα [12]. Intracerebroventricular administration (i.c.v.) of TNFα exerted a synergistic effect with GLE (40 mg/kg, i.g.) (Fig. 5.2), while TNFα antibody inhibited the hypnotic effect of GLE (80 mg/kg, i.g.). Coadministration of TNFα with GLE significantly increased delta activity during NREM sleep, which can be inhibited by TNFα antibody (Fig. 5.3). These results suggest that the sleep-promoting effect of *G. lucidum* extract was through multiple targets, including (but not limited to) central benzodiazepine receptor and immune cytokine. In contrast to benzodiazepines, which reduces slow wave sleep (SWS) and REM sleep, *G. lucidum* increases sleep depth and left REM sleep intact, implying that immunomodulatory effect may play a role in the hypnotic effect of *G. lucidum*.

Table 5.1 Effect of flumazenil on the increase in delta activity during NREM induced by GLE in pentobarbital-treated rats

	Delta activity during NREMs (*volts*^{2} $\times$*10*^{3})					
Groups	0–10 min	10–20 min	20–30 min	30–40 min	40–50 min	50–60 min
Control	6.43 ± 0.88	7.98 ± 0.95	8.32 ± 1.12	7.18 ± 1.08	6.63 ± 0.85	4.22 ± 0.80
Flumazenil	5.63 ± 0.71	7.64 ± 1.69	8.88 ± 1.93	7.76 ± 1.64	8.13 ± 0.94	5.18 ± 1.11
GLE	7.72 ± 0.96	10.54 ± 0.56*	11.32 ± 0.35*	13.53 ± 1.52*	9.77 ± 1.51	8.01 ± 1.44
GLE+FLU	7.30 ± 0.74	9.59 ± 0.87	10.96 ± 1.22	9.98 ± 2.95	6.45 ± 0.70	5.66 ± 0.51

Each value represents the mean ± S.E.M. for 5 rats. *$P < 0.05$ vs. control (Student–Newman–Keuls test)

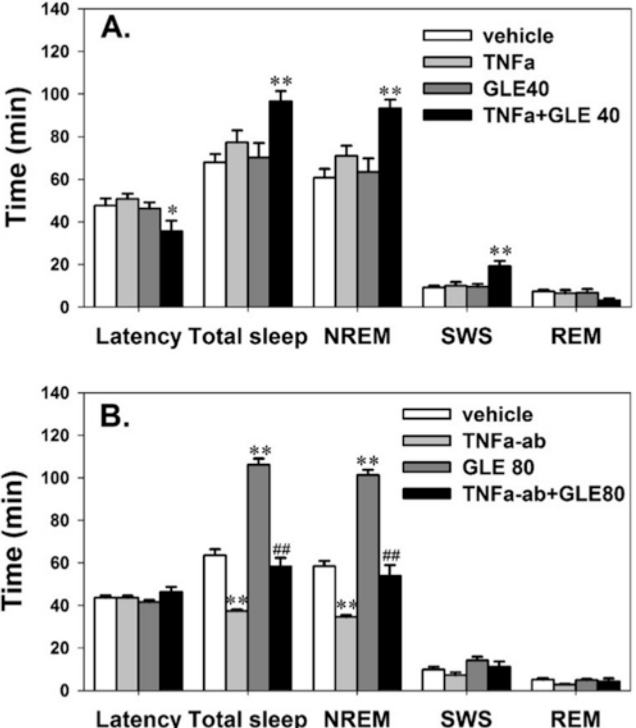

Fig. 5.2 Effects of TNF-α and TNF-α antibody in GLE-treated rats. (**a**) Synergic effects of GLE (40 mg/kg, i.g.) with TNF-α (12.5 ng/rat, i.c.v.) on the sleep parameters in freely moving rats. Sleep latency [F (3, 16) = 5.340, $P < 0.05$], total sleep [F (3, 16) = 5.795, $P < 0.01$], NREM sleep [F (3, 16) = 9.166, $P < 0.01$], SWS [F (3, 16) = 7.921, $P < 0.01$], and REM [F (3, 16) = 2.014, $P > 0.05$] were assessed. Data are presented as mean ± S.E.M. (n = 5). GLE 40: GLE 40 mg/kg (i.g.). $*P < 0.05$ and $**P < 0.01$ vs. vehicle (Student–Newman–Keuls test). (**b**) Effect of TNF-α antibody on hypnotic activity of GLE (80 mg/kg, i.g.) in freely moving rats. Sleep latency [F (3, 16) = 2.884, $P > 0.05$], total sleep [F (3, 16) = 127.274, $P < 0.01$], NREM sleep [F (3, 16) = 114.785, $P < 0.01$], SWS [F (3, 16) = 2.829, $P > 0.05$], and REM [F (3, 16) = 1.973, $P > 0.05$] were assessed. Data are presented as mean ± S.E.M. (n = 5). TNFα-ab: TNF-α antibody (2.5 μg/rat, i.c.v.); GLE 80: GLE 80 mg/kg (i.g.). $**P < 0.01$ vs. vehicle. $^{##}P < 0.01$ vs. group treated with GLE 80 mg/kg (Student–Newman–Keuls test). (Reproduced with permission from Ref. [12])

5.3 Neuroprotective Effects

Neuroprotection plays a vital role in the treatment of neurodegenerative diseases and neural impairment and is among the most investigated effects of *Ganoderma*. The most investigated neurodegenerative diseases are Alzheimer's disease (AD) and Parkinson's disease (PD). Both AD and PD are common and debilitating degenerative diseases. *G. lucidum* polysaccharides (GLP, 30 mg/kg, once per day for 90 days) could serve as a regenerative therapeutic agent for the treatment of cognitive decline associated with neurodegenerative diseases in APP/PS1 mice

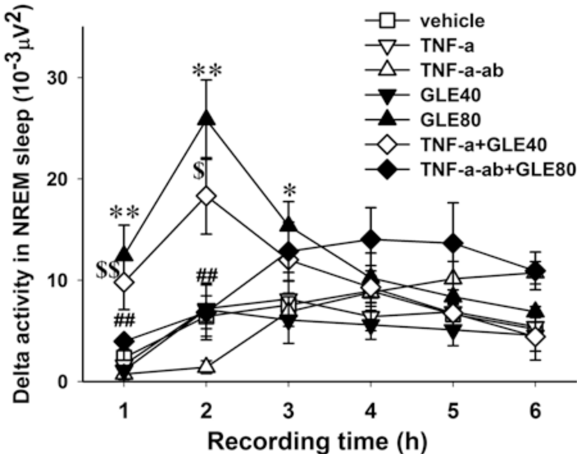

Fig. 5.3 EEG delta activity during NREM sleep. 0–1 h: [F (6, 33) = 8.417, P < 0.01], 1–2 h: [F (6, 33) = 11.717, P < 0.01], 2–3 h: [F (6, 33) = 3.396, P < 0.05], 3–4 h: [F (6, 33) = 1.649, P > 0.05], 4–5 h: [F (6, 33) = 2.443, P < 0.05], and 5–6 h: [F (6, 33) = 3.292, P < 0.05] were assessed. Data are presented as mean ± S.E.M. (n = 5~10). TNF-α (12.5 ng/rat, i.c.v.); TNF-α-ab: TNF-α antibody (2.5 μg/rat, i.c.v.); GLE 40: 40 mg/kg; GLE 80: GLE 80 mg/kg. $*P < 0.05$ and $**P < 0.01$ vs. vehicle; $^{\#\#}P < 0.01$ vs. group treated with GLE 80 mg/kg; $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ vs. group treated with GLE 40 mg/kg (Student–Newman–Keuls test). (Reproduced with permission from Ref. [12])

[15]. It has been reported that GLP have significant neuroprotective effects against oxidative stress-induced neuronal apoptosis by regulating the expression of apoptosis-associated proteins to inhibit oxidative stress-induced neuronal apoptosis [16]. Apart from the effects on neurodegenerative diseases, *Ganoderma* also has neuroprotective effects on spinal cord injury [17] and cerebral ischemic injury in rats [18].

AD is the most common and progressive developed neurodegenerative disease among elderly population, characterized by continuous cognitive decline and worsening of daily living performance, as well as the loss of functional neurons and synapses. Guo YJ et al. (2006) showed that microinjection of $A\beta_{25-35}$ into the hippocampus induced learning and memory dysfunction in rats. These abnormal behavior was reversed by GLP (50 mg/kg for 7 days) in the Morris water maze test [19]. In addition, neurons showed ultrastructural pathological changes as cell hyperplasia, endoplasmic reticulum dilatation, chromatin agglomeration, and astrocyte hypertrophy after injection of $A\beta_{25-35}$ into the hippocampus. The lesions were significantly reduced in the polysaccharide group, and the ultrastructure was almost normal. The number of astrocytes was significantly decreased as compared with that of the AD group [19]. In addition to neuronal apoptosis, synaptic degeneration had been recognized as an important mode of neurodegeneration. Several synaptic density proteins including synaptophysin, synaptotagmin, and PSD-95 are reduced in the AD brain. The immunoreactivity of synaptophysin (SYP) is significantly reduced in neurites of $A\beta_{25-35}$-treated neurons, and the distribution of SYP is

unevenly distributed, and these changes are reversed by the treatment of *G. lucidum* aqueous extract (GLA) (Fig. 5.4) [20]. By full-length $A\beta_{1-42}$, these authors confirm the results obtained from $A\beta_{25-35}$. Numbers of TUNEL-positive neurons were increased in neurons treated with $A\beta_{1-42}$, and exposure to GLA significantly reduced the number of apoptotic bodies (Fig. 5.5) [20]. Zhang Y et al. (2012) reported that

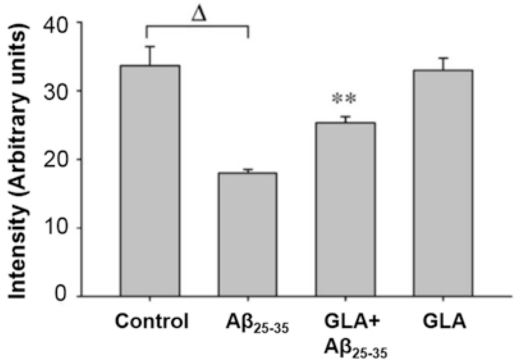

Fig. 5.4 GLA inhibits Aβ-induced downregulation of synaptophysin immunoreactivity. Cultured cortical neurons were treated with 500 μg/ml of GLA for 1 h and then subjected to $A\beta_{25-35}$ (10 μM) for 24 h. Quantitative analysis of mean intensity of synaptophysin immunofluorescence staining (mean pixel intensity). $\Delta P < 0.001$ vs. control; $**P < 0.05$ treated with Aβ peptide per se. Significant difference between different groups was compared by one-way ANOVA for multiple comparison and Student–Newman–Keuls test as post hoc test. Pictures are representative of three independent experiments. Scale bar = 20 μm. (Reproduced with permission from Ref. [20])

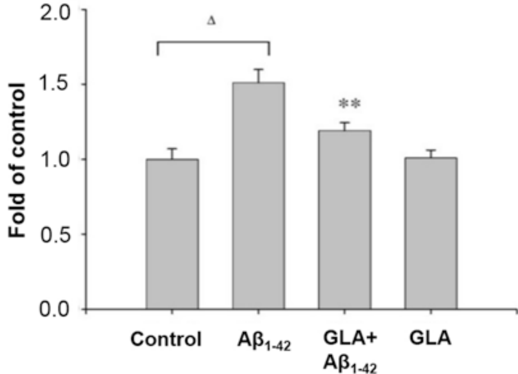

Fig. 5.5 GLA inhibits $A\beta_{1-42}$-triggered apoptosis. Quantitative analysis of the percentage of apoptotic neurons. Cultured cortical neurons were treated with 500 μg/ml of GLA for 1 h and then subjected to $A\beta_{1-42}$ (20 μM) for 24 h. TUNEL staining for DNA breaks was performed to distinguish normal and apoptotic cells under fluorescent microscopy. Results were expressed as mean ± SE from at least three independent experiments. $^{\Delta}P < 0.001$ vs. control; $** P < 0.05$ vs. the group treated with Aβ peptide only by one-way ANOVA for multiple comparison, and Student–Newman–Keuls as post hoc test. (Reproduced with permission from Ref. [20])

Ganoderma triterpenes significantly improve the learning and memory of naturally aging rats in Morris water maze and found that this mechanism may be related to the improvement of the total antioxidant capacity of brain tissue [21]. Huang SC et al. (2017) reported that GLP promote cognitive function and neural progenitor proliferation in transgenic AD mice. These authors further showed that treatment of GLP potentiated the activation of fibroblast growth factor receptor 1 (FGFR1) and downstream extracellular signal-regulated kinase (ERK) and AKT cascades [15]. In addition to the neuroprotective effect, cognition improvement effect may contribute to the therapeutic action of *G. lucidum* on the AD. Kaur R et al. (2017) reported that *Ganoderma* species have anti-amnesic effects in the memory deficit animal model induced by scopolamine and the mechanism of action involved to the anti-acetylcholinesterase and antioxidant effects of them [22]. Choi YJ et al. (2015) also showed the anti-amnesic effects of fermented *G. lucidum* water extracts on scopolamine-induced memory impairment in rats [23].

A pilot study performed by Wang GH et al. (2018) explored the feasible efficacy and safety of the *G. lucidum* spore powder (GLSP) for the treatment of patients with AD. This study recruited 42 eligible patients treated with GLSP for 6 weeks. The primary outcome was measured by Alzheimer's Disease Assessment Scale-Cognitive (ADAS-cog). The secondary outcomes were measured by the World Health Organization Quality of Life Questionnaire (WHOQOL-BREF) and Neuropsychiatric Index (NPI). The adverse events were also recorded during the treatment period. Interestingly, at the end of the treatment, GLSP (250 mg/capsule, 4 capsules each time, 3 times a day) for 6 weeks did not show more encouraging outcomes in symptoms improvement. The authors considered that it may be due to relative short term of GLSP intervention, and further clinical trials with larger sample size and longer treatment course are needed in the future to validate therapeutic effect for AD in human patients [24].

PD is the second common neurodegenerative disease characterized by motor symptoms of tremor, bradykinesia, rigidity, and postural instability resulting from massive and progressive death of dopaminergic neurons in the substantia nigra compact. In addition to the classic treatment such as levodopa administration, a bunch of Chinese herbs or herb extracts are indicated to delay the degeneration of dopamine neurons and symptoms. The neurotoxin methyl-4-phenylpyridine (MPP^+) is the active parkinsonian neurotoxin metabolite of 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which selectively destroys dopaminergic pathways by way of inhibiting the activity of mitochondrial electron transport chain complex I and induces parkinsonian syndrome in humans, monkeys, and mice. Oil from *G. lucidum* spores showed neuroprotective effect on pathological changes in the substantia nigra and on behaviors of MPTP model of PD [25]. However, the underlying mechanism of this neuroprotective effect has not been fully elucidated. Rotenone, another neurotoxin, can damage dopaminergic neurons with the mechanism similar to MPP^+. Guo SS (2016) found that GLP possess neuroprotective properties against MPP^+ and rotenone neurotoxicity through suppressing oxidative stress in primary mesencephalic dopaminergic cell culture owing to its antioxidant activities. Furthermore, GLP dramatically decreased the relative number of apoptotic

cells and increased the declining mitochondrial membrane potential (ΔΨm) induced by MPP^+ and rotenone in a dose-dependent manner [26]. Recently, Ren ZL et al. (2018) reported that *G. lucidum* extract (GLE), prepared with methanol by low-temperature extraction of the dried fruiting bodies, ameliorates MPTP-induced PD symptoms and protects dopaminergic neurons from oxidative stress via regulating mitochondrial function, autophagy, and apoptosis [27]. AMPK/mTOR and PINK1/Parkin pathways are the key molecular pathways that regulate autophagy and mitochondrial functions in PD. The AMPK is a major metabolic energy sensor that negatively regulates mTOR, leading to ULK1 (unc-51-like kinase 1)-AMPK interaction to stimulate autophagy. PINK1 and Parkin act as a ubiquitous core signaling pathway coupling mitochondrial stress to mitochondrial surveillance to regulate mitochondrial dynamics, mitophagy, and biogenesis. Mitochondrial quality control depending on them became the key mechanisms underlying PD pathology. The anti-PD-like effects of GLE may involve the activation of both the AMPK/mTOR and PINK1/Parkin signaling pathway and directly or indirectly contribute to the mitophagy and mitochondrial biogenesis essential for the survival of neuronal cells (Fig. 5.6) [27]. These findings implied that *G. lucidum* may serve as a functional food or agent for neural protection, although there is no clinical trial with *Ganoderma* as single agent or combined with other ingredients in the patients with PD [28].

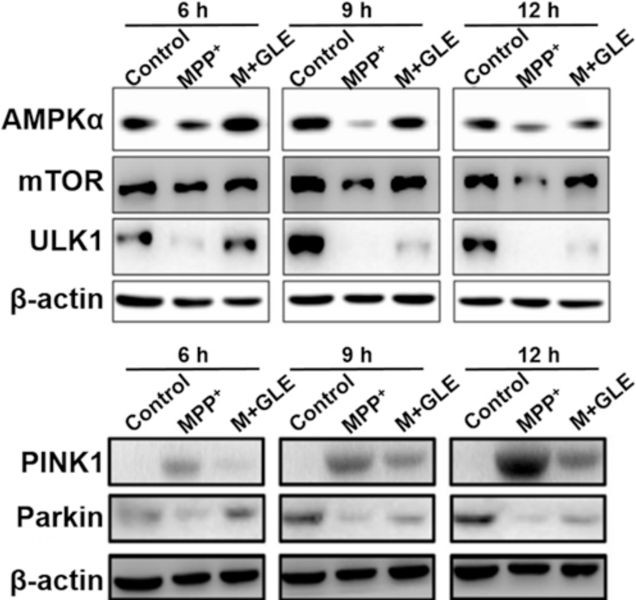

Fig. 5.6 GLE administration regulated autophagy induced by MPP+ in neuro-2a cell lines (part of Fig. 4 in [27]). Representative images of Western blot with antibodies against AMPKα, mTOR, and ULK1 (upper) and PINK1 and Parkin expression levels (lower). (Reproduced with permission from Ref. [27])

Spinal cord injury is a central nervous system disorder that causes major changes in spinal cord dysfunction or loss due to direct spinal cord injury. The spinal cord trauma model was created by the occlusion of the spinal cord with an aneurysm clip [29]. Gokce et al. (2015) reported that GLP (400 mg/kg for 7 days) can protect the spinal cord from experimental spinal cord injury. The results indicated that the increases in caspase-3 activity, tumor necrosis factor-alpha levels, myeloperoxidase activity, malondialdehyde levels, and nitric oxide levels were reversed after the administration of GLP [17]. Zhou ZY et al. (2010) showed the neuroprotective effects of GLP in middle cerebral artery occlusion (MCAO) in Sprague-Dawley (SD) rats and oxygen and glucose deprivation (OGD) in primary cultured rat cortical neurons. Moreover, GLP decreased the percentage of apoptotic neurons; relieved neuronal morphological damage; suppressed overexpression of active caspase-3, caspase-8, and caspase-9 and Bax; and inhibited the reduction of Bcl-2 expression [18]. Zhang W et al. (2014) found that pretreatment with *G. lucidum* (40 mg/kg) for 3 and 7 days reduced neuronal loss in the hippocampus, diminished the content of malondialdehyde in the hippocampus and serum, decreased the levels of tumor necrosis factor-alpha and interleukin-8 in the hippocampus, and increased the activity of superoxide dismutase in the hippocampus and serum. These results suggest that pretreatment with *G. lucidum* was protective against cerebral ischemia/reperfusion injury through its anti-oxidative and anti-inflammatory actions [30].

5.4 Antinociceptive and Analgesic Effects

Nociception is the form of somatic sensation that detects noxious, potentially tissue-damaging stimuli. Pain begins with peripheral nociceptors and has both a localizing somatic sensory component and an aversive emotional and motivational component [31]. In the present, most of researches focused on the antinociceptive effects of *Ganoderma* in hot-based plate test and tail flick test, as well as chemical-based acetic acid writhing test or formaldehyde test. Koyama K et al. (1997) tested the antinociceptive components of *G. lucidum* and found that CH_2Cl_2 extract of *G. lucidum* contains ganoderic A, B, G, and H and has antinociceptive effects [32]. Wan F et al. (1992) reported that *Ganoderma sinense* have analgesic effects in the hot scalding test and acetic acid-induced writhing test. The analgesic effect of *Ganoderma sinense* extract at 510 mg/kg was similar to that of 10 mg/kg indomethacin [33].

It was reported that water-soluble extract of *G. lucidum* decreases pain significantly in patients with post-herpetic neuralgia and herpes zoster infection [34]. Another pilot clinical trial also demonstrated that administration of an herbal formula containing *G. lucidum* (0.75 g/dose as dry weight) decreased herpes zoster pain and no patient developed post-herpetic neuralgia [35]. To our knowledge, *Ganoderma* commonly showed analgesic and anti-inflammatory action simultaneously in these tests [1]. The underlying mechanism of analgesic effect of *Ganoderma* is largely unknown, and further work needs to be done to explore the role of immunoregulatory effect played in these effects.

5.5 Antiepileptic Effects

Epilepsy is one of the most common widespread noncommunicable diseases of the nervous system, characterized by sudden abnormal discharge of neurons in the brain. Epilepsy is a chronic condition that causes cerebral transient dysfunction [29]. There have been a large number of researches focused on the antiepileptic effects of *G. lucidum* spores (GLS). Epilepsy caused by brain injury has been confirmed by many animal experiments and clinical observations. Ionic glutamate receptor (NMDAR) mediated excitatory amino acids and stimulates the inflow of Ca^{2+}, which closely associated with epilepsy susceptibility. Wang H et al. (2006) found that GLS (150 mg/kg) can significantly decrease the NR1, the function unit of NMDAR, in the cortex and hippocampus of rats and exert antiepileptic action by attenuating excitability of the nervous system [36]. Zhao S et al. (2007) found that GLS (300 mg/kg) can delay the onset of epilepsy in rats induced by pentylenetetrazol (PTZ, 35 mg/kg) from the 17th day of administration. These underlying mechanisms involved suppression of NF-kappa B protein expression and enhancement of IGF 1 activity [37]. Similarly, Zhang JG et al. (2012) found that GLS (300 mg/kg) protect brain from injury caused by epilepsy in the amygdala kindling model, which was related to the reduction of TNF-α and IL-6, the cytokines associated with the development of epilepsy [38].

N-Cadherin is a member of the cadherin family which plays an important role in targeting the growth of axons and the construction of correct synaptic connections [29]. With cultured primary hippocampal neurons, Wang SQ et al. (2013) found that GLS may protect hippocampal neurons from magnesium ion (Mg^{2+})-free extracellular medium-induced spontaneous seizure discharge by promoting neurotrophin-4 expression and inhibiting N-cadherin expression. Furthermore, the expression of neurotrophin-4 was significantly increased, while the expression of N-cadherin was decreased in the GLS-treated group compared with the model group [39]. Wang SQ et al. (2014) also found that the *G. lucidum* polysaccharides (GLP) have antiepileptic effect in the primary hippocampal neurons with mechanism differed from GLS by inhibition of intracellular calcium accumulation and stimulation of expression of CaMKII α in epileptic hippocampal neurons [40]. Yang ZW et al. (2016) found the antiepileptic effects of ganoderic acids, the chemical constituents of GLS, in vitro by indirectly inhibiting mossy fiber sprouting and adjusting the synaptic reconstructions dependent on the BDNF and TRPC3 [41]. By downregulating the expression of neural cell adhesion molecule (NCAM-1), GLS can inhibit the growth of axons and the transmembrane signal transduction of central nervous system and have antiepileptic effects [29].

In 2018, there is a retrospective study firstly investigating the feasibility effect and safety of *G. lucidum* spore powder (GLSP) for treating patients with epilepsy. Eighteen eligible patients with epilepsy were included and assigned to receive GLSP treatment (1000 mg each time, 3 times a day) for 8 weeks. The primary outcome included weekly seizure frequency. The secondary outcomes consisted of each seizure episode and quality of life, measured by the Quality of Life in Epilepsy

Inventory-31 (QOLIE-31), and the adverse events (AEs). Results indicated that GLPS reduced the weekly seizure frequency significantly, while each seizure episode and quality of life were not ameliorated. Further studies are still needed to warrant above results [42].

5.6 Antidepressant Effects

Depression is a type of mood disorder characterized by significant and prolonged mood depression, slow thinking, impaired cognitive function, diminished will activity, and somatic symptoms as the main clinical features [29]. Matsuzaki H et al. (2013) reported that a water-soluble extract from a culture medium of *G. lucidum* mycelia (MAK, 0.3 and 1 g/kg) exerts antidepressant-like potential, such as decreased immobility time in the forced swim test (FST) (Fig. 5.7) with no significant change in the locomotor activity in the open-field test (OFT) (Fig. 5.8). The antidepressant effects of MAK are most likely due to the antagonism of 5-HT_{2A} receptors [43]. Similarly, studies showed that extract of *G. lucidum* mycelium exerts antidepressant-like effects in the FST [44] and tail suspension test (TST) [29], which is an antidepressant activity similar to fluoxetine. In a clinical trial, 48 breast cancer patients treated with endocrine therapy received GLSP (1000 mg each time, 3 times a day) for 4 weeks. At the end of treatment, outcome assessment showed that the scores of cancer-related fatigue index and the hospital anxiety and depression scale were significantly reduced by GLSP treatment. This is the first clinical study investigating the effect of *Ganoderma* on mood in patients with cancer and showed a good effect on the depression [45].

To date, there are no *Ganoderma*-related drugs approved by the US Food and Drug Administration (FDA), which might result from its unclear active

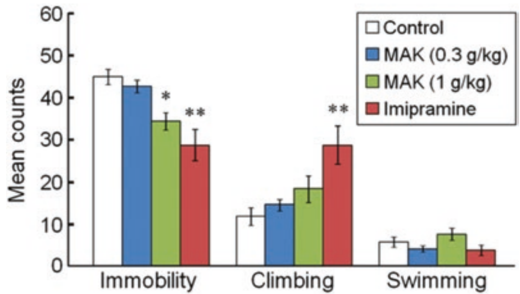

Fig. 5.7 Effects of MAK on the duration of immobility, climbing, and swimming behaviors in the forced swimming test. Behaviors were scored every 5 s for a 5-min observation period. Results are the mean ± S.E.M. The number of rats per group was as follows: control group, n = 8; MAK (0.3 g/kg)-treated group, n = 6; MAK (1 g/kg)-treated group, n = 6; imipramine-treated group, n = 5. $*P < 0.05$, $**P < 0.01$ compared with the control group, one-way analysis of variance followed by Tukey's test. (Reproduced with permission from Ref. [43])

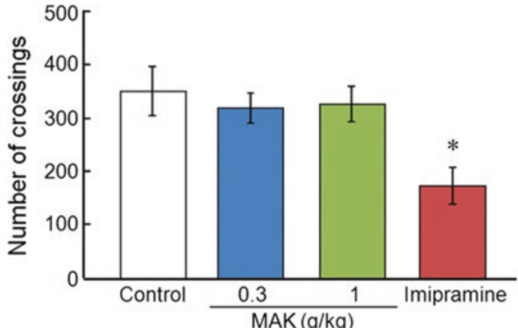

Fig. 5.8 Effects of MAK on locomotor activity in the open-field test. Results are the mean ± S.E.M. The number of crossings recorded for a 30-min period. The number of rats per group was as follows: control group, n = 6; MAK (0.3 g/kg)-treated group, n = 5; MAK (1 g/kg)-treated group, n = 5; imipramine-treated group, n = 5. $*P < 0.05$ compared with the control group, one-way analysis of variance followed by Tukey's test

pharmaceutical ingredients and drug action [28]. More large-scale and well-designed clinical trials should be performed in the future to translate the effects of *Ganoderma* in patients with CNS diseases.

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Chapter 6
Preventive and Therapeutic Effect of *Ganoderma* (Lingzhi) on Brain Injury

Yazhu Quan, Ang Ma, and Baoxue Yang

Abstract Neurological dysfunction and death are common events leading to acute and chronic neurodegenerative diseases. Neurodegenerative disorders such as Alzheimer's and Parkinson's disease account for a significant and increasing proportion of morbidity and mortality in the developed world. *Ganoderma lucidum* (*G. lucidum*, Lingzhi), one of highly nutritious and significantly effective medicinal herbs, has been used for clinical applications for thousands of years. Several researches have shown that it has a wide range of brain damage protection, such as amelioration of Alzheimer's disease, therapeutic effect on epilepsy, and the protective effect on neural cells in stroke injury. This chapter reviews the neuroprotective effects of *G. lucidum* and its extracts on brain injury diseases, including Alzheimer's disease, Parkinson's disease, stroke, epilepsy, and other neurodegenerative diseases, and the potential clinical applications.

Keywords *Ganoderma* · Alzheimer's disease · Parkinson's disease · Stroke · Epilepsy

6.1 The Protective Effect of *Ganoderma lucidum* on Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is insidious onset. It is characterized by memory impairment, aphasia, misuse, loss of recognition, impairment of spatial skills, executive dysfunction, and personality. Current estimates suggest that 44 million people live with dementia worldwide at present [1]. The number of global dementia cases has doubled in the last 20 years

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and is predicted to reach 75.6 million in 2030 and 135 million in 2050 [2]. Currently, many drugs such as cell metabolic activators are used clinically to improve brain cell function in patients with Alzheimer's disease, and there is no specific therapeutic drug and method. However, several references have shown that *Ganoderma lucidum* (*G. lucidum*) has a certain therapeutic effect on Alzheimer's disease.

Researchers have found several potential pathogenic mechanisms of AD including β-amyloid (Aβ) aggregation and deposition, tau hyperphosphorylation, neurofibrillary tangles formation, inflammatory processes, oxidative stress, neurovascular dysfunction, mitochondrial dysfunction, and so on [3]. *G. lucidum* can exert anti-AD effects in many aspects (Fig. 6.1).

Aβ is a normal metabolite of the body and is hydrolyzed by β-amyloid precursor protein (APP). Normally, the production and degradation of Aβ are in equilibrium. When APP hydrolysis is abnormally metabolized, Aβ is more produced and/or degraded less, which leads to a large amount of Aβ deposition. The toxic effects of abnormal deposition of Aβ on neurons have an important influence on the pathogenesis of AD, of which the immunoreactivity of synaptophysin is significantly reduced

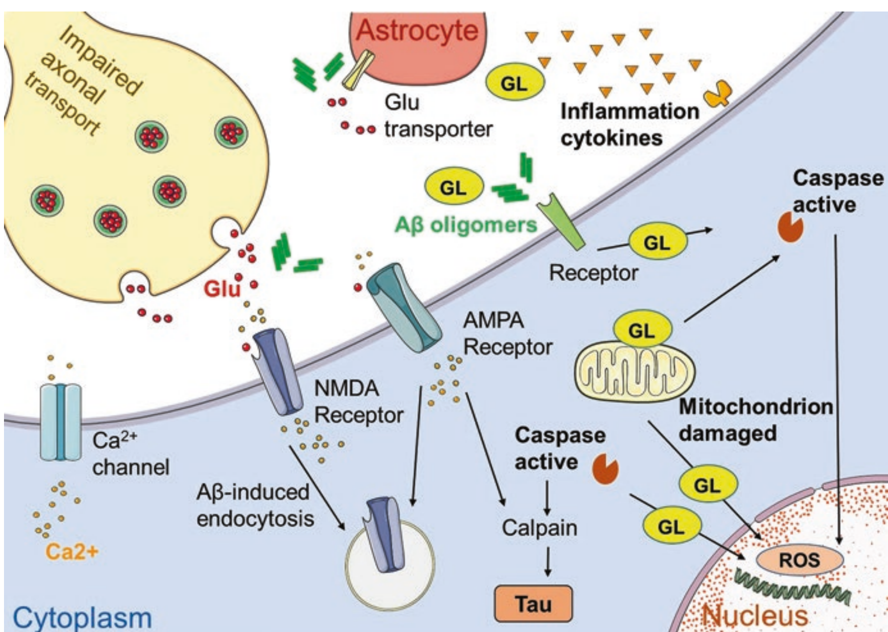

Fig. 6.1 Alzheimer's disease pathogenesis and *G. lucidum* mechanisms. Abnormal accumulation of Aβ and tau as oligomers and neurofibrillary tangles impair neuronal function. Aβ aggregates can induce the proliferation and activation of astrocytes and microglia, leading to the inflammation cytokines and reactive oxygen species (ROS). In addition, Aβ aggregates can activate caspases through several pathways, including cell death receptors, calpain activation, and mitochondrial damage, leading to neuronal apoptosis. *G. lucidum* can protect neurons against Aβ peptide in many ways, including inhibiting the activation and reactivity of astrocytes and reducing inflammation cytokines, improving mitochondrial function and playing an antioxidant activity, maintaining Ca^{2+} homeostasis, counteracting the Aβ-induced apoptosis, etc. GL: *Ganoderma lucidum*

in neuritis of $A\beta_{25-35}$-treated neurons. The distribution of synaptophysin is unevenly distributed. It is aggregated to form a stained plaque along the axon [3]. Synaptophysin, a membrane protein distributed on synaptic vesicles, is closely related to synaptic structure and function, reflecting the distribution and density of synapses, and is one of the important markers of synaptic remodeling and synaptic function [4]. As we know, synapse is the key structure for learning memory function and neural information transmission in the brain. Synaptic loss is closely associated with decreased cognitive function in AD.

Lai CS et al. (2008) found that synaptophysin was evenly distributed in the control group and the *G. lucidum* aqueous extract (GLA)-treated group. Pretreatment with GLA (500 μg/mL) inhibited the downregulation of synaptic vesicular protein immunoreactivity induced by $A\beta_{25-35}$ as well as preserved the neurite network at high dose. GLA significantly attenuated the $A\beta_{25-35}$-induced DEVD-cleavage activity, resulting in reducing $A\beta_{25-35}$ toxicity [3, 5]. Similarly, Yuan DJ et al. (2011) found GLP (50 and 75 mg/kg) significantly increased synaptophysin expression and synaptic number density (Nv) and areal density (Sv) in rat hippocampus compared to the model group [4]. It suggested that *G. lucidum* may exert anti-AD effects by increasing the decreased synapses and synaptophysin in the hippocampus of AD rats. These results suggest a protective effect of *G. lucidum* on nerve cells against Aβ peptide.

High concentration of Aβ is toxic to differentiated mature neurons, and its abnormal aggregation can cause abnormal activation of glial cells, which secrete complement and cytokines, cause inflammatory reactions, damage normal nerve cells, and further cause a large number of abnormal accumulation of Aβ, resulting in a vicious circle. Studies have shown that there is a chronic inflammatory response in the brain tissue of patients with AD, which is characterized by a remarkable increase in the activation of reactive astrocytes. Chronic inflammatory response is an important pathological change in the development and progression of AD [6].

Research found that pretreatment of BV2 cells with GLP followed by LPS stimulation could inhibit LPS-induced pro-inflammatory cytokines IL-1β, IL-6, and inducible nitric oxide synthase (iNOS) expressions and also upregulated the anti-inflammatory cytokine TGF-β expression. The morphological modulations associated with the LPS stimulation were inhibited by GLP pretreatment such as cell area, perimeter, Feret's diameter, and circularity. Pretreatment with GLP for BV2 and primary microglia significantly reduced the phagocytosis events [3, 7].

Guo YJ et al. (2006) observed the changes of GFAP expression in astrocytes and the effects of GLP on AD rats. GFAP is a marker of astrocyte activation. They found that after using GLP (50 mg/kg), the number of GFAP-positive cells was reduced, the cell bodies were fine, the processes were slender, and the pathological changes in the hippocampus were observed to be alleviated [8].

Wang BH et al. (2007) studied the changes of IL-6 expression in the hippocampus of rats with AD and the intervention effect of GLP. They observed a significant increase in IL-6 expression in the model group compared with the control group, and IL-6 expression was significantly reduced after treatment with GLP (50 mg/kg). GLP treatment can inhibit the early inflammatory reaction in AD model rats and

reduce the release of IL-6, which plays an immunomodulatory role and promotes the repair of chronic inflammation in brain tissue [9]. It is suggested that one of the roles of *G. lucidum* may be to inhibit the activation and reactivity of astrocytes in AD rats through immunoregulatory mechanisms and to attenuate the effect of activated astrocytes releasing inflammatory cytokines.

In addition to inflammatory response, Aβ can cause apoptosis of brain neuronal cells by inducing Ca^{2+} influx, destroying Ca^{2+} homeostasis, and causing damage to mitochondria and many other links. As we know, dysfunction of the mitochondrial respiratory chain, being direct intracellular source of reactive oxygen species (ROS), is important in the pathogenesis of number of aging associated human disorders.

G. lucidum may play a protective role in neurodegenerative diseases by improving mitochondrial function in aged rats. Research shows that the extract of *G. lucidum* (50 and 250 mg/kg) significantly enhanced the activities of pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (α-KGDH), succinate dehydrogenase (SDH), and complex I and II in the brain of aged rats when compared to that of the aged control animals.

The level of the lipid peroxidation was significantly lowered in the *G. lucidum*-treated group with respect to that of aged control. The enhanced lipid peroxidation can cause opening of mitochondrial membrane permeability transition (MPT) pores that attributes to cell death. *G. lucidum* extract might effectively improve brain mitochondrial function and protect cells from death in aged rats which can be partially correlated to its antioxidant activity [10].

During brain tissue aging, unsaturated fatty acids on the cell membrane of neurons are oxidized to produce large amounts of reactive oxygen species. Oxidative damage of free radicals plays an important role in the pathogenesis of AD. Because the brain has high oxygen consumption, is rich in various unsaturated fatty acids, and relatively has lack of antioxidant enzymes, it is very vulnerable to free radical attack. It is currently believed that reactive oxygen species damage is one of the important mechanisms of brain damage in AD patients [11]. Studies have found that free radicals are involved in the characteristic pathological damage of AD such as senile plaques, neurofibrillary tangles, and neuronal death and can induce membrane lipid peroxidation and produce malondialdehyde (MDA). The amount of MDA produced is parallel to the lipid peroxidation reaction.

Guo YJ et al. (2006) investigated the effects of *G. lucidum* polysaccharides (GLP) on the morphology and antioxidant capacity of hippocampus in rats with Alzheimer's disease. In their research, the AD model rats were induced by β-amyloid ($Aβ_{25–35}$) injection in bilateral hippocampus. The model rats were intraperitoneally injected with GLP (50 mg/kg) for 7 days. On the eighth day, the learning and memory behavior was measured with the Morris water maze, and the time when the rats found the platform was used as an indicator. The results showed that the GLP group significantly shortened the time to find the platform compared with the model group, indicating that GLP can improve learning and memory impairment in AD model rats. Electron microscopy showed the cytoplasmic edema of the pyramidal cells, the expansion of the endoplasmic reticulum pool, and the proliferation of astrocytes. The lesions in the GLP group were significantly reduced, the ultrastructure was

normal, and the hippocampal astrocytes were significantly reduced compared with the AD group. Further studies found that while improving the learning and memory ability of AD model rats, GLP can significantly increase SOD activity and decrease MDA content in hippocampus of model rats. Superoxide dismutase (SOD) is an important antioxidant enzyme in the body. It has the function of scavenging oxygen free radicals and protecting cells from damage. SOD activity and MDA content in the hippocampus indirectly reflect the oxidative stress in the brain. It is suggested that with the changes of MDA content and SOD activity in the brain, the antioxidant capacity is enhanced, which hinders the degenerative damage caused by free radicals to the hippocampus [12]. Similar experiments done by Yan T et al. (2011) confirmed the above results [13].

G. lucidum triterpenoids (GLT) also have effects on the activities of catalase (CAT), acetylcholinesterase (ACHE), SOD, and MDA in brain tissue of AD model rats. Compared with the control group, the CAT and SOD activities in the brain tissue of the model group decreased, the ACHE activity increased, and the MDA content increased. These changes in the GLT (0.25, 0.5, 1.0 g/kg) group had been alleviated. In addition, GLT can significantly improve the degeneration of neurons in hippocampal CA1 region of brain tissue of model rats. It indicates that GLT has the ability to enhance free radical scavenging and anti-lipid peroxidation and promote the recovery of cholinergic nervous system function in AD model rats [14, 15].

Yang HM et al. (2009) observed the effects of *G. lucidum* polysaccharide peptide (GLPP) on the ultrastructure of hippocampus, SOD activity, MDA content, and spatial learning and memory ability in hippocampus of Alzheimer-like rats. Sixty male Wistar rats were randomly divided into control group, model group, normal saline (NS) group, and GLPP group with 15 rats in each group. Except the control group (normal circadian rhythm), the other groups were continuously exposed to light (illuminance 400 Lux) for 24 h for 30 days. In the meantime, the GLPP group was intragastrically administered with GLPP (250 mg/kg) once a day, and the NS group was given the same volume of normal saline. After 30 days of illumination, the spatial learning and memory ability, hippocampal SOD activity, and MDA content of the rats in each group were tested. The SOD activity in the hippocampus of the model group was significantly decreased, and the MDA content was increased, indicating that continuous light can reduce the free radical scavenging ability of hippocampus in AD rats, and the oxidative stress response is enhanced. The possible reason is that continuous light caused the disorder of sleep rhythm in rats, then the disorder of sleep rhythm induced oxidative stress and the oxidative stress in hippocampus increased, which led to a series of AD-like pathological changes. These results showed that the model group had lower SOD activity and increased MDA content in the hippocampus compared with the control group. The GLPP group had higher SOD activity and decreased MDA content compared with the model group. The SOD activity and MDA content in the NS group were lower, and there was no significant difference compared with the model group. Transmission electron microscopy showed that the mitochondrial axons and synapses in the hippocampus of the control group and GLPP group were basically normal. In the hippocampus of model group and NS group, mitochondria were swollen, mitochondrial

membrane structure was destroyed, and mitochondrial ridges were blurred or even disappeared. These results indicate that GLPP can protect the membrane structure of neurons and the structure and function of mitochondria and synapses by improving the activity of antioxidant enzymes in hippocampus of AD rats, and improve the memory function of AD rats [16].

In addition, *G. lucidum* spore (GLS) could alleviate oxidative stress and mitochondrial dysfunction in rat hippocampus of intracerebroventricular (ICV) injection of streptozotocin (STZ). Three groups of Sprague-Dawley rats were preadministrated with GLS at doses of 2.0, 4.0, and 8.0 g/kg, respectively, for 3 weeks before the ICV STZ injury. Thereafter, the rats were operated with ICV STZ (1.5 mg/kg) bilaterally on days 1 and 3. The results showed that ICV STZ model rats exhibited a significant increase of MDA, a significant decrease of glutathione reductase (GR), and reduced glutathione (GSH), ATP, and cytochrome oxidase (CytOx), accompanied with marked impairments in spatial learning and memory and severe damage of hippocampal neuron. Preadministration with GLS at dose of 8.0 g/kg in ICV STZ rats significantly reversed these abnormalities (Tables 6.1 and 6.2). It indicated that preadministration with GLS might protect hippocampus from oxidative impairment and energy metabolism disturbance of ICV STZ [17].

Moreover, apoptosis is an important pathological mechanism of the pathogenesis of AD. One of the main neuropathological features of AD is the senile plaque formed by abnormal deposition of Aβ, and its toxic effect is characterized by neuronal apoptosis. Apoptosis causes a large number of neurons to be lost, causing dam-

Table 6.1 Effects of GLS on the parameters of oxidative stress in the hippocampus of rats [17]

Group	GSH(mg GSH/g Pr)	GR(U/g Pr)	MDA(nmol/mg Pr)
Normal control	$15.83 \pm 2.43^{\#}$	$30.25 \pm 4.20^{\#}$	$0.32 \pm 0.09^{\#}$
Model group	$8.18 \pm 1.79^{*}$	$18.99 \pm 2.31^{*}$	$0.86 \pm 0.14^{*}$
H-GLS	$15.70 \pm 0.87^{\#}$	$30.05 \pm 1.51^{\#}$	$0.34 \pm 0.04^{\#}$
M-GLS	$12.85 \pm 1.38^{*,\#}$	$23.18 \pm 1.49^{*,\#}$	$0.56 \pm 0.14^{*,\#}$
L-GLS	$8.27 \pm 0.63^{*}$	$18.03 \pm 1.65^{*}$	$0.83 \pm 0.13^{*}$

Values are mean ± S.D., $n = 7$
$*p < 0.05$ vs. normal control group
$\#p < 0.05$ vs. model group

Table 6.2 Effects of GLS on the levels of CytOx and ATP in the hippocampus of rats [17]

Group	CytOx (U/μg min)	ATP (μg/mL)
Normal control	$0.596 \pm 0.050^{\#}$	$2.128 \pm 0.136^{\#}$
Model group	$0.112 \pm 0.007^{*}$	$0.016 \pm 0.006^{*}$
H-GLS	$0.515 \pm 0.026^{*,\#}$	$1.567 \pm 0.042^{*,\#}$
M-GLS	$0.306 \pm 0.017^{*,\#}$	$0.044 \pm 0.005^{*}$
L-GLS	$0.136 \pm 0.005^{*}$	$0.014 \pm 0.005^{*}$

Values are mean ± S.D., $n = 5$
$*p < 0.05$ vs. normal control group
$\#p < 0.05$ vs. model group

age to the brain, which may lead to obstacles to advanced memory function. It has been found that GLP reduce the apoptosis rate of hippocampal cells by using flow cytometry [18].

Immediate early gene (IEG) is involved in the regulation of Aβ-induced apoptosis, and IEG overexpression is closely related to the sensitivity of Aβ-induced apoptosis. C-fos is a representative member of the IEG family. It plays an important role in regulating the signal transduction and regulation of important brain function activities. Normally, c-fos can have basal levels of expression in nerve cells, but the levels are low and difficult to detect. Recent studies have found that c-fos in the cerebral cortex and hippocampus of AD patients are overexpressed, and it is speculated that the c-fos gene which is continuously expressed is associated with apoptosis. Li G et al. (2007) found that GLP (50 mg/kg) can significantly improve the spatial learning and memory ability of AD rats and can significantly reduce the expression of c-fos gene in hippocampus of model rats. It suggested that the apoptosis of the cells induced by Aβ may be reversed with the change of c-fos expression in the brain [19].

Fas is one of the most important proapoptotic genes found so far and may be a signal indicating early and indirect expression of apoptosis. Caspase is a "death protease." Studies have shown that caspases act not only as effectors in the process of neuronal apoptosis in the cortex and hippocampus but also directly on neuronal cells, forming AD-associated proteins. Zhang YP et al. (2008) found that continuous intraperitoneal injection of GLP (50 mg/kg) for 7 days significantly improved the spatial learning and memory ability of the AD model induced by $A\beta_{25-35}$ in the hippocampus and significantly decreased the expression of caspase-3 and FasL in the hippocampus of model rats [20]. GLP may protect hippocampal neurons by counteracting the Aβ-induced apoptosis, thereby playing an anti-AD effect.

In addition, the number of neurons in the hippocampus of the AD model rats decreased, the cell damage was obvious, the cell arrangement was extremely disordered, and the size and shape were irregular. The number of apoptotic-positive cells in hippocampus was significantly increased, and there were a lot of deep-dyed brown-yellow granules in the nucleus. GLT has significantly improved the changes mentioned above [21]. These results indicate that *G. lucidum* has a preventive effect on AD, and its mechanism is related to reducing neuronal necrosis and inhibiting apoptosis.

The key enzyme inhibition is currently the most established strategy for the treatment of AD. A research found that *G. lucidum* extract demonstrated an inhibitory effect against AChE, BChE, and α-glucosidase [22]. Zhang Y et al. (2011) also got similar results. They found that in the brain tissue of the AD model group, Glu content and AchE activity increased and chAT activity, Na^+-K^+-ATPase activity, and brain tissue total antioxidant capacity (T-AOC) activity decreased. GLT significantly improved the changes mentioned above [23–25].

6.2 The Protective Effect of *G. lucidum* on Parkinson's Disease

Parkinson's disease (PD) is a complex neurodegenerative disorder characterized mainly by the selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), which leads to disabling motor abnormalities such as rigidity, resting tremor, and disturbance in balance. PD mainly occurs in middle-aged and elderly people over 65 years old, and the incidence rate increases with age. It is the second most common neurodegenerative disease, affecting more than 6.5 million people worldwide [3]. Despite decades of endeavors to seek therapies that can slow disease progression, PD remains an incurable disorder. Currently available treatments can only manage the symptoms but are unable to influence its initiation and progression. The current most effective symptomatic therapy for PD is levodopa administration, but the efficacy declines as the disease progresses.

It is widely believed that PD is the result of a combination of genes, environment, and aging factors. However, the specific pathogenesis of the disease is still unclear. The studies indicate that mitochondrial dysfunction, oxidative stress, neuroinflammation, and excitotoxic damage are involved in the pathogenesis of PD. Several references have showed that *G. lucidum* has a certain protective effect on PD (Fig. 6.2).

Zhu WW et al. (2005) studied the effects of *G. lucidum* spore oil (*GLSoil*) on the behavior and substantia nigra pathological changes of MPTP-induced PD mouse model and whether *GLSoil* can alleviate the selective damage of MPTP to DA neurons in the substantia nigra. The result shows that the use of *GLSoil* (1.5 g/kg) can significantly reduce the involuntary tremor of the forelimbs and other actions induced by MPTP, extend the pole time, and also increase the amount of DA and metabolites in the striatum. The number of TH-positive cells and the TH protein expression were significantly increased in the *GLSoil* group compared with non-medication group. TH is the first enzyme that catalyzes tyrosine to form dopamine in vivo and is also the rate-limiting enzyme. The results of *GLSoil* on MPTP mouse model in behavioral changes, neurotransmitter content, pathological damage, etc. are highly suggestive that *GLSoil* can effectively alleviate the selective damage to substantia nigra DA neurons with exact neuroprotective effects [3, 26].

Although the exact etiology and pathogenesis of PD still unknown, oxidative stress is confirmed as a pivotal contributing factor to the selective degeneration of dopaminergic neurons in SNpc. It has been shown that excessive production of the reactive oxygen species (ROS) induces mitochondrial dysfunctions, including decrease in mitochondrial membrane potential ($\Delta\Psi m$) and respiratory chain complex I activity, and mitochondrial DNA abnormality. Moreover, selective susceptibility to oxidative stress of the nigrostriatal pathway contributes to DNA damage in dopaminergic neurons of PD patients [27]. Several references have shown that *G. lucidum* can protect mitochondria from damage, reduce ROS production, and exert antioxidant effects on PD.

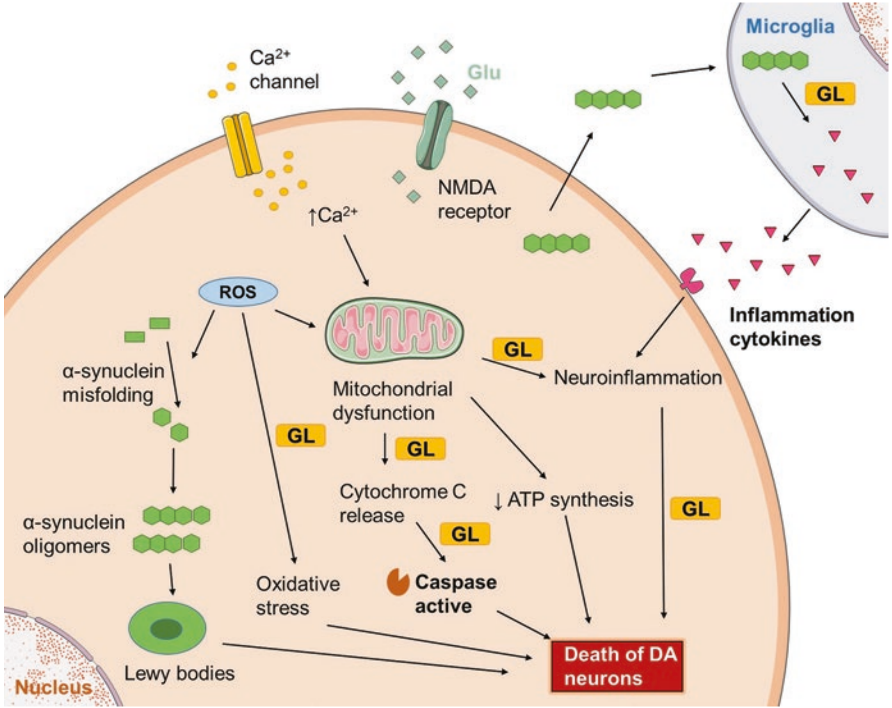

Fig. 6.2 Parkinson's disease pathogenesis and *G. lucidum* mechanisms. The neuropathological mechanisms responsible for pathogenesis of PD include protein misfolding, disrupted protein handling, mitochondrial dysfunction, oxidative stress, impaired calcium handling, and neuroinflammation. Besides, *G. lucidum* can protect dopaminergic neurons in above ways, especially in regulating the neuroimmune response, attenuating oxidative stress injury and reducing the apoptosis of DA. GL: *Ganoderma lucidum*

Guo SS et al. (2016) found that GLP possesses neuroprotective properties against MPP^+ and rotenone neurotoxicity by inhibiting oxidative stress in primary mesencephalic dopaminergic cell cultures due to its antioxidant activity. MPP^+ and rotenone were both powerful inhibitors of complex I activity in isolated brain mitochondria, and they can induce parkinsonism both in vitro and in vivo. The result shows that through primary mesencephalic cultures, GLP could protect dopaminergic neurons against MPP^+ and rotenone at the concentrations of 25, 50, and 100 μg/mL in a dose-dependent manner. The interesting thing is, even if no toxins were added to cell culture system, GLP treatment increased the survival rate of tyrosine hydroxylase (TH) immunoreactive neurons, as well as the length of neurites of dopaminergic neurons. These toxin effects were reversed by GLP in a dose-dependent manner. Furthermore, GLP treatment dramatically reduced the ROS formation, decreased the apoptotic cell number, and increased the mitochondrial membrane potential which was declined by MPP^+ and rotenone [3, 27].

Mitochondrial movement is critical for cellular function and survival, especially in neurons with a polarized structure. Ren ZL et al. (2018) investigated whether *G. lucidum* extract (GLE) has protective effects on mitochondrial dysfunction. Mice were injected with MPTP to induce parkinsonism. Then the mice were administered GLE (400 mg/kg/d, i.g.) for 4 weeks. The result shows that GLE administration significantly improved locomotor performance and increased tyrosine hydroxylase expression in the SNpc of MPTP-treated mice. In in vitro experiment, treatment of neuroblastoma neuro-2a cells with MPP^+ (1 mmol/L) caused mitochondrial membrane potential collapse, radical oxygen species accumulation, and ATP depletion. Application of GLE improved mitochondrial movement dysfunction in cultured primary mesencephalic neurons. It suggested that GLE could attenuate the impairment of mitochondrial transport and movement and protected DA neuron synaptic function [28].

In addition, Bao C et al. (2014) used a brain-injected 6-hydroxydopamine (6-OHDA) to establish a rat model of Parkinson's disease to observe the effects of *G. lucidum* spore powder (25 g/kg) on oxidative stress. The results showed that *G. lucidum* spore powder can significantly reduce the content of MDA and NO in brain tissue of PD model rats, increase glutathione (GSH) content, increase the activity of SOD and GSH-PX, and reduce oxidative stress injury induced by 6-OHDA [29].

Neuroinflammation is involved in the development of PD. Study has showed that activated microglia and astrocytes were present around the injured neurons in substantia nigra pars compacta of PD patients [3].

Zhang R et al. (2011) investigated whether *G. lucidum* extracts (GLE) could protect against dopaminergic neuron degeneration and attenuate the inflammatory responses of microglial cells to exogenous or endogenous stimulus. The result shows that GLE (50~400 μg/mL) significantly prevent the production of microglia-derived pro-inflammatory and cytotoxic factors in a dose-dependent manner and downregulate the TNF-α and IL-1β expressions on mRNA level as well [30]. Zhu WW et al. (2007) found that *G. lucidum* spore oil (1.5 g/kg) can alleviate the expression of TNF-α and IL-1β mRNA in the substantia nigra of AD model which is caused by neurotoxin MPTP [31]. Bao C et al. (2014) found that *G. lucidum* spore oil can inhibit the production of inflammatory cytokines TNF and IL in the mouse model of MPTP, inhibit the activation of iNOS, and effectively regulate the neuro-immune response [29].

LPS is a powerful inflammatory response inducer. The substantia nigra region is the most abundant region of microglia in the brain. Injection of LPS into the substantia nigra of rats activates microglia, releasing inflammation-related factors leading to degeneration of DA neurons in the substantia nigra. Yang HH et al. (2006) explored the effects of *G. lucidum* spore powder on the degeneration of dopaminergic neurons induced by LPS. After stereotactic injection of LPS into the substantia nigra of rats, *G. lucidum* spore powder (400 mg/kg) was administered intragastrically every day for 14 days. The result showed that *G. lucidum* spore powder was effective in improving the rotational behavior of LPS rats. In addition, it can increase the number of TH-positive cells and the expression of TH mRNA in the substantia

nigra [32]. These results suggest that GL effectively protects dopaminergic neurons against inflammatory damage induced by microglial activation.

Mitochondrial dysfunction, oxidative stress, and inflammatory change are considered to lead to cell dysfunction and death by apoptosis or autophagy. Researchers have found that *G. lucidum* has a direct role in protecting neurons from apoptosis. After administering *G. lucidum* spore powder (4 g/kg) daily for 3 days, it has a protective effect on the substantia nigra lesions of experimental PD rats induced by 6-hydroxydopa by reducing the apoptosis of substantia nigra neurons [33]. Further studies indicate that it may be related to the reduction of caspase-3 expression by *G. lucidum* spore powder [34]. Besides, *G. lucidum* spore oil can increase the expression of antiapoptotic gene BcL-2, inhibit the transcription of proapoptotic gene Bax, and effectively reduce the apoptosis of DA [29].

In addition, Ren ZL et al. (2018) found that GLE (400 mg/kg) counteracted the decline in NIX (also called BNIP3L) expression and increase in the LC3-II/LC3-I ratio evoked by MPP^+. Moreover, GLE reactivated MPP^+-inhibited AMPK, mTOR, and ULK1. Similarly, GLE was sufficient to counteract MPP^+-induced inhibition of PINK1 and Parkin expression. GLE suppressed MPP^+-induced cytochrome C release and activation of caspase-3 and caspase-9 [28].

G. lucidum can also affect neurotransmitters in PD rats. Research has showed that *G. lucidum* spore powder can reverse the decrease of substantia nigra and striatum neurotransmitters in PD rats, including dopamine, 3,4-bishydroxyphenylacetic acid, homovanillic acid, serotonin, and norepinephrine [35].

6.3 The Protective Effect of *G. lucidum* on Ischemic Stroke

Ischemic stroke is the most common form of stroke and the third leading cause of death and the primary cause of disability all over the world. Ischemic stroke is caused by a temporary or permanent decrease in blood flow to the blood supply area of an artery due to embolism or bleeding. Despite many therapeutic trials, stroke is still the major cause of death in the world. The present treatment for stroke is to perfuse with recombinant tissue plasminogen activator. However, a narrow therapeutic time window and risk of hemorrhage have hindered the success of this treatment. Therefore, a useful and safe-to-use protective agent is particularly important in treating and alleviating the unfavorable outcomes of stroke.

Ischemic stroke involves different pathophysiological cascades, including oxidative stress, energy depletion, calcium overload, immune mediator production, glutamate release, ion homeostasis, acidosis, activation of apoptotic pathways, neuronal cell excitotoxicity, etc. [36] During ischemia, reduced glucose and oxygen transport to the brain causes cellular bioenergetic failure, which may lead to oxidative stress, inflammation, blood-brain barrier dysfunction, and eventually neuronal cell death, particularly in the hippocampus. Our research and other references show that *G. lucidum* can play a protective role in ischemic stroke.

In the treatment of stroke, restoration of the blood supply can reduce more extensive brain injured. However, reperfusion carries certain risks. Oxidative stress usually results in reperfusion of the infarcted brain tissue causing reperfusion injury. Thus, reducing the release of oxidative stress factors is considered to be an efficacious therapeutic strategy for ischemic stroke. Zhao HB et al. (2005) found that *Ganoderma* total sterol (GS) protects rat cerebral cortical neurons from hypoxia/reoxygenation (H/R) injury. The research shows that GS can significantly reduce the volume of cerebral infarction, cerebral edema, and neurobehavioral scores in rats, reduce the pathological changes of cortical brain tissue in injured rats, inhibit the production of MDA in brain tissue, and increase the activity of SOD. Administration of GS (50 and 100 mg/kg) to rats has protective effects against focal cerebral ischemia-reperfusion injury. It indicates that its mechanism may be related to the role of antioxidant damage [37]. Further research indicates that GS increased neuronal viability after H/R and also increased the activity of Mn-SOD, but not Cu/Zn-SOD, and inhibits IκBα degradation and blocked the translocation of NF-κB. The translocation of NF-κB to the nucleus is preceded by the phosphorylation and proteolytic degradation of IκB. The application of oxidant stressors such as H_2O_2 to cell cultures stimulates IκB degradation and increases NF-κB activity. It is suggested that GS inhibited H/R-induced IκBα degradation and then blocked activation of NF-κB, possibly by changing cell redox balance, and GS may enhance the antioxidant capacity by enhancing Mn-SOD activity, thereby resisting I/R oxidative damage. In addition, they found that GS1 had a stronger protective effect on neurons than GS at the same dose in the neuroprotective effect of GS on H/R [3, 38]. Zhang W et al. (2014) also showed that pretreatment with GLA (40 mg/kg) for 3 days and 7 days reduced the loss of neurons in the rat hippocampus and decreased the contents of MDA in the hippocampus and serum in the model of I/R. It also increased SOD activity in the hippocampus and serum, indicating that pretreatment with GLA has a protective effect on cerebral I/R injury through its antioxidant effects [3, 39].

Mitochondria play an important role in the metabolism of eukaryotic cells, which is the site of synthesis of ATP and participates in the regulation of cytosolic calcium homeostasis. During the ischemic period, due to energy depletion, the energy-dependent ion pump on the cell membrane is damaged, causing intracellular Ca^{2+} overload. Mitochondria can take up Ca^{2+} in the cytosol and act as an intracellular buffer. Oxidative stress and high mitochondrial matrix Ca^{2+} induce mitochondrial permeability transition (MPT). MPT can cause the release of cytochrome C. The release of mitochondrial cytochrome C is an important pre-event of apoptosis [36]. Studies have found that *G. lucidum* not only has antioxidant effects but also protects against cerebral I/R injury in improving mitochondrial function and reducing neuronal apoptosis. Wang XZ et al. (2009) investigated the effects of *G. lucidum* on neuronal apoptosis rate and mitochondrial transmembrane potential after cerebral I/R in rats. The result shows that GL can significantly reduce the apoptosis rate of rat brain tissue and promote mitochondrial transmembrane potential ($\triangle\Psi$m). $\triangle\Psi$m is a sensitive indicator for observing mitochondrial function. It is suggested

that GL can effectively stabilize the mitochondrial membrane structure, prevent the dissipation of △Ψm, regulate cell energy metabolism, reduce peroxide accumulation, and thus inhibit the occurrence of neuronal apoptosis [40].

Zhou ZY et al. (2010) found that oral administration of GLPS (100, 200, and 400 mg/kg) significantly reduced cerebral infarct area, attenuated neurological functional deficits, and reduced neuronal apoptosis in ischemic cortex. In oxygen glucose deprivation OGD model, GLSP (0.1, 1, and 10 μg/mL) effectively reduced neuronal cell death and relieved cell injury. Moreover, GLPS decreased the percentage of apoptotic neurons, relieved neuronal morphological damage, suppressed overexpression of active caspase-3, caspase-8, and caspase-9 and Bax, and inhibited the reduction of Bcl-2 expression. These findings indicate that GLPS protects against cerebral ischemic injury by inhibiting apoptosis by downregulating caspase-3 activation and modulating the Bcl-2/Bax ratio [41].

The research of Xuan M et al. (2015) showed that *G. lucidum* mycelia (MAK) significantly inhibited superoxide production, neuronal cell death, and vacuolization in the ischemic penumbra, with a decrease in the number of TUNEL or cleaved caspase-3-positive cells. Pretreatment with MAK (0.3 or 1 g/kg) for 1 week significantly reduced H/I-induced neurological deficits and infarct volume (Fig. 6.3). In addition, MAK decreases the expression of receptor-interacting protein kinase 3 (RIP3) mRNA and protein expression. For necroptosis pathway, the kinase activity of RIP3 is required. These results indicated that MAK conferred resistance to apoptosis and necrotic cell death and reduces cerebral ischemic injury induced by H/I [3, 42].

In addition, studies have shown that the molecular modification of *G. lucidum* polysaccharide, such as *G. lucidum* polysaccharide sulfate [43] and hydroxymethylated *G. lucidum* polysaccharide [44], also provides significant neuroprotective effect. And the mechanisms may be involved in the regulation of the HSP70/PI3K/Akt signaling pathway and the reduction of inflammatory responses secondary to reperfusion.

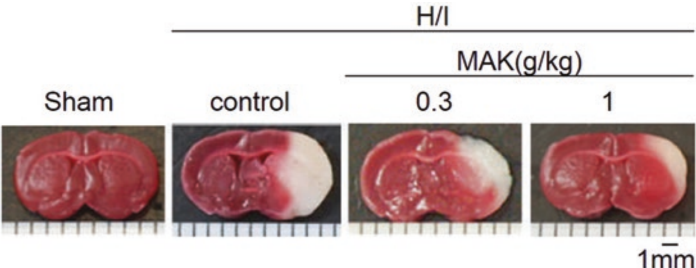

Fig. 6.3 Effects of chronic pretreatment with *G. lucidum* mycelia on ischemic stroke [42]. Representative data of triphenyltetrazolium chloride (TTC) staining from the coronal brain sections at 24 h of reoxygenation after H/I in the mice. Scale bar =1 mm. (cited from Ref 42)

6.4 The Protective Effect of *G. lucidum* on Epilepsy

Epilepsy is a sudden, abnormal discharge of neurons in the brain and a chronic condition that causes transient brain dysfunction. There are around 60 million epileptic patients worldwide, and nearly 80% of them are in developing regions. It is a condition that can seriously affect quality of life, and a global campaign against epilepsy is required. Epilepsy can be managed by drugs, for example, 75% of patients respond well to phenobarbital treatment. After 1 year of treatment with sodium valproate, 42% of patients were seizure-free and 84% of patients had a decrease in seizure frequency of, at least, 50%. However, most of drugs have side effects on brain function, e.g. mood alteration or neurocognitive function reduction in neuron excitation and inhibition of normal activity. Recent efforts have shown that *G. lucidum* and its spore (GLS) have antiepileptic effects in in vivo and in vitro studies.

The pathological mechanism of epilepsy can be divided into neurotransmitter abnormalities, ion channel dysfunction, glial dysfunction, immune and inflammatory factors, and molecular genetic mechanism [3]. *G. lucidum* can affect multiple aspects of the pathogenesis of epilepsy. In this part, we will introduce the antiepileptic effect of bioactive substances of *G. lucidum* including polysaccharide and *G. lucidum* spore powder (Fig. 6.4).

Seizures or initial brain injury can lead to accumulation of free radicals. Glutathione that is a nonenzymatic antioxidant molecule is abundant in tissues. GSH-Px is one of the major components of an enzymatic protection system that exerts anti-lipid peroxidation in cells. Zhu KL et al. (2015) found that GLP (150 mg/kg)

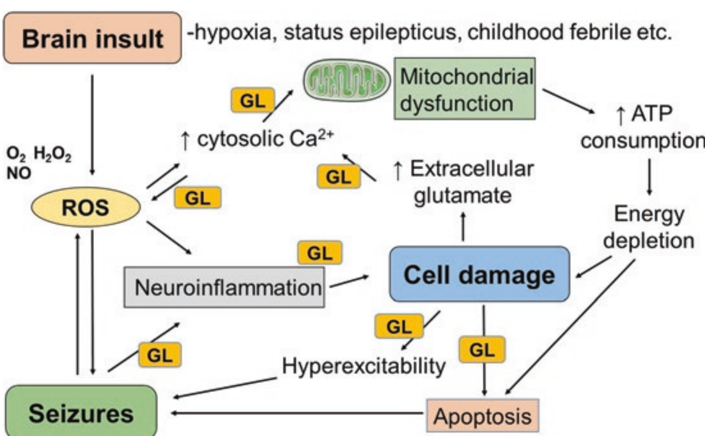

Fig. 6.4 Epilepsy pathogenesis and *G. lucidum* mechanisms. Seizures lead to accumulation of free radicals. Seizures alone, or through cell damage, cause hyperexcitability and increased extracellular glutamate concentration, which results through increased cytosolic Ca^{2+} concentration and consequently overstimulated Ca^{2+} signaling pathways in mitochondrial dysfunction, increased ATP consumption, and energy depletion. In addition, cell death in itself is also considered as a cause of seizures. *G. lucidum* can affect the pathogenesis of epilepsy in above aspects. GL: *Ganoderma lucidum*

can reduce the expression of GSH-Px [45]. Besides, after the intervention of *G. lucidum* spore powder, the activity of superoxide dismutase and total antioxidant capacity in the brain of rats and the expression of cytochrome C in mitochondria were significantly higher than those in the nonintervention group, and the MDA content was significantly lower than that in the nonintervention group [46]. It indicated that *G. lucidum* could reduce excessive free radicals produced in the brain of epileptic rats, relieve free radical damage to mitochondrial membrane, enhance the antioxidant capacity, and maintain the integrity of mitochondria structure and function.

Seizures alone, or through cell damage, cause hyperexcitability and increased extracellular glutamate concentration, which through increased cytosolic Ca^{2+} concentration and consequently overstimulated Ca^{2+} signaling pathways results in mitochondrial dysfunction, increased ATP consumption, and energy depletion.

Ionic glutamate receptors (NMDAR), in which NMDAR1 is the functional unit of the receptor, mediate excitatory amino acids, stimulate Ca^{2+} influx, and are closely related to epilepsy susceptibility. The study of Wang H et al. (2006) have shown that continuous gavage of *G. lucidum* spore powder (150 mg/kg) for 28 days can effectively reduce the content of NMDAR1 in rat cerebral cortex and hippocampus, thereby reducing Ca^{2+} influx, weakening the excitability of neurons, and inhibiting the onset of epilepsy [47].

It has been found that there are many neurotransmitters related to epileptogenesis, such as gamma-aminobutyric acid (GABA), glycine, glutamic acid (Glu), aspartic acid, dopamine, norepinephrine, serotonin, etc. Glutamate is one of the most excitatory neurotransmitters in the brain and plays an important role in maintaining central excitability. Excessive glutamate can cause disturbances in neuronal functional activity, which can induce seizures. The action of glutamate on its excitatory neurons is achieved by binding to glutamate receptors. The clearance of extracellular Glu is primarily dependent on high-affinity excitatory amino acid transporters (EAATs). Zhu et al. (2015) found that *G. lucidum* polysaccharide increased the expression of excitatory glutamate transporter GLAST (EAAT1), GLT1 (EAAT2), and EAAC1 (EAAT3) in the brain of epileptic rats, accelerated the clearance of excitatory amino acid Glu (glutamate), and reduced neuronal excitability and nervous system damage [48].

In addition, Zhu KL et al. (2013) found that *G. lucidum* spore powder (150 mg/kg) effectively reduced the content of somatostatin (SS) in the cortex and hippocampus of rats with epilepsy, increased the content of 5-HT in brain tissue, weakened the excitability of neurons, and reduced the onset of epilepsy [49].

Ion channels are important substances that regulate the excitability of neuronal cells. The ion channels associated with epilepsy mainly include sodium, potassium, and calcium channels. Among them, calcium channels play a critical role in neuronal firing and circuit excitability. The accumulation of elevated levels of calcium, a major signaling molecule in the central nervous system, in neurons is regarded as one of the characteristics of epilepsy. Therefore, maintaining the homeostasis of calcium ions is an important target in the prevention and treatment of epilepsy.

Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) is a protein kinase that is involved in the regulation of neuronal activity in various ways, such as neurotransmitter synthesis and release, and activity-dependent neuronal modifications. CaMK II α plays an important role in Ca^{2+} transfer in many types of neurons. It can bind Ca^{2+} and forms Ca^{2+}/CaM complexes. A reduction in CaMK II α activity can lead to the onset of epilepsy. According to study by Wang SQ et al. (2014), the extracellular fluid that contained no Mg^{2+} significantly increased the amount of calcium in the cytoplasm of neurons. The concentration of calcium reached a peak at about 30 s. However, when GLP was added to Mg^{2+}-containing extracellular fluid, Ca^{2+} fluorescence intensity in hippocampal neurons was significantly reduced. It indicates that GLP can inhibit Ca^{2+} accumulation in neuronal cytoplasm caused by Mg^{2+}-free medium. Moreover, GLP treatment can increase CaMKIIα expression in epileptic hippocampal neurons protecting epileptic neurons. These results demonstrate that GLP could inhibit the Ca^{2+} accumulation in neurons and subsequent stimulation of CaMK II α expression, which indicates a beneficial role for GLP in the prevention or treatment of epilepsy [3, 50].

Long-term recurrent seizures can lead to brain damage, activate pro-inflammatory cytokines and immune responses, increase nerve excitability, destroy the blood-brain barrier, and improve epilepsy susceptibility. Studies have shown that the central nervous system and peripheral immune media are involved in the development of epilepsy. It is currently believed that cytokines such as IL-1, IL-2, IL-6, IL-21β, IL-210, TNF-α, IFN, and serum-soluble IL-2R are associated with epilepsy [51]. Several references have indicated that *G. lucidum* can exert antiepileptic effects through anti-inflammatory effects.

Wang WQ et al. (2007) found that *G. lucidum* spore powder can attenuate the expression of c-fos in the cerebral cortex and hippocampus of experimental rats, and the number of positive cells is reduced. It indicated that *G. lucidum* spore powder can inhibit the expression of c-fos in brain tissue of rats with epilepsy and block the delayed response gene (LRG) to achieve antiepileptic effect [52]. In addition, they also found that G. lucidum spore powder can effectively reduce the levels of cytokines IL-1β [52], IL-2 [53], and IL-6 [54] in brain tissue of rats with epilepsy, correct immune dysfunction, and play an antiepileptic effect.

The concentration of TNF-α in the hippocampus is closely related to the severity of seizure activity, mainly involved in the occurrence of epilepsy by affecting the central nervous system immune inflammatory response. Zhao S et al. (2005) found that *G. lucidum* spore powder (300 mg/kg) can reduce the content of TNF-α in hippocampus of rats with epilepsy, indicating that *G. lucidum* spore powder can play an immunomodulatory role by inhibiting the expression of TNF-α secreting neurons [55].

Similarly, Zhang JG et al. (2012) found that in the model of amygdala-ignited epilepsy, daily *G. lucidum* spore powder (300 mg/kg) was applied to protect brain damage caused by epilepsy. This effect is associated with a reduction in the levels of TNF-ɑ and IL-6 associated with epileptic seizures [56].

Li J et al. (2008) observed the effect of *G. lucidum* spore powder on IL-10 in rats with epilepsy. The results showed that the content of IL-10 in the brain and blood of

the rats in the intervention group of *G. lucidum* spore powder (150 mg/kg) was lower than that in the epileptic model group, indicating that *G. lucidum* spore powder can reduce the expression of IL-10 and inhibit epileptic discharge, thereby exerting immunomodulatory effects [57].

Neurotrophic factors can promote protein synthesis and neuronal development, maintain functional neuronal integrity, and have a protective effect on epilepsy. NGF is one of the neurotrophic factors that can promote the normal survival, growth, and differentiation of neurons, maintain the normal function of the nervous system, and accelerate the repair of nervous system injury. Zhao CX et al. (2013) found that the number of NGF cells in the hippocampus of rats with epilepsy treated with *G. lucidum* spore powder (50 mg/kg) was significantly reduced, indicating that *G. lucidum* spore powder can protect nerve cells by changing the amount of NGF in rat brain [58].

Brain-derived neurotrophic factor (BDNF) is the neurotrophin that occurs earlier in seizures, has the largest change in content, and has the longest duration. BDNF can increase the activity of various neurons, reduce the incidence of apoptosis, and promote neuronal regeneration and differentiation and maturation. It is closely related to the occurrence and seizure of epilepsy. Wang H et al. (2006) found that *G. lucidum* spore powder (150 mg/kg) can promote the expression of BDNF in the brain of epileptic rats, protect neurons and promote the recovery of damaged neurons, and exert antiepileptic effect [59].

Neurotrophin-4 (NT-4), which is a member of the neurotrophin (NT) family, is expressed widely in the brain. Long-term use of NT-4 has been shown to inhibit hippocampal neuronal death by up to 50%. N-cadherin is a member of the cadherin family, which plays an important role in targeting the growth of axons and the construction of correct synaptic connections. Abnormal expression of N-cadherin leads to neurodevelopmental disorders (such as schizophrenia and epilepsy) and behavioral defects in animal models [3]. Wang SQ et al. (2013) found that after GLS treatment, the number of normal hippocampal neurons increased and the morphology was preserved well. In addition, NT-4 expression was significantly increased while N-cadherin expression was decreased in the GLS-treated group as compared to the model group, suggesting that GLS protected hippocampal neurons by promoting the expression of NT-4 and inhibiting N-cadherin expression [3, 60].

Epilepsy is associated with cell damage and necrotic or delayed apoptotic cell death. Cell death in itself is again considered as a cause of seizures. Caspase-3 is one of the most important injury factors in the process of epileptic brain injury. It promotes apoptosis and normal cell death and is a key protease for mammalian apoptosis. Li J et al. (2012) found that *G. lucidum* spore powder (150 mg/kg) may reduce the expression of caspase-3 in the brain of epileptic rats by regulating the level of apoptosis-related proteins, thereby reducing nerve cell damage and protecting neurons [61].

In rats with seizures, the expression of NF-κB can produce a large amount of reactive oxygen species and free radicals in the rat brain, which can activate NF-κB again, accelerate the expression of apoptosis-related genes, and induce neuronal apoptosis. Zhao S et al. (2007) used PTZ to replicate the rat model of chronic

epilepsy, perfused the rats with *G. lucidum* spore powder (300 mg/kg) daily, and found that the incubation period of seizures could be prolonged from the 17th day. Its mechanism involves inhibiting the expression of NF-κB in rat brain, which is involved in promoting neuronal cell survival and inhibiting apoptosis [62].

Apoptosis is a process of cell autonomic death regulated by multiple genes, and the bcl-2 gene family plays an important role in the regulation of apoptosis. Bcl-2 and bax are closely related to apoptosis. The bcl-2 protein product inhibits cell apoptosis, while the bax protein product promotes apoptosis. Studies have shown that the active ingredient of *G. lucidum* spore powder (150 mg/kg) can upregulate the expression of bcl-2 and downregulate the expression of bax in epilepsy model rat brain, thereby exerting antiapoptotic neuroprotective effect [63, 64].

6.5 The Protective Effect of *G. lucidum* on Other Neurological Diseases

Wang JL et al. (2006) studied the effects of *G. lucidum* on Huntington disease. They established a rat model of HD by 3-nitropropionic acid to detect the effect of *G. lucidum* spore powder on HD rats. The results showed that *G. lucidum* spore powder (4 g/kg) can reduce the degree of striatum lesions in HD model rats, reduce the apoptotic rate, maintain the expression of striatum BDNF-positive neurons, improve the behavior of rats, and improve memory [65].

In addition, *G. lucidum* can also prevent motor neuron diseases. Zhang W et al. (2005) found that *G. lucidum* spores can promote the survival of injured spinal motor neurons and spinal dorsal nucleus neurons in rats by establishing an animal model of spinal cord motor neurons. Subsequent studies have also found that *G. lucidum* spores also have a significant effect on axonal regeneration of injured spinal motor neurons [66, 67].

At present, a large number of experimental studies have shown that the active ingredients of *G. lucidum* and its extracts have obvious neuroprotective effects. It has good clinical application prospects for cerebrovascular disease, Alzheimer's disease, Parkinson's disease, and epilepsy. In the future, *G. lucidum* can be further studied for other diseases of the nervous system such as myasthenia gravis and peripheral neuropathy.

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Chapter 7
Protective Effect of *Ganoderma* (Lingzhi) on Cardiovascular System

Jia Meng and Baoxue Yang

Abstract Cardiovascular diseases (CVDs) are disorders of the heart and blood vessels and include coronary heart diseases, cerebrovascular diseases, rheumatic heart diseases, and other conditions. CVDs are one of the most major causes of morbidity and mortality around the world, taking the lives of 17.9 million people every year. Several investigations have shown the influence of *Ganoderma lucidum* (*G. lucidum*, Lingzhi) on some metabolic markers, such as low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), blood pressure, and oxidative damage. *G. lucidum* potentially reduces the risk of suffering cardiovascular diseases. Some studies found that *G. lucidum* prevented from heart damage in a variety of disease models, such as streptozotocin (STZ)-induced diabetic, high-fat-diet-induced diabetic, isoprenaline (ISO)-induced myocardial hypertrophy, acute ethanol-induced heart toxicity, and transverse aortic constriction (TAC) models. This chapter summarizes putative preventive and therapeutic effects of *G. lucidum* on cardiovascular diseases and the potential clinical use of *G. lucidum* involved in these effects.

Keywords *Ganoderma* · Atherosclerosis · Diabetic cardiomyopathy · Hypertension · Antioxidant activity

7.1 Effect of *G. lucidum* on Hypertension

Hypertension is the leading risk factor for cardiovascular disease (CVD) and mortality worldwide with an estimated number of 1.56 billion affected individuals by 2025. Medical guidelines define hypertension as a blood pressure higher than 130 over 80 mmHg according to guidelines issued by the American Heart Association

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(AHA) in November 2017. Increased systemic vascular resistance, increased vascular stiffness, and increased vascular responsiveness to stimuli are key factors to the pathophysiology of hypertension [1, 2]. Diuretics and β-adrenoceptor antagonists are the primary recommended therapy. Other currently used drugs include α1-adrenoceptor antagonists, calcium-channel antagonists (CCAs), angiotensin-converting enzyme (ACE) inhibitors, angiotensin II (Ang II) receptor antagonists, and combination therapy [3]. However, improvements in the therapy of isolated systolic hypertension are desirable. Future prospects in the treatment of hypertension include the drugs with less side effects and more adaptiveness for long-term use.

Yearul et al. [2] reported that the powder of the fruiting bodies of *G. lucidum* administrated by p.o. significantly lowered the systolic blood pressure of the spontaneously hypertensive rats (SHR) after the 4-week feeding period [4]. However, the exact mechanism for the effect was unknown.

In an effort to understand the mechanism of the antihypertensive effects actions of *G. lucidum*, Seung Y et al. [5] used the water extract of the mycelia from *G. lucidum* to treat anesthetized rabbits and rats. The extract decreased systolic and diastolic blood pressure, inhibited the activity of renal efferent sympathetic nerve, but did not decrease heart rate in these animals although there was clear effect of lowering blood pressure in dose-dependent manner. Experimental results suggest that the hypotension induced by *G. lucidum* was secondary to the effect on the central nerve system, in which the extract suppressed the sympathetic outflow. The effect of *G. lucidum* on blood pressure might be due to its inhibiting sympathetic nerve activity [5].

Xing et al. [6] investigated the effect of a commercial *G. lucidum* products named Tiaozhiling (TZL), which was composed of *G. lucidum* bacteria on SHR [6]. In the experimental group, 16 rats were given TZL 300 mg/kg daily by gavage. In the control group, 15 animals were given the same amount of 1% normal saline. The experiment lasted for 6 weeks. At the end of the experiment, the weight of heart, brain, and kidney was observed, and the incidence of stroke (hemorrhage and embolism) was determined by pathological examination. The results showed that TZL protected the integrity of endotheliocyte of artery, prevented thrombosis and hemorrhage, decreased stroke rate, and reduced hypertrophy of heart, brain, and kidney caused by hypertension. The results of transmission electron microscope showed that TZL maintained the normal arrangement of cardiomyocytes and myofibrils. This may be the main mechanism that TXL alleviated hypertensive damage.

Transverse aortic constriction (TAC) in the mouse is a commonly used experimental model for pressure overload-induced cardiac hypertrophy and heart failure. TAC initially leads to compensated hypertrophy of the heart, associated with a temporary enhancement of cardiac contractility [7]. Over time, the response to the chronic hemodynamic overload becomes maladaptive, resulting in cardiac dilatation and heart failure (Fig. 7.1a). The murine TAC model (Fig. 7.1b) was first established by Rockman et al. [8] and has been extensively used as a valuable tool to mimic human cardiovascular diseases and investigate fundamental mechanisms involved in the cardiac hypertrophic response and heart failure development.

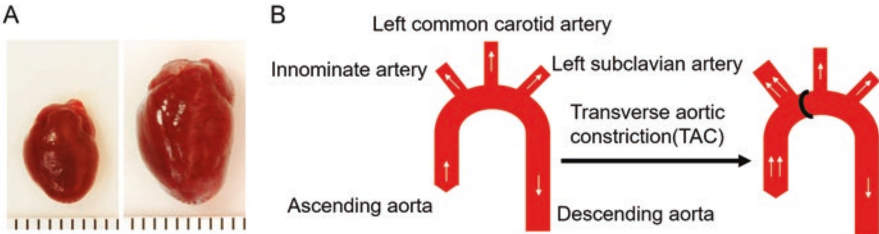

Fig. 7.1 Transverse aortic constriction (TAC) mouse model. (**a**) Representative whole heart images of sham-operated mouse heart and TAC-operated mouse heart. Each line = 1 mm. (**b**) Anatomic diagram and TAC ligation loci of aorta

Yang's group studied the effect of *Ganoderma* spore oil on pressure overload-induced cardiomyopathy in TAC mice model [9]. Their results demonstrated a potential cardioprotective role of *Ganoderma* spore oil. TAC mice were orally administered *Ganoderma* spore oil every other day for a period of 14 days. Mice were anesthetized with 2% isoflurane inhalation for transthoracic echocardiography and invasive hemodynamic assessment. Left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), left ventricular end-diastolic diameter (LVED), and cardiac output were measured by transthoracic echocardiography. The ejection fraction of healthy sham mice was 65.23%, while TAC mice were found to exist an ejection fraction of 43.26%. TAC mice treated with the antihypertensive medication approximated the normal range, while delivery of *Ganoderma* spore oil recovered the stroke volume to normal range. Consistent with these results, the fractional shortening of TAC mice was within the abnormal range of 20–25% and was brought to the normal range of 25–45% by the treatment with *Ganoderma* spore oil. The TAC mice also showed increased LVED, while mice treated with *Ganoderma* spore oil exhibited meliorative levels of left ventricular hypertrophy. *Ganoderma* spore oil delivery also recovered the physiologic cardiac output levels at 24.1 ml/min, which led to improved vascular perfusion in TAC mice.

These results suggest that *Ganoderma* spore oil increases the heart function to resist the damage of the body. As for the detailed mechanism, analysis of total RNA expression using cardiac tissue samples from these mice confirmed reduced expression of genes associated with heart failure, including a novel circular RNA circ-Foxo3. To verify the role of Foxo3 involved in this process, they cultured mouse cardiac fibroblasts and stimulated the cells using hydrogen peroxide, following treatment with *Ganoderma* spore oil. Relative to control groups, treatment with *Ganoderma* spore oil decreased circ-Foxo3 levels in a concentration- and time-dependent manner. The results above provided evidence for *G. lucidum* reducing hypertension in laboratory animals as a potential cardioprotective agent needing further preclinical exploration.

Furthermore, in a Chinese clinical trial, Zhang et al. [10] provided evidence that *G. lucidum* may attenuate refractory hypertension as an adjuvant therapy [10].

There were 40 patients with primary hypertension at stage II participating in the study. They all had received long-term treatment with captopril (25 mg, tid) or nimodipine (20 mg, tid), but their blood pressure still remained above 120/90 mmHg. In this term, they were diagnosed as refractory hypertension. Patients were randomized to either treatment group (27 subjects) or placebo group (13 subjects). The treatment group took *G. lucidum* tablets provided by Wakan Shoyaku Institute of Health Medicine in Japan. Each tablet contained 55 mg *G. lucidum* extract equivalent to 1.375 g of *G. lucidum* bacteria, the main component of which was *Ganoderma* polysaccharide. The treatment group took two tablets each time and three times a day for a total dose of 330 mg/day for 3 months. All the subjects received conventional hypotensor at the same time *G. lucidum* tablets were taken as an adjunctive treatment. The results showed that *G. lucidum* assisted in lower blood pressure, including the arteries, arterioles, and capillaries. And the blood viscosity got improved significantly as well especially the whole blood viscosity, plasma viscosity, and hematocrit at low shear rate. Since plasma $NO2^-$ was commonly used as an indicator of plasma NO changes and in this investigation, they found that nitrite levels in hypertensive patients receiving the auxiliary treatment of *G. lucidum* plasma were significantly higher than those in the control group, and there was a significant positive correlation among NO, capillary density, and capillary diameter. Therefore, in the treatment of refractory hypertension, when conventional antihypertensive treatment is ineffective, the addition of *G. lucidum* cannot only reduce blood pressure but also improve microcirculation, the mechanism of which is related to the increase of NO concentration in plasma.

7.2 Effect of *G. lucidum* on Atherosclerosis

Atherosclerosis is a chronic arterial disease and a major cause of vascular death. Fatty streaks in arterial walls gradually develop into atheroma and characteristic plaques. The acute rupture of these atheromatous plaques causes local thrombosis, leading to partial or total occlusion of the affected artery [11]. The clinical consequences of these plaques depend on the site, degree, and speed of vessel occlusion. The major clinical manifestations of atherosclerosis include ischemic heart disease (IHD), ischemic stroke, and peripheral arterial disease (PAD) [12]. Currently, it is widely believed that atherosclerosis is kind of chronic inflammation resulting from interaction between modified lipoproteins, monocyte-derived macrophages, T cells, and the normal cellular elements of the arterial wall. The inflammatory process ultimately leads to the development of complex lesions and formation of plaques, which protrude into the arterial lumen. Plaque rupture and thrombosis usually result in the acute clinical complications of myocardial infarction and stroke. Among the many genetic and environmental risk factors that have been identified, elevated level of serum LDL cholesterol is a unique risk factor sufficient to drive the development of atherosclerosis, even in the absence of other known risk factors [13].

There is now a consensus that atherosclerosis represents a state of heightened oxidative stress characterized by lipid and protein oxidation in the vascular wall. In the oxidative modification hypothesis, LDL oxidation is considered as an early event in atherosclerosis and is involved in the whole course of the disease.

An emerging consensus also emphasizes the important roles of oxidative events played in vascular disease in addition to LDL oxidation, which include the production of reactive oxygen and nitrogen species by vascular cells as well as oxidative modifications contributing to important clinical manifestations of coronary artery diseases such as endothelial dysfunction and plaque disruption. Usually, inflammation needs to be considered as a primary process of atherosclerosis accompanied by oxidative stress event [14, 15].

Berger et al. [16] found that *G. lucidum* lowered cholesterol in vitro and in vivo in two animal models with some differences between hamsters and minipigs. At the very beginning, they found organic fractions containing *G. lucidum*-oxygenated lanosterol derivatives inhibited cholesterol synthesis in T9A4 hepatocytes. Then in in vivo experiments, they found 5% *G. lucidum* did not affect LDL in hamsters but decreased 9.8% TC and 11.2% HDL. Both concentrations of *G. lucidum* (2.5% and 5%) reduced hepatic microsomal ex vivo 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase activity. In minipigs, 2.5% *G. lucidum* decreased TC, LDL, and HDL cholesterol by 20%, 27%, and 18%, respectively; meanwhile, *G. lucidum* increased fecal cholestanol and coprostanol and decreased cholate. There is great chance that oxygenated lanosterol derivatives in *G. lucidum* lower cholesterol by decreasing cholesterol synthesis. Fibrous components and glucans in *G. lucidum* were likely responsible for the observed alterations in fecal neutral sterols and bile acids in both animal species, ultimately affecting cholesterol absorption and bile acid recycling and contributing to cholesterol lowering [16].

An exploratory trial also confirmed the cholesterol-lowering effect of *G. lucidum*. Xing et al. [6] verified the effect of Tiaozhiling (TZL), a commercial *G. lucidum* products composed of *G. lucidum* bacteria, on 160 patients with hyperlipidemia [6]. 50 mL of TZL was taken orally twice a day for 2 months, and they found that the total effective rate of lowering TC, LDL-C, and TG was 71.4%, 71.4%, and 48.4%, respectively. The total effective rate of increasing HDL-C was 87.5%. What's more, TZL lowered blood pressure, blood glucose, and elevated ALT in some of the patients. There was no side effect. So *G. lucidum* may be particularly suitable for those who suffered from coronary heart disease, hypertension, and diabetes complicated by hyperlipidemia.

In another research, 26 patients received 1.44 g of *G. lucidum* daily or matching placebo for 12 weeks in a randomized, double-blind, crossover study with placebo-controlled run-in and crossover periods. Body weight, blood pressure, metabolic parameters, urine catecholamines, cortisol, antioxidant status, and lymphocyte subsets were measured after each period. There showed no significant difference in body mass index (BMI) or blood pressure when treated with *G. lucidum* or placebo. However, *G. lucidum* treatment lowers plasma insulin and homeostasis model assessment-insulin resistance. Total triglyceride decreased and HDL-cholesterol increased in *G. lucidum* group but not in placebo group in the first treatment period.

Urine catecholamines and cortisol, plasma antioxidant status, and blood lymphocyte subsets showed no significant difference across treatments. Results indicate that *G. lucidum* might have mild lower TC and HDL effects and potentially improve atherosclerosis [17].

Rubel R et al. [18] investigated the effect of *G. lucidum* supplementation on iNOS-mediated NO production in macrophages and found that *G. lucidum* mycelium inhibits inducible nitric oxide synthase expression in macrophages. In this study, the research object was *G. lucidum* mycelium. The *G. lucidum* supplementation caused significant reduction of $CD3^+$ (12.83%) and $CD8^+$ (14.30%) spleen lymphocytes population. $CD4^+$ spleen cells were also reduced slightly but without statistical significance. There was a significant increase of IFN-γ concentration by 15.36% in the plasma from mice fed with *G. lucidum*. IL-12p70 was also increased but without statistical significance. In vitro experiments, human monocytic cell (THP-1)-derived macrophages were incubated with lipopolysaccharide (LPS) for 24 h. In LPS-treated macrophages, NO production was significantly increased resulting from elevated iNOS mRNA expression and iNOS activity. Meanwhile, the superoxide anion level was also elevated. Treatment with *G. lucidum* extract (100 μg/ml) completely abolished LPS-induced iNOS mRNA expression and NO production in macrophages. These results suggest that *G. lucidum* may exert a therapeutic effect against atherosclerosis via ameliorating iNOS-mediated NO overproduction in macrophages [18].

There also is a study suggesting that water-soluble extract of *G. lucidum* inhibits atherosclerosis through the inhibition of platelet aggregation. Fifteen healthy volunteers and 33 patients with atherosclerotic diseases received water-soluble extract of *G. lucidum* 1 g three times a day for 2 weeks, and the platelet aggregation induced by ADP was tested. The results showed that the first and the second phases of aggregation of platelets of the healthy volunteers were inhibited obviously in the case of water-soluble extract of *G. lucidum* added to the platelets at different concentrations in vitro, and the speed of platelet aggregation was slowed down. The inhibitory effect was dose-dependent. After the patients had taken *G. lucidum* 1 g three times a day for 2 weeks, platelet aggregation induced by ADP in final concentration of 2 μmol/L and 3 μmol/L was significantly inhibited, and the maximum platelet aggregation inhibition rates were 31.49% and 17.7%, respectively. Length and weights (wet and dry) of the extracorporeal thrombi were also reduced, respectively, after oral administration of *G. lucidum*. However, the mechanism remained to be further investigated [19]. Kwok et al. [20] set a randomized double-blind study. There were healthy volunteers who received oral *G. lucidum* capsules 1.5 g or placebo daily for 4 weeks. There was no significant difference between groups. Although *G. lucidum* inhibited the aggregation of platelet, the use of *G. lucidum* preoperatively was unlikely to increase the risk of surgical bleeding in healthy patients [20].

In addition to plasma LDL level, the inflammatory response of vascular smooth muscle is also an important factor in the development of atherosclerosis. Cytokines of the interleukin-1 (IL-1) family play a pivotal role in regulating the immune-inflammatory responses. Chan-Jung Liang et al. [21] found that G. lucidum polysaccharides (GLPP) reduced lipopolysaccharide (LPS)-induced interleukin-1β

expression in cultured smooth muscle cells and in thoracic aortas in mice. In human arterial smooth muscle cells (HASMCs), pretreatment with 10 μg/mL GLPP decreased LPS-induced extracellular-regulated protein kinases (ERK), p38, c-Jun N-terminal kinase (JNK), and protein kinase B (Akt) phosphorylation. In addition, the phosphorylation and nuclear translocation of nuclear factor (NF-)κB p65 elevated by LPS were also decreased. Furthermore, they found that IL-1β was strongly expressed in thoracic aortas in LPS-treated mice. Oral administration of GLPP decreased the expression of IL-1β in thoracic aortas. The level of IL-1β expression in GLPP-treated or in LPS/GLPP-treated group was lowered, similar to that of the saline-treated group in toll-like receptor 4-deficient ($TLR4^{-/-}$) mice. They demonstrated that GLPP treatment effectively blocked IL-1β expression *in vitro* and *in vivo*. The effect of GLPP-lowering IL-1β expression in LPS-treated HASMCs might be mediated through inhibition of ERK phosphorylation, NF-κB activation, and TLR4 receptor pathway. These findings suggest that GLPP has the anti-inflammatory property and could be used in the prevention of vascular diseases and inflammatory responses [21].

The abnormal accumulation of smooth muscle cells (SMCs) in the arterial intima is an important event in the development of atherosclerosis. The identification of the key mechanisms involved in SMC function will help in understanding cellular responses to vascular injury. The degrees of proliferation of SMCs that have migrated from the media to form the neointima are considered as the main character of pathological conditions. In addition, elevated platelet-derived growth factor (PDGF) level is one of the most important risk factors for atherosclerosis and cardiovascular morbidity. Chen et al. [22] found that GLPP prevent PDGF-stimulated smooth muscle cell proliferation in vitro and neointimal hyperplasia in the endothelial-denuded artery in vivo. In in vivo studies, the femoral artery of C57BL/6 mice was endothelial-denuded and the mice were fed a diet daily containing 100 mg/kg GLPP. On day 14, both cell proliferation in the neointima and the neointima/media area ratio were significantly reduced. HASMCs were incubated in serum-free medium with or without 10 μg/ml of GLPP for 18 h, then with different mitogen-activated protein kinase (MAPK) inhibitors for 6 h, and were stimulated with 30 ng/ml of PDGF for 24 h. The effect of GLPP and MAPK inhibitors on the proliferation of PDGF-treated HASMCs was measured by BrdU incorporation staining. So GLPP treatment attenuated the proliferation of PDGF-stimulated HASMCs in vitro and cell proliferation in the endothelial-denuded artery of mice in vivo. The effects of GLPP on PDGF-stimulated HASMCs included arresting of cell cycle progression; downregulation of expression of cyclin-dependent kinases D1 (cyclin D1), cyclin-dependent kinases E2 (cyclin E), cyclin-dependent kinases 2 (CDK2), and cyclin-dependent kinases 4 (CDK4); and upregulation of expression of the CDK inhibitor cyclin-dependent kinase inhibitor 1B (p27Kip1). The antiproliferative effect of GLPP might therefore be mediated by regulation of JNK phosphorylation (Fig. 7.2) [22].

Hyperhomocysteinemia (HHcy) is an independent risk factor for cardiovascular disease. Homocysteinemia (Hcy) inhibits the activity of glutathione oxidase and promotes the formation of peroxides, causing production of a large number of

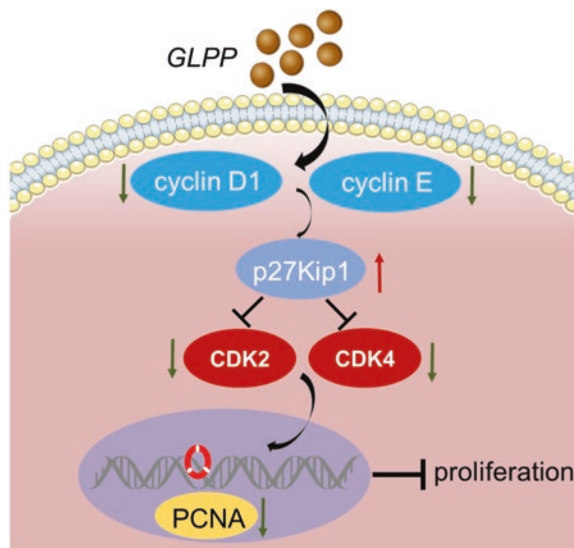

Fig. 7.2 Schematic mechanism of GLPP prevents platelet-derived growth factor-stimulated smooth muscle cell proliferation. For the detail, see text. Arrows or lines represent the activation or inhibition relationship between two molecules. GLPP treatment of PDGF-stimulated HASMCs induced arrest of cell cycle progression; downregulation of expression of cyclin D1, cyclin E, CDK2, and CDK4; and upregulation of expression p27Kip1

hydroxyl groups, superoxide anions, hydrogen peroxide, and other oxygen active substances, which would damage endothelial cells and reduce the elasticity of blood vessels. In addition, HHcy also induces the expression of cell adhesion molecules and chemokines, which promote the early event of atherosclerosis formation. In the early stage of atherosclerosis formation, monocytes adhere to vascular endothelial cells and migrate into the endothelial to absorb lipids and convert into foam cells. Hence, endothelial dysfunction is considered as one of the most important pathological status in HHcy-related cardiovascular diseases. Yang's group [23] found that *Ganoderma* triterpenes could protect endothelial injury induced by HHcy via reducing oxidative stress and downregulating canonical TGF-β/Smad and non-Smad-dependent signaling pathway to regulate Smad and Snail-mediated gene transcription [23].

After stimulating with 800 mM Hcy over 48 h, BAECs were found lost their cobblestone appearance and presented elongated and spindle-like morphology compared with untreated cells by observing cell morphology. Western blot results showed that the expression of VE-cadherin reduced in a dose-dependent manner in Hcy-treated BAECs compared with control group. The EndMT process triggered by Hcy is involved with the activation of TGF-β/Smad, PI3K/AKT, and MAPK signaling. The accumulation of ROS induced by Hcy could be eliminated by pretreatment of GT at 25 mg/ml. GT could improve SOD activity at a lower concentration (6.25 mg/ml) and reversed NADPH oxidase 4 (Nox4) expression at a higher concentration (25 mg/ml). These data hinted that GT attenuated oxidative stress induced by Hcy. Meanwhile, GT treatment rescued the abnormal morphologic changes of BAECs caused by Hcy and the formation of spindle-like shape induced by TGF-β1, suggesting that GT have an essential role in preventing EndMT. The pretreatment of

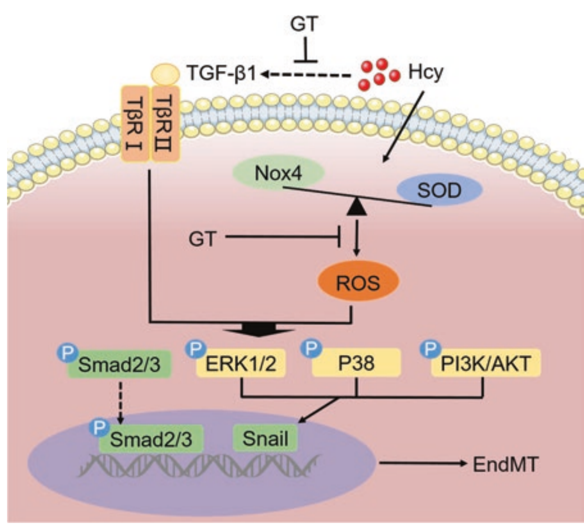

Fig. 7.3 Schematic mechanism of endothelial cells dysfunction exposure to HHcy. For the detail, see text. Arrows or lines represent the activation or inhibition relationship between two molecules. GT attenuated Hcy-induced oxidative stress, and GT treatment decreased the protein levels of phosphorylated Smad2/3, ERK1/2, AKT, GSK-3β, and Snail induced by Hcy

GT could lessen the expression of vimentin, PAI-1, and TGF-β1 without remarkable dose-dependent effect. Compared with Hcy group, the protein levels of phosphorylated Smad2/3, ERK1/2, AKT, GSK-3β, and Snail were significantly decreased by preincubation with 25 mg/ml GT. Taken together, canonical TGF-β/Smad and non-Smad-dependent signalings are corporately involved in the protective effect of GT in EndMT [23]. The detailed mechanisms proposed in this study were illustrated in Fig. 7.3.

Many studies have confirmed that *G. lucidum* has multiple inhibitory effects on atherosclerosis, and *G. lucidum* is likely to become a clinical drug for the treatment of atherosclerosis in future.

7.3 Effects of *G. lucidum* on Diabetic Cardiomyopathy

The incidence and prevalence of diabetes mellitus are increasing rapidly in modern society. The majority of patients with diabetes succumb ultimately to heart disease, many of which accompanied with atherosclerotic disease and hypertension. This disorder is a complex diabetes-associated process characterized by significant changes in the physiology, structure, and mechanical function of the heart [24]. In the early stages, these pathophysiologic changes appear reversible with tight metabolic control, but as the disease processes, the changes are irreversible and contribute to an excess risk of heart failure among diabetic patients independently of common comorbidities. Therapeutic agents specifically targeting these pathophysiologic changes are in the early stages of development. Although glycemic control and early administration of neurohormonal antagonists are still the main treatment at present, newer treatment targets are still underexplored (Fig. 7.4) [25].

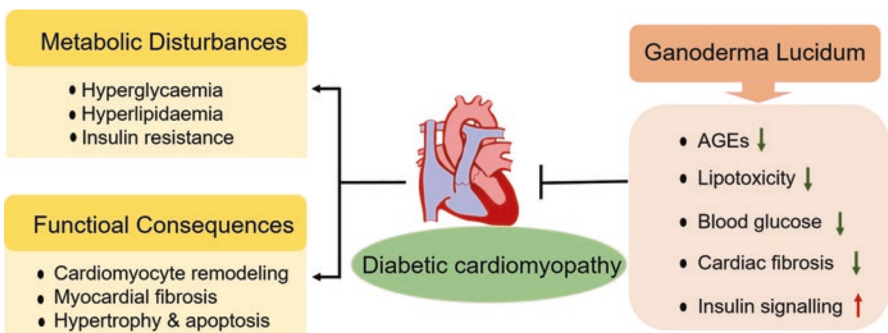

Fig. 7.4 Schematic depiction of the multiple potential mechanisms implicated in the pathophysiology of diabetic cardiomyopathy and the summary of existing *G. lucidum* studies on diabetic cardiomyopathy

Streptozotocin (STZ)-induced diabetes is a well-documented model of experimental diabetes. STZ diabetes provides a relevant example of endogenous chronic oxidative stress due to hyperglycemia. In STZ rats, lipid peroxides are increased and nonenzymatic antioxidant levels and antioxidant enzyme activities are decreased significantly in the plasma and livers [26]. Effect of GLPP treatment on STZ-induced diabetic rats was studied. Blood glucose, blood insulin level, lipid peroxidation, and nonenzymatic and enzymatic antioxidants in the plasma and liver were tested. In one study, the GLPP were prepared from the fruiting bodies of *G. lucidum* by boiling water extraction. The diabetic rats were treated with GLPP (60, 120, 180 mg/kg) for a month. In the diabetic control group, the fasting blood glucose increased significantly, while blood insulin was significantly decreased. Administration of GLPP to diabetic rats significantly decreased fasting blood glucose and elevated blood insulin levels dose-dependently. Nonenzymatic antioxidants, measured as vitamin C (vC), vitamin E (vE), and glutathione (GSH), and antioxidant enzymes, measured as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx), were significantly decreased in both the plasma and liver of STZ-induced diabetic rats. Polysaccharides treatment significantly restored the decreased nonenzymatic antioxidant levels and antioxidant enzymes activities around normal levels in dose-dependent manner. These results demonstrated that the orally administered *G. lucidum* would retard hyperglycemia process and could effectively normalize the impaired oxidative stress in the plasma and liver of the diabetic rats. The promising antioxidant and anti-hyperglycemia effects of *G. lucidum* provided in this study may offer new insights in the treatment of diabetes and its complication [27].

In another study, GLPP treatment decreased blood glucose, while increased insulin level in high-fat-diet and STZ diabetic rats. SD rats were fed with high-fat diet for 4 weeks and injected with STZ (30 mg/kg) to establish diabetic model. The diabetic rats were randomly divided into diabetes group, GLPP group (600 mg/kg),

metformin group (600 mg/kg), combination group (GLPP 300 mg/kg + Met 300 mg/kg), and normal control group. The levels of fasting blood glucose, insulin, and advanced glycation end products (AGEs) and the activity of catalase (CAT) and glutathione peroxidase (GSH-Px) were detected after 12 weeks of treatment. In combination group, the fasting blood glucose was lowered significantly, insulin level was raised in plasma, the activity of CAT and GSH-Px was improved in myocardium, the concentration of AGEs in serum was decreased, and the expression of AGEs and connective tissue growth factor (CTGF) in thoracic aorta was reduced. The possible mechanism may be related to inhibit the oxidative stress of thoracic aorta through downregulation of AGEs and CTGF [28].

It was also found that GLPP attenuated myocardial collagen cross-linking in diabetic rats, which was related to the decreased level of AGE and elevated activities of antioxidant enzymes. In this study, type 2 diabetic rats were studied. It was found that diabetic rats showed distinctive performances of type 2 diabetes mellitus characterized as elevated blood glucose, HbA1c, blood fat and decreased insulin. Diabetic rats were randomly divided into four groups ($n = 10$ per group): the diabetic control (DC group) and three drug groups taking different doses of GLPP (GLPP-L: 200 mg/kg, GLPP-M: 400 mg/kg, and GLPP-H: 800 mg/kg). The dose of GLPP-M group was in line with Chinese traditional phytotherapy. For three drug groups, administration was conducted once daily over a 16-week period by gastric gavage. The normal controls rats received the same volume of physiological saline once a day over the same period. After 16 weeks of treatment, blood glucose and HbA1c were increased significantly in the diabetic control, while insulin was significantly decreased compared to the untreated normal control rats. Administration of middle or high dose of GLPP significantly decreased blood glucose and HbA1c, but there was no significant difference of the insulin levels between the DC group and the GLPP-L group. Myocardial fibrosis, which is characterized by excessed collagen accumulation and altered ventricular compliance in myocardium, is one of the major threating cardiac complications of diabetes. In this study, GLPP attenuated myocardial collagen cross-linking in diabetic rats. The content of the total hydroxyproline is an index of total collagen quantity. DC group tended to have a higher hydroxyproline concentration than the NC group and suggested that there had been more collagen accumulation and obvious myocardial fibrosis in DC group. After treatment with GLPP, the total hydroxyproline concentration showed a significant decrease dose-dependently. Meanwhile, there was a significant increase of AGE in myocardial and serum in DC group. The accumulation of AGE and AGE-related collagen was considered as the main factor for the decrease of cardiac compliance in diabetes mellitus. After treatment, the content of AGE was significantly reduced in the GLPP-M and GLPP-H groups, which implied that treatment with moderate doses of GLPP attenuated the enhanced AGE activation in diabetic rats efficiently [29].

In addition to the cardioprotective effect on type 1 and type 2 diabetes, *G. lucidum* has also been shown to play a role in other diabetic models like alloxan-induced diabetic rats. The object of this study was ethyl acetate and n-butanol fractions of *G. lucidum* aqueous extract. *G. lucidum* was subperitoneally given to alloxan-induced

diabetic rats for 2 weeks. Fasting blood glucose was significantly decreased in the *G. lucidum* administration group. At the end of the 2 weeks, the animals were sacrificed and blood samples were taken from all the groups for the determination of hematological parameters. The preliminary phytochemical screening of the two fractions of *G. lucidum* aqueous extract revealed the presence of alkaloids, flavonoids, and saponins. The LD_{50} was 1265 and 471 mg/kg for ethyl acetate and n-butanol fractions of the *G. lucidum* aqueous extract, respectively. Both ethyl acetate and n-butanol fractions of *G. lucidum* aqueous extract were found to have potent antidiabetic effects [30].

In another alloxan- and steroid-induced fasting diabetic rat model, Long-Evans rats were administered alloxan monohydrate (150 mg/kg) intraperitoneally for 3 days. Rats with plasma glucose levels higher than 12 mmol/L were chosen for further study. The *G. lucidum* extracts were orally administered at doses of 200, 400, 600, and 800 mg/kg once a day, respectively, for 7 days. Metformin (150 mg/kg) was used as a standard antidiabetic drug. Glucose levels were measured at the first day and last day of treatment. The animals were again intramuscularly injected with dexamethasone (2 mg/kg) for 3 days, and glucose levels were monitored simultaneously. The results showed that the *G. lucidum* dose-dependently reduced the plasma glucose levels in alloxan- and steroid-induced fasting diabetic rats. The maximum reduction of fasting plasma glucose levels were 55.57% and 36.01% in alloxan-induced and 51.41% and 32.02% in steroid-induced diabetic rats, respectively. Metformin (150 mg/kg), used as a positive control, decreased fasting blood glucose levels by 60.02% and 51.12% in the alloxan-induced and steroid-induced diabetic rats, respectively. What's more, plasma insulin levels and HbA1c were significantly augmented and reduced respectively in alloxan- and steroid-induced diabetic rats. Additionally, the same dose of the extracts also significantly reduced the levels of total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-c), as well as increased the level of high-density lipoprotein cholesterol (HDL-c). This research demonstrated that *G. lucidum* would improve hyperglycemia, hyperlipidemia, and insulin sensitivity in both alloxan and corticosteroid-induced diabetic rats. The bioactive chemicals responsible for those activities are most probably the GLPP [31]. In the clinical studies of patients with refractory hypertension cited in the former [10], researchers found that the blood glucose levels were also significantly reduced in the patient after oral administration of *G. lucidum* (a total dose of 330 mg *G. lucidum* extract each day, the main component of which was GLPP), and this change was even more significant in patients with previously high blood glucose levels. But their blood sugar did not reach the level at which diabetes was diagnosed. Therefore, usage of *G. lucidum* may be beneficial for the management of diabetes.

There are some evidences that *G. lucidum* may be protective against diabetic cardiomyopathy. However, there was also evidence from a small number of randomized controlled trials revealing that the use of *G. lucidum* for treatment of cardiovascular risk factors in people with type 2 diabetes mellitus is useless. Five trials with a total of 398 participants were eligible for inclusion. Results from two studies showed that *G. lucidum* was not associated with statistically or clinically significant

reduction in HbA1c, total cholesterol, LDL, or BMI. No improvement for fasting plasma glucose was found. The results after measurement of postprandial blood glucose level were found inconsistent. As the minimal clinically important differences are unknown, the clinical significance of this effect is unclear. There was no significant difference between groups for blood pressure or triglycerides. People who took *G. lucidum* for 4 months were 1.67 times more likely to have an adverse event than those who took a placebo, but the side effects were not serious. Evidence from a small number of randomized controlled trials did not support the use of *G. lucidum* to treat cardiovascular risk factors in type 2 diabetes. Future research into the efficacy of *G. lucidum* should be further studied [32].

To sum up, *G. lucidum* alleviates the progression of diabetic cardiomyopathy, and its specific mechanism involves the upregulation of insulin signaling, lowering the blood glucose, reduction of the expression of AGEs, and retarding lipotoxicity and myocardial fibrosis. There is a great chance that *G. lucidum* can be developed into a drug for the treatment of diabetic cardiomyopathy (Fig. 7.4).

7.4 *Ganoderma lucidum* Protects the Heart through Antioxidant Stress

Reactive oxygen species (ROS) at physiological levels are now appreciated to function as signaling molecules to regulate a wide range of processes in the cardiovascular system and to contribute to the maintenance of cardiovascular homeostasis [33]. In the failing heart, oxidative stress occurs in the myocardium and is closely related to left ventricular dysfunction. ROS negatively affects myocardial calcium handling, causes arrhythmias, and promotes myocardial remodeling by inducing hypertrophic signaling, apoptosis, and necrosis. Furthermore, a complicated pro- and anti-oxidant systems that orchestrate region-specific ROS production and removal keeps the strict balance of vasculature oxidative. ROS also regulates multiple vascular cell functions, including endothelial and smooth muscle cell growth, proliferation, migration, and apoptosis. ROS also involves in vascular tone, host defenses, and genomic stability. However, excessive levels of ROS promote vascular disease via direct and irreversible oxidative damage to macromolecules, as well as making destruction to vascular wall [34].

The most recognized function of *G. lucidum* is antioxidant effect, and the oxidative stress is involved in many cardiovascular disease progresses. Many studies have reported that *G. lucidum* metabolites modulate multiple systems response acting as an antioxidant agent.

Alcohol is one of the major risk factors for the cardiovascular disorders. Liu et al. found that the hot water extract of the *G. lucidum* has cardiac protective effects against heart toxicity induced by acute and massive ethanol. *G. lucidum* was administered (p.o.) to ICR mice at different doses of 10, 25, and 50 mg/kg. The experimental results showed that *G. lucidum* could inhibit the lipid peroxidation and superoxide

scavenging activity in mouse heart homogenate in a dose-dependent manner. Since the superoxide scavenging activity and anti-lipid peroxidation levels were always used as indicators of heart protection in vivo, in this study, *G. lucidum* was found to inhibit lipid peroxidation and decreased malonic dialdehyde (MDA) formation significantly. These findings indicated that the decrease in MDA formation may play a crucial role in the prevention of heart injuries induced by ethanol. It is concluded that the antioxidative activity may therefore protect the heart from superoxide-induced damage [35].

ISO, a synthetic β-adrenoreceptor agonist, has been used to cause myocardial necrosis, particularly in subendocardial regions of left ventricles and interventricular septum. The lesions caused by subcutaneous injection of ISO resemble those produced by myocardial infarction (MI) in human being [36]. Several mechanisms of ISO-induced myocardial necrosis have been reported. The imbalance between oxygen supply and demand in myocyte and myocardial overactivity (due to increased heart rate and contractility) are considered to be the basis of myocardial injury. There are other probable mechanisms associated with myocardial necrosis, increased cyclic adenosine monophosphate, increased intracellular Ca^{2+} overload, and depletion of high-energy phosphate stores and oxidative stress [37]. In brief, ISO causes myocardial damage mainly through oxidative stress and is a frequently used model for myocardial hypertrophy and heart failure.

In Janardhanan's research, cardiac toxicity was assessed by determining the activities of creatine kinase (CK) and lactate dehydrogenases (LDH) after subcutaneous injection of ISO (85 mg/kg) at an interval of 24 h for 2 days. *G. lucidum* (100 and 250 mg/kg, p.o.) was given to the rats once daily for 15 days prior to the ISO challenge. Similarly, α-tocopherol (100 mg/kg, p.o.) was used as the standard control. The animals were sacrificed 24 h after last ISO administration.

In the model group, administration of ISO elevated CK and LDH activities significantly. Treatment of *G. lucidum* significantly protected the elevation of CK and LDH activities caused by ISO. There were approximately 1.98- and 5.5-fold decreases for CK and 1.23- and 2.07-folds for LDH in the case of *G. lucidum* 100 and 250 mg/kg, respectively. The activities of CK and LDH in α-Toc group were approximately 1.60- and 1.71-fold decreased, respectively, than that of ISO control. The activities of cardiac mitochondrial innate antioxidant activities such as MnSOD and GPx were lowered significantly in the ISO group than that of the normal group, while after the treatment of *G. lucidum* at 250 mg/kg, the cardiac antioxidant status was significantly protected. The activities of MnSOD and GPx in the *G. lucidum*-treated (250 mg/kg) group were approximately 1.18- and 1.24-fold increased than that of the ISO control. The mean values of the activities of MnSOD and GPx were higher in *G. lucidum* (100 mg/kg) group, but there was no significant difference. The activities of Krebs cycle dehydrogenases and mitochondrial complexes I, II, III, and IV as well as the level of ROS and mitochondrial membrane potential (ΔΨmt) were evaluated because of ISO treatment, while GLPP significantly protected mitochondria by preventing the decline of antioxidant status and ΔΨmt as well as by directly scavenging the free radicals [38].

Mo et al. also invested the effect of *Ganoderma* triterpenoid using ISO-induced myocardial injury mouse model. C57BL/6 mice were subcutaneously injected with ISO (100 mg/kg/day) for 5 days to cause myocardial injury. Aqueous-ethanolic extract of the fruiting body of *G. lucidum* was suspended in water and delivered orally (300 mg/kg/day) 2 h before each ISO injection. Two days after the completion of ISO injections, they found myocardial infarction and fibrosis were evident in the infarcted area in ISO-treated mice, but *G. lucidum*-treated mice showed no detectable myocardial damage, hinting that *G. lucidum* was resistant to the cardiotoxicity of ISO (Fig. 7.5). They next identified the active constituents in *G. lucidum* are ganoderic acids (GAs). They then treated the ISO- induced mice model with GAs. 8-OHdG, a marker of oxidative damage to DNA, was attenuated in the GAs-treated group. Fas and FasL expression was not detected in the myocardium of GAs-treated ISO-induced mice. Then they investigated the protective mechanism used by GAs in cultured H9C2 cells. The cells were pretreated with GAs for 4 h before a H_2O_2 (200 μM, 30 min) treatment. The elevation of ROS and apoptotic levels by H_2O_2 was completely suppressed by GAs. H_2O_2-induced cleavage of

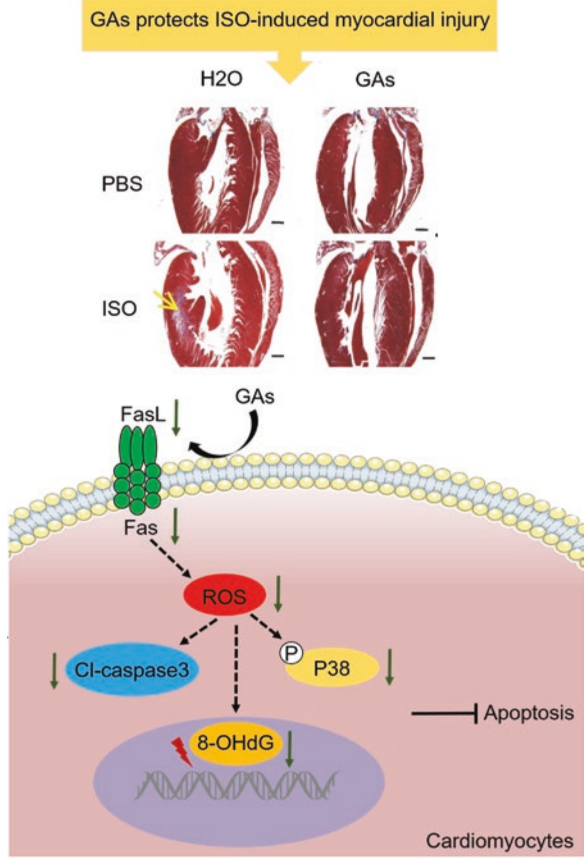

Fig. 7.5 Schematic mechanism of *Ganoderma* triterpenoid, GAs, protects ISO-induced myocardial injury. For the detail, see text. Arrows or lines represent the activation or inhibition relationship between two molecules. Myocardial infarction and fibrosis in ISO-treated mice were eliminated by GAs treated. Fas and FasL were downregulated in the myocardium of ISO + GAs-treated mice. Apoptotic cardiomyocytes decreased due to decreased ROS release and downregulation of cl-caspase3 and p-P38

caspase-3 and p-38 was also inhibited in H9C2 cells pretreated for 4 h with GAs (150 μg/mL). Thus, *Ganoderma* triterpenoid dissipated the cellular reactive oxygen species imposed by H_2O_2 and prevented cell death [39].

Ischemia reperfusion is also a major factor in myocardial injury caused by oxidative stress. Burkova et al. used a model of total 45-min ischemia and 30-min reperfusion of isolated rat heart by the Langendorff technique. The administration (15 days) of *G. lucidum* extract attenuated reperfusion contracture and decreased creatine kinase levels in the rat's isolated heart during reperfusion. However, the extract did not relieve the pressure developed by the left ventricle, reduction in the heart rate, and contraction and relaxation rates caused by reperfusion. The extract had no effect on the incidence of ventricular arrhythmia. There is great chance that *G. lucidum* extract could develop into a drug that prevents irreversible cardiomyocyte damage during ischemia and heart reperfusion [40]. Adriamycin, also known as doxorubicin, exists extensively fatal cardiotoxicity. *G. lucidum* specimens were cut into pieces, dried, and powdered. The powdered sample was extracted by ethanol using Soxhlet apparatus. Wistar rats were administered with adriamycin (1.5 mg/kg) for 3 weeks to induce cardiotoxicity. Another group was treated with adriamycin and *Ganoderma* extract (250 mg/kg) for 30 days. Adriamycin treatment resulted in increased serum levels of cardiotoxicity marker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK). Besides increasing the lipid peroxidation (LPO), adriamycin significantly reduced antioxidant enzymes, glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) in heart. Level of reduced glutathione (GSH) was also reduced significantly. However, the alterations in *G. lucidum* extract-treated rats were not significant. The study showed that *G. lucidum* extract exhibits significant antioxidant property and protects heart from free radical induced by Adriamycin [41].

Aging is also associated with increased oxidative damage at multiple cellular levels, declined in cellular energy production, and enhanced free radical status. Janardhanan et al. invested the effect of *G. lucidum* ethanolic extract on the activities of tricarboxylic acid cycle enzymes and mitochondrial complexes I–IV of the electron transport chain in aged rats orally administrated with 70% ethanolic extract (50 and 250 mg/kg) of *G. lucidum* once daily for 15 days. The activities of Krebs cycle dehydrogenases and mitochondrial electron transport chain complex IV were enhanced significantly in aged rat hearts. The profound activity of the extract may be correlated to the antioxidant property of *G. lucidum*. The results of the study revealed that *G. lucidum* is effective to ameliorate the age-associated decline of cardiac cellular energy status [42].

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Chapter 8
Preventive and Therapeutic Effect of *Ganoderma* (Lingzhi) on Diabetes

Qian Liu and Lu Tie

Abstract As extracts from *Ganoderma lucidum* (*G. lucidum*, Lingzhi) have been reported to be an alternative adjuvant treatment for diabetes, numerous of work have been carried out on it. Among the many biologically active constituents of *Ganoderma*, polysaccharides, proteoglycans, proteins, and triterpenoids have been shown to have hypoglycemic effects. Based on our research and other references, this article discusses the antidiabetic effect of *Ganoderma* mediated by protecting pancreas islet; inhibiting protein tyrosine phosphatase 1B, a promising therapeutic target of diabetes; decreasing lymphocyte infiltration; and increasing the antibody detection of insulin in diabetic mice. This review summarizes researches about the hypoglycemic action effects of polysaccharides, proteoglycans, proteins, and triterpenoids from *Ganoderma* as a guide for future research on diabetes and its complications. In addition, clinical studies with diabetic indexes are reviewed.

Keywords *Ganoderma* · Lingzhi · Polysaccharides · Diabetes · Complication

8.1 Introduction

Diabetes, also called diabetes mellitus (DM), is a group of metabolic diseases characterized by high blood glucose levels over a prolonged period [1]. It occurs when pancreas islet produces very little or no insulin or when the body does not respond appropriately to insulin.

There are three main types of diabetes: type 1 (T1DM), type 2 (T2DM), and gestational diabetes (GDM). T1DM is characterized by loss of the insulin-producing β cells of the pancreatic islets, resulting in insulin deficiency [2, 3]. Patients with T1DM are insulin-dependent, which means they must take artificial insulin daily or

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other steps to manage high blood glucose and stay alive. There currently have no cure and T1DM could not be prevented [2]. According to the National Institute of Diabetes and Digestive and Kidney Diseases, T2DM is the most common type of diabetes mellitus [4], accounting for 85–90% of all cases of diabetes [5]. T2DM is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion [6]. T2DM affects the way the body uses insulin. While the body still makes insulin, unlike in T1DM, cells in the body do not respond to it as effectively as they once did. It is believed that the defective responsiveness of body tissues to insulin is involved in the insulin receptor. In the early stage of T2DM, the predominant lesion is reduced insulin sensitivity. At this stage, high blood glucose can be controlled by improving insulin sensitivity or reducing the glucose production of liver. While there is currently no cure for T2DM, the condition can be managed through lifestyle modifications and medication. Without managing the blood glucose, T2DM can cause long-lasting (chronic) health problems or complications. GDM is a condition in which a woman without diabetes develops high blood glucose levels during pregnancy. GDM resembles T2DM in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness [7].

Ganoderma has been claimed to be effective in the prevention and treatment of many diseases [8]; however, almost all the data on the benefits of *Ganoderma* are based on laboratory and preclinical studies. Few clinical studies were conducted, while results were negative. Nevertheless, those researches have proved that *Ganoderma* is likely to have some benefits for many diseases. *Ganoderma* comprises probably 400 different biologically active constituents principally polysaccharides, triterpenoids, proteins, enzymes, steroids, sterols, nucleotides, fatty acids, vitamins, and minerals, which have been proved to have several therapeutic properties to control various diseases [9]. Some pharmacological active compounds of *Ganoderma* have been reported to have a hypoglycemic effect in human as well as in animals, such as *Ganoderma lucidum (G. lucidum)* polysaccharide (*GL*-PS) [10]; Fudan-Yueyang G. lucidum (FYGL) [11], a proteoglycan-reported antihyperglycemic extract from *G. lucidum* fruiting bodies; triterpenoids [12]; and Ling Zhi-8 (LZ-8) [13], a protein extracted from *G. lucidum* (Fig. 8.1). This review summarizes most of the researches about the hypoglycemic action effects of those active compounds from *Ganoderma* as a guide for future research.

8.2 Protective Effects of *Ganoderma* in T1DM

To identify antidiabetic effect of *Ganoderma*, several toxins, including streptozotocin (STZ) and alloxan [14], are used to induce hyperglycemia in animal models. Erna and colleagues [15] treated STZ-induced T1DM male Wistar rats with *Ganoderma* hydroethanolic extract (GWA), a *Ganoderma* extract containing β-glucan, proteins, and phenols. Biochemical analysis indicated a decrease of plasma glycemic and lipid levels in GWA-treated diabetic rats. Histopathological analysis from pancreas of GWA-treated diabetic rats showed a 50% preservation of

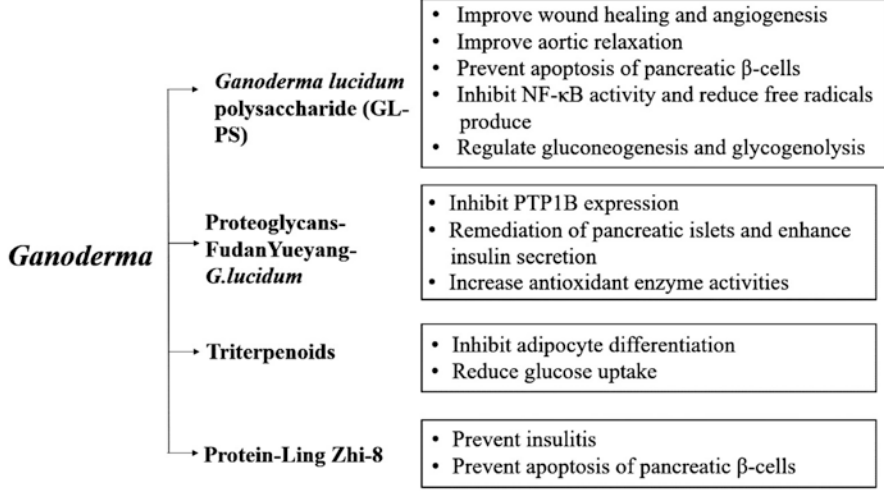

Fig. 8.1 Schematic diagram of *Ganoderma* antidiabetes properties

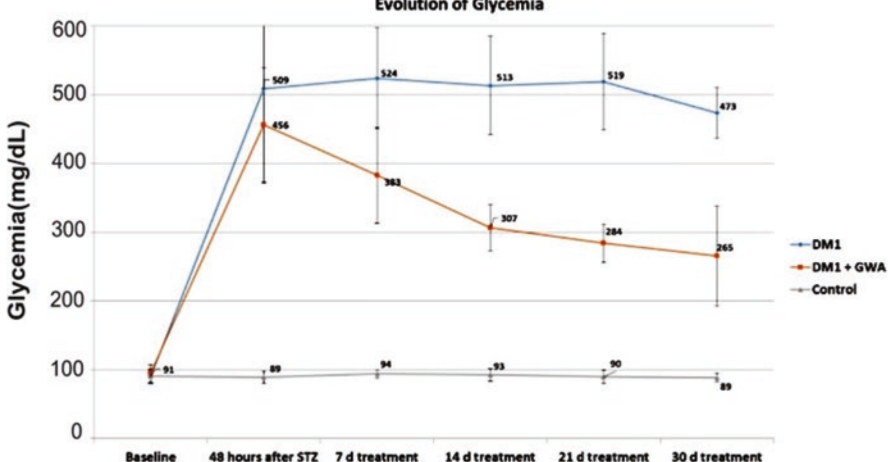

Fig. 8.2 Blood glucose level changes during the study treatments. Blood glucose level evolution during 30 days from Control, DM1, and DM1 + GWA groups. $P < 0.01$ between DM1 and DM1 + GWA groups [15]

pancreatic islet compared to the diabetic control group (Fig. 8.2). Those results indicated that the extract from *Ganoderma* possesses hypoglycemic and hypolipidemic activities and reverses the pancreatic islet damage induced by diabetes.

Kino K et al. (1990) [13] tested Ling Zhi-8 (LZ-8) for in vivo effect against T1DM in the nonobese diabetic mouse. LZ-8 is a polypeptide consisting of 110 amino acid residues with an acetylated amino terminus [16, 17]. LZ-8 was the first immunomodulatory protein which was obtained from the mycelial extract of

G. lucidum with the help of chromatographic and electrophoretic techniques. Intraperitoneal administration of LZ-8 twice weekly into the mice (10.3–12.6 mg/kg body weight) from 4 weeks of age prevented insulitis and protected β cells. No cumulative incidence of diabetes mellitus was observed in the LZ-8-treated group, compared with 70% and 60% incidences observed in an untreated group.

Zhang HN et al. (2003) [18] found that *G. lucidum* polysaccharides protect pancreatic cell against alloxan-induced damage by inhibiting NF-κB activity. *GL*-PS are found in 10–50% of dry matter of fruit bodies. Over 200 different polysaccharides have been searched from spores, fruiting bodies, and mycelia including β-D-glucans, α-D-glucans, α-D-mannans, and polysaccharide-protein complexes [9]. In alloxan-induced diabetic mice model, *GL*-PS was found to increase serum insulin and reduce serum glucose level dose-dependently. The study showed that *GL*-PS could inhibit the production of free radicals which is induced by alloxan in the isolated pancreatic islets. Pretreatment of islets with *GL*-PS for 12 h and 24 h could also significantly reverse alloxan-induced islets viability loss. *GL*-PS also had the capacity to improve oxidative stress state of diabetic mice [19–21]. *GL*-PS can be used as efficient protective agents against the free radical and reactive oxygen species (ROS)-induced cell damage.

Li F et al. (2011) [22] demonstrated that *GL*-PS might increase the renewal of β cells in the pancreas or permit the recovery of partially destroyed β cells and stimulate pancreatic insulin secretion. Zheng J et al. (2012) [23] showed that plasma concentrations of fasting glucose, triacylglyceride, total cholesterol, and nitric oxide were significantly decreased in *GL*-PS-treated groups compared to diabetic control group. Moreover, in *GL*-PS-treated groups, pancreatic superoxide dismutase, catalase, and glutathione peroxidase were significantly increased; the mRNA expressions of B-cell lymphoma-2 (Bcl-2) and pancreatic duodenal homeobox-1 (PDX-1) in pancreas were upregulated, but Bax, inducible nitric oxide synthase (iNOS), and caspase-3 were downregulated in *GL*-PS-treated groups. Those results suggested that *GL*-PS had a hypoglycemic effect in STZ-induced diabetic rats through preventing apoptosis of pancreatic β cells and enhancing β-cell regeneration.

8.3 Protective Effects of *G. lucidum* in T2DM

T2DM and related phenotypes such as obesity are insulin resistant [24]. The selective inbreeding of animals that spontaneously develop a type 2 diabetes-like phenotype has generated many of the strains used today [25]. Much can also be learned from animals with single gene mutations, as evidenced by the advances in knowledge generated from the study of the *db/db*, *ob/ob*, and so on [26]. Xiao C et al. (2012) [27] and Wang F et al. (2015) [28] figured out that *GL*-PS may be associated with decreased mRNA expression levels of several key enzymes involved in gluconeogenesis and/or glycogenolysis. The study of Xiao C et al. (2012) [27] reported the hepatic mRNA levels of glycogen phosphorylase (GP), fructose-1,6-bisphosphatase (FBPase), phosphoenolpyruvate carboxykinase (PEPCK), and

glucose-6-phosphatase (G6Pase) were significantly attenuated in both *GL*-PS-treated groups compared with the diabetic control group, while the study of Wang F et al. (2015) [28] found upregulation of lipid metabolism-related genes (Acox1, ACC, Insig-1, and Insig-2) and glycogen synthesis-related genes (GS2 and GYG1) in *GL*-SP group compared to model control group.

In the study of Xiao C et al. (2017) [29], they further demonstrated that the main bioactive in *GL*-PS was F31, which was determined to be a β-heteropolysaccharide with the weight-average molecular weight of 15.9 kDa. The possible action mechanism of F31 may be associated with downregulation of the hepatic glucose-regulated enzyme mRNA levels via adenosine monophosphate-activated protein kinase (AMPK) activation, improvement of insulin resistance, and decrease of epididymal fat/body weight (BW) ratio (Fig. 8.3). These results strongly suggest that F31 has antidiabetic potential.

Those results indicate that *GL*-PS consumption could provide a beneficial effect in terms of lowering the blood glucose levels by promotion of glycogen synthesis and inhibition of gluconeogenesis. Meanwhile, *GL*-PS treatment was also associated with the improvement of blood lipid compositions by regulation of cholesterol homeostasis in the type 2 diabetic rats.

Yang Z et al. (2018) [11] found that FYGL could decrease blood glucose, reduce body weight, and ameliorate insulin resistance in *ob*/*ob* mice. FYGL, a neutral hyperbranched proteoglycan ingredient extracted from *G. lucidum*, has hypoglycemic activity in vivo and inhibitory potency on protein tyrosine phosphatase 1B (PTP1B) in vitro [30]. PTP1B is one of main causes involved in type 2 diabetes. It dephosphorylates insulin receptor substrate (IRS) and dysregulates insulin signaling pathway, thus inducing insulin resistance. After FYGL treatment, it was observed that PTP1B expression decreased while phosphorylation of PTP1B increased, which targets the insulin signaling pathway in skeletal muscles. FYGL had excellent cell permeability and had a positive effect on insulin-stimulated glucose uptake by using the 2-deoxyglucose (2-DG) method. FYGL could inhibit PTP1B expression at the mRNA level, phosphorylating insulin receptor substrate-1 (IRS1), as well as activating phosphatidylinositol-3 kinase (PI3K) and protein kinase B (Akt). FYGL increased the phosphorylation of AMPK and consequently upregulated the expression of glucose transporter type 4 (GLUT4), promoting GLUT4 transportation to the plasma membrane in PTP1B-transfected L6 cells.

Teng BS et al. (2012) [31] and Wang CD et al. (2012) [32] suggested that FYGL regulated the tyrosine phosphorylation level of the IR 13-subunit as a result of the inhibition of the PTP1B expression and activity. FYGL also controlled the plasma biochemistry indexes relative to the type 2 diabetes-accompanied metabolic disorders.

Pan D et al. (2013) [33] pointed out that FYGL lead to not only a reduction in glycated hemoglobin level but also an increase in insulin and C-peptide level, whereas a decrease in glucagon level showed a potential for the remediation of pancreatic islets. FYGL was an effective antidiabetic agent by enhancing insulin secretion and suppressing hepatic glucose output along with increases of adipose and skeletal muscle glucose disposal in the late stage of diabetes. Moreover, the antioxi-

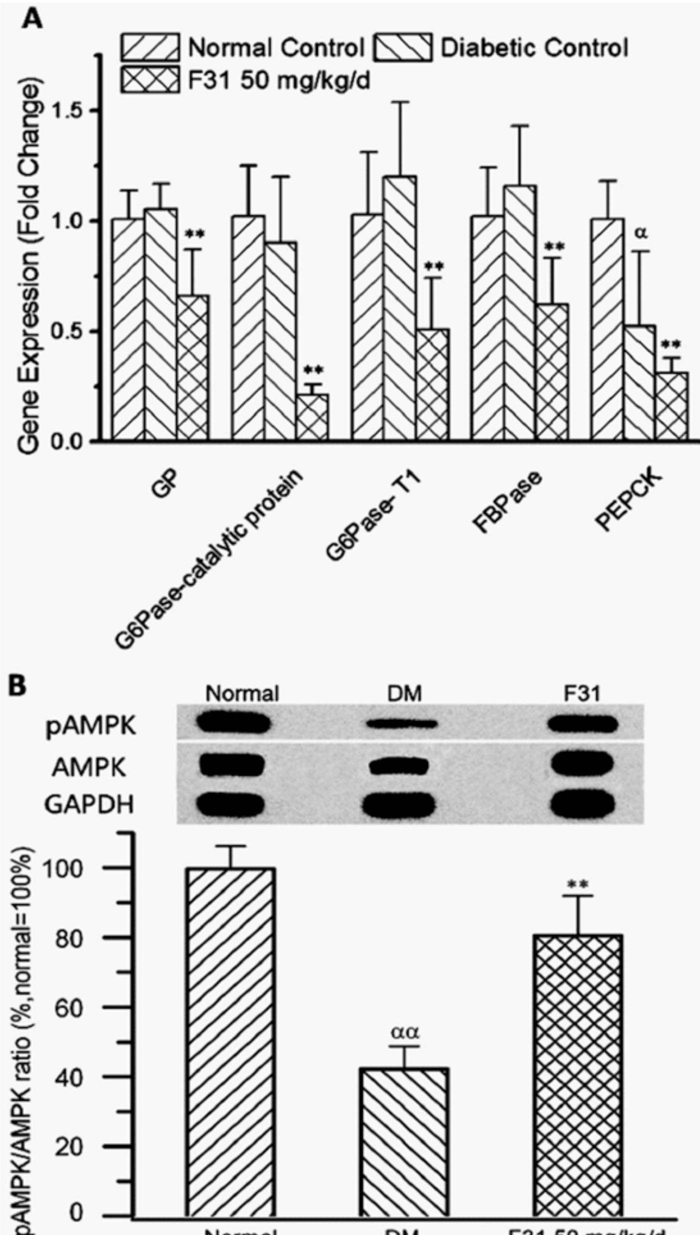

Fig. 8.3 Effects of F31 on Hepatic mRNA expression of GP, FBPase, PEPCK, and G6Pase. (**a**) Effects of F31 on mRNA expression of GP, FBPase, PEPCK, and G6Pase. (**b**) Protein levels expression of pAMPK/AMPK in liver tissue treated by F31 (values represent means ± SD (n = 8/ group). $*P < 0.05$, $**P < 0.01$ vs. diabetic control. $\alpha\alpha P < 0.01$ vs. normal control) [29]

dant enzyme activities were also increased by FYGL treatment. Furthermore, FYGL is beneficial against oxidative stress, thereby being helpful in preventing the diabetic complications.

Anita Thyagarajan-Sahu et al. (2011) [34] evaluated the effect of dietary supplement ReishiMax (RM), containing triterpenes and polysaccharides extracted from medicinal mushroom *Ganoderma*, on adipocyte differentiation and glucose uptake in 3 T3-L1 cells. The data revealed that RM inhibited adipocyte differentiation through suppressing the expression of adipogenic transcription factors peroxisome proliferator-activated receptor-γ (PPAR-γ), sterol regulatory element binding element protein-1c (SREBP-1c), and CCAAT/enhancer binding protein-α (C/EBP-α). RM also abrogated expression of enzymes and proteins responsible for lipid synthesis, transport, and storage: fatty acid synthase (FAS), acyl-CoA synthetase-1 (ACS1), fatty acid binding protein-4 (FABP4), fatty acid transport protein-1 (FATP1), and perilipin. RM induced AMPK and increased glucose uptake by adipocytes. This study suggests that RM can ameliorate insulin resistance in diabetes by controlling adipocyte differentiation and glucose uptake.

8.4 Protective Effects of *Ganoderma* in Diabetic Complications

All forms of diabetes increase the risk of long-term complications. Typically, complications develop chronically for many years, but there also have acute complications such as hypoglycemia and hyperglycemia, diabetic coma, and nonketotic hyperosmolar coma. The primary complications of diabetes result from the damage in small blood vessels such as damage to the eyes [35], kidneys [36], nerves [37], and wound healing [38]. Damage to the eyes, called diabetic retinopathy, is caused by damaging the blood vessels in the retina of the eye, which can result in gradual vision loss and eventual blindness. Damage to the kidneys, called diabetic nephropathy [39], can lead to chronic kidney disease, which develops to the end of the stage and may require dialysis or kidney transplantation. Damage to the nerves, called diabetic neuropathy [40], is the most common complication of diabetes. Proximal diabetic neuropathy results in painful muscle atrophy and weakness. Diabetic foot ulcer is an example of diabetic damage to wound healing. It is difficult to treat, occasionally requiring amputation [41, 42]. Diabetes also have damage to macrovascular. Diabetes doubles the risk of cardiovascular disease [41], and about 75% of deaths in diabetics are due to coronary artery disease [43]. Other macrovascular diseases include stroke and peripheral artery disease.

Zhu KX et al. (2014) [44] investigated PSG-1, a kind of polysaccharide purified from *Ganoderma atrum*. This study evaluated the protective effect of PSG-1 on diabetes-induced endothelial dysfunction in rat aorta. Rats were fed a high-fat diet for 8 weeks and then injected with a low dose of streptozotocin to induce T2DM. The diabetic rats were orally treated with PSG-1 for 4 weeks. Result showed that administration PSG-1 significantly reduced levels of fasting blood glucose, improved

endothelium-dependent aortic relaxation, and increased levels of PI3K, phosphorylation of AKT, endothelial nitric oxide synthase (eNOS), and nitric oxide in the aorta from diabetic rats compared to untreated diabetic rats. These results indicated that *Ganoderma atrum* polysaccharide improved aortic relaxation in diabetic rats via PI3K/Akt pathway.

Studies from our lab (2012) [45] reported that *GL*-PS dose-dependently rescued the delay of wound closure in diabetic mice. 50 and 250 mg/kg/day of *GL*-PS treatment significantly increased the mean perfusion rate around the wound and rescued the delayed wound healing in STZ-induced type 1 diabetic mice. The study further demonstrated that *GL*-PS administration suppressed manganese superoxide dismutase (MnSOD) nitration and increased MnSOD and glutathione peroxidase (GPx) activities (Fig. 8.4). Moreover, *GL*-PS attenuated the redox enzyme p66Shc expression and phosphorylation dose-dependently in diabetic mice skin.

In another study, Cheng PG et al. (2013) [46] found that the hot aqueous extract of *Ganoderma* could evaluate the wound healing activity in streptozotocin-induced diabetic rats. The extract of *Ganoderma* was standardized based on chemical contents (w/w) of total polysaccharides (25.1%), ganoderic acid A (0.45%), and adenosine (0.069%). The antioxidant activity in serum of rats treated with aqueous extract of *Ganoderma* was significantly higher, whereas the oxidative protein products and lipid damage were lower when compared to those of the controls. These findings strongly support the beneficial effects of standardized aqueous extract of *Ganoderma* in accelerating wound healing in STZ-induced diabetic rats.

The study of Pan D et al. (2014) [47] showed that FYGL (250 mg/kg) not only dose-dependently reduced the blood glucose concentration (23.5%, $P < 0.05$), kidney/body weight ratio (23.6%, $P < 0.01$), serum creatinine (33.1%, $P < 0.01$), urea nitrogen (24.1%, $P < 0.01$), urea acid contents (35.9%, $P < 0.01$), and albuminuria (30.7%, $P < 0.01$) of diabetic nephropathy (DN) mice compared to the untreated DN mice but also increased the renal superoxide dismutase (75.3%, $P < 0.01$), glutathione peroxidase (35.0%, $P < 0.01$), and catalase activities (58.5%, $P < 0.01$) compared to the untreated DN mice. The decreasing of renal malondialdehyde content and 8-hydroxy-2′-deoxyguanosine expression was also observed in FYGL-treated DN mice compared to the untreated DN mice, along with an amelioration of renal morphologic abnormalities. This study suggests a potential nutritional supplement for the prevention and therapy of DN for FYGL confers protection against the renal functional and morphologic injuries by increasing activities of antioxidants and inhibiting accumulation of oxidation.

Zhu KX et al. (2016) [48] evaluated the beneficial effect of PSG-1 on liver function in T2DM. In the study, PSG-1 decreased the activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) while increasing hepatic glycogen levels. PSG-1 caused upregulation of PPAR-γ, GLUT4, and PI3K mRNA expression in the liver of diabetic rats. Moreover, the concentrations of short-chain fatty acids (SCFA) were significantly higher in the liver, serum, and feces of diabetic rats after treating with PSG-1 for 4 weeks. These results indicated that PSG-1 might improve the liver function in T2DM rats and regulate the hepatic glucose uptake by inducing GLUT4 translocation through PI3K/Akt signaling pathways.

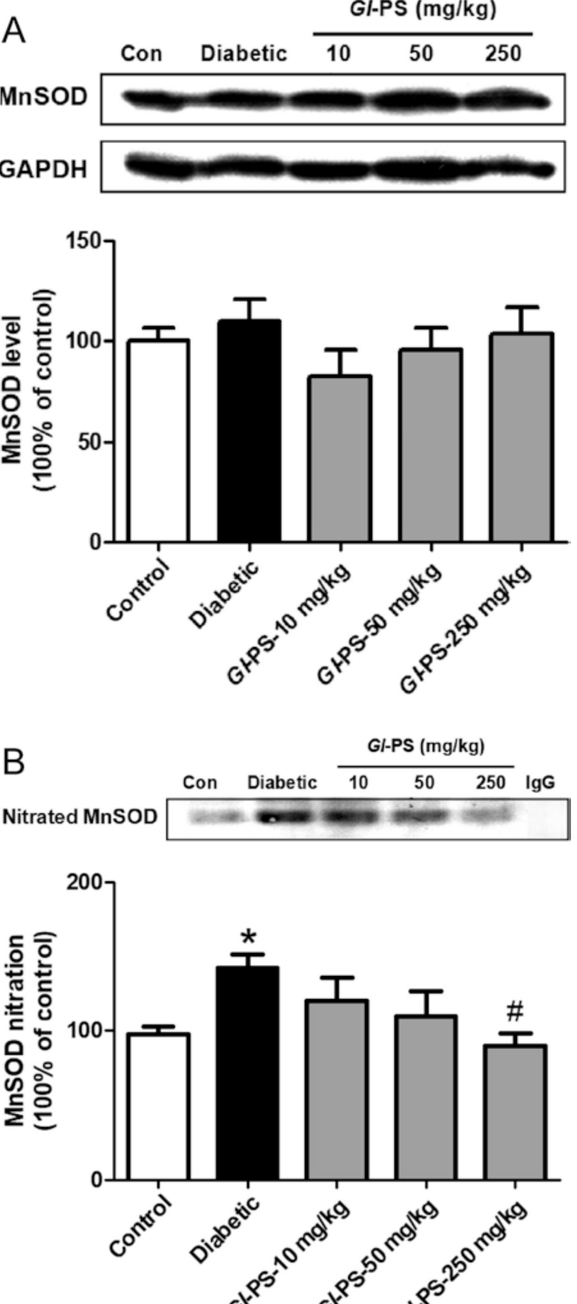

Fig. 8.4 Effects of *GL*-PS on MnSOD nitration/inactivation in diabetic mice (**a**) Effect of *GL*-PS on MnSOD protein expression in diabetic mice skin. Cutaneous MnSOD was analyzed by Western blot. Data were expressed as mean ± SEM and were shown as a percentage of the control. $n = 5$~12 per group. (**b**) Effect of *GL*-PS on MnSOD nitration in diabetic mice skin. Proteins extracted from skin homogenates were immunoprecipitated with anti-MnSOD antibody and resolved in 12% SDS–PAGE followed by Western blot analysis with the antibody against nitrotyrosine. Data were expressed as mean ± SEM and were shown as a percentage of the control. $n = 4$~6 per group. $*P < 0.05$ vs. control mice, $\#P < 0.05$ vs. diabetic mice [45]

8.5 Clinical Study of *G. lucidum* in Relation to Diabetes

There are few clinical studies exploring the effects of *G. lucidum* on diabetes.

In 2011, Li SH and colleagues [49] conducted a trial to explore the treatment effect of Bozhi Glycopeptide Injection on diabetic foot. Bozhi Glycopeptide Injection is a sterilized aqueous solution obtained by extracting the dried mycelium powder of *Ganoderma* capense (Lloyd) Teng from the GC1 strain by liquid fermentation culture. The components are polysaccharide and peptide. The granule glycopeptide has showed clinical treatment effect on antiaging, antioxygen free radical, and immunomodulatory, which are mainly used for progressive muscular dystrophy, atrophic myotonia, and various vertigo and various diseases caused by immune dysfunction, such as tumors, hepatitis, and so on. To investigate its effect on diabetic foot, 66 patients with diabetic foot enrolled to this trial. The patients were divided into observation group and control group, 38 cases in each group. Compared with control group, which received routine treatment, the observation group received Bozhi Glycopeptide Injection at a therapeutic dose of 4 mL/time, once for 2 days, and one course for 1 month, except for routine treatment. There were statistically significant differences observed between the two groups in the treatment of cold, pain, numbness, intermittent claudication, and ulcer gangrene ($P < 0.05$). The improvement of the observation group was significantly better than that of the control group ($P < 0.05$). This study showed that Bozhi Glycopeptide had a clear clinical efficacy in the treatment of diabetic foot.

There were studies suggesting that *G. lucidum* had antioxidant effects and possibly beneficial effects on blood pressure, plasma lipids, and glucose, but those have not been confirmed with mild hypertension or hyperlipidemia. Chu TT et al. (2012) [50] conducted the trial to assess the cardiovascular, metabolic, antioxidant, and immunomodulatory responses to therapy with *Ganoderma* in patients with borderline elevations of blood pressure and/or cholesterol in a controlled crossover trial. Twenty-six patients participated in the clinical trial, who received 1.44 g of *Ganoderma* daily or matching placebo for 12 weeks in a randomized, double-blind, crossover study. Body weight, blood pressure, metabolic parameters, urine catecholamines and cortisol, antioxidant status, and lymphocyte subsets were measured after each period. Data from 23 evaluable subjects showed there were no changes in body mass index (BMI) or blood pressure when treated with *Ganoderma* or placebo. Plasma insulin and homeostasis model assessment-insulin resistance was lower after treatment with *Ganoderma* than after placebo. Triacylglycerol decreased and high-density lipoprotein cholesterol increased with *Ganoderma* but not with placebo in the first treatment period, but significant carry-over effects prevented complete analysis of these parameters (Fig. 8.5). Urine catecholamines and cortisol, plasma antioxidant status, and blood lymphocyte subsets showed no significant differences across treatments. These results suggest that *Ganoderma* may have a mild antidiabetic effect and may ameliorate diabetic dyslipidemia.

Klupp NL and colleagues (2016) [51] conducted a prospective, double-blind, randomized, placebo-controlled trial to evaluate the efficacy and safety of *G. lucidum*

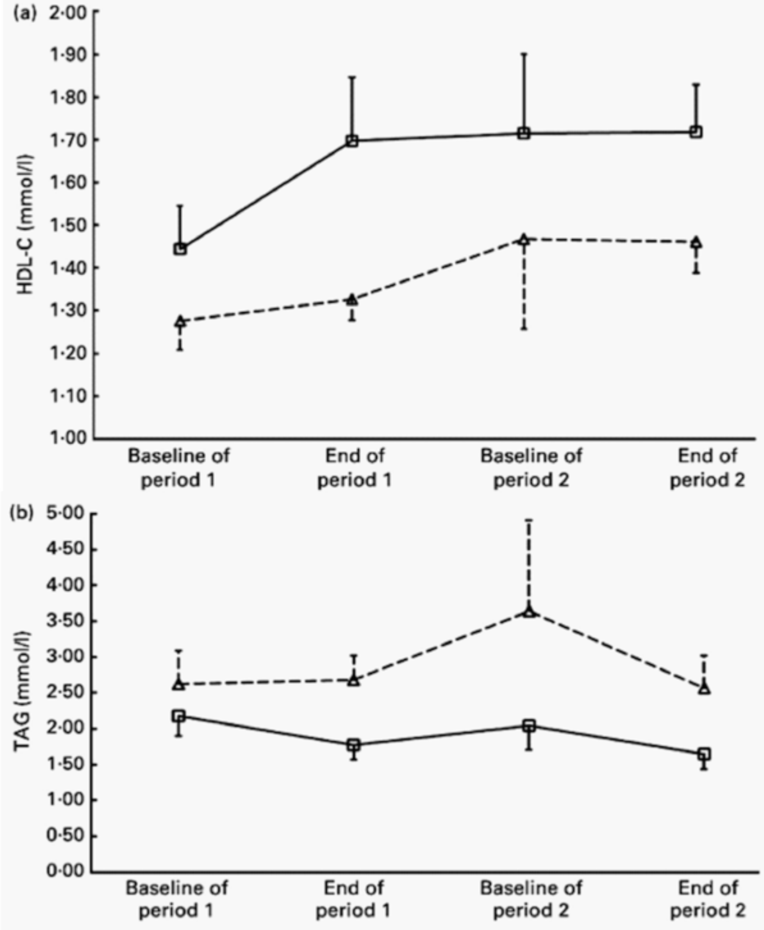

Fig. 8.5 Changes in (**a**) HDL cholesterol (HDL-C) and (**b**) TAG during the study treatments. Values are means, with their standard errors represented by vertical bars for subjects randomized to the first group (Lingzhi as the first treatment followed by placebo (−A–, n 13)) and the second group (taking placebo in the first period, then switched to the Lingzhi treatment (–K–, n 10)). There was no significant difference between the baseline and after 12 weeks' treatment of the two groups, while the data showed a significant carry-over effect in these two parameters, HDL-C and TAG [50]

for the treatment of hyperglycemia and other cardiovascular risk components of metabolic syndrome using (Trial ID: ACTRN12606000485538). Eighty-four participants with T2DM and metabolic syndrome were randomized to three intervention groups: *Ganoderma*, *Ganoderma* with Cordyceps sinensis, and placebo. The dosage was 3 g/day of *G. lucidum*, with or without Cordyceps sinensis, for 16 weeks. The primary outcome measure was blood glucose (glycosylated hemoglobin [HbA1c] and fasting plasma glucose [FPG]); a group of secondary outcome mea-

sures were also tested. The two intervention groups' data were combined. The data from this randomized clinical trial showed there were no evidence to support the use of *G. lucidum* for treatment of cardiovascular risk factors in people with diabetes mellitus or metabolic syndrome.

To observe the effect of *G. lucidum* granules on glucose metabolism in patients with type 2 diabetes mellitus (T2DM) and its mechanism of action on inflammatory factors, Tong L. and colleagues (2018) [52] conducted a prospective, randomized, placebo-controlled trial. 58 patients with T2DM were randomized into 2 groups. Both groups received routine treatment. 29 patients in the treatment group were treated with *G. lucidum* granules; 29 patients in the control group were treated with placebo. Both groups were treated for 12 weeks. Except for regular outcome measures, the level of inflammatory-related factors, such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), adiponectin (APN), and leptin (LP), was also measured. After treatment, the levels of PG, HbA1c, HOMA-IR, and LP in the treatment group were lower ($P < 0.05$) than before. What's more, the 2 h PG, HbA1c, and LP in the treatment group were lower than those in the control group ($P < 0.05$). After treatment, the APN of the treatment group was higher ($P < 0.05$). The APN of the treatment group was higher than that of the control group ($P < 0.05$). These results suggested that *G. lucidum* granules can significantly improve the blood glucose index and insulin resistance of patients with T2DM. The mechanism may be related to the effective regulation of APN and LP levels.

Despite few clinical trial researches, the effect of *G. lucidum* on diabetes is still a research hot point.

8.6 Conclusion

Ganoderma has a leading place in present-day medicinal mushroom development which has been utilized for centuries in East Asia to prevent or treat various diseases. It was also used as a tonic of traditional Chinese medicine in promoting good health, perpetual youth, vitality, and longevity. Studies on *Ganoderma* have shown many interesting biological activities effects.

Antihyperglycemic effects of *Ganoderma* have been extensively studied and have shown potential therapeutic activities. A variety of *G. lucidum* molecules, such as polysaccharides, triterpenoids, proteoglycans, and proteins, exhibits antidiabetic effects. Not only do those molecules possess antihyperglycemia properties, but they also regulate lipid profiles and immune responses. *G. lucidum* polysaccharides and FYGL decrease the levels of total cholesterol, triglycerides, and low-density lipoprotein cholesterols in the body, which may help prevent diabetic complications, such as atherosclerosis and hyperlipidemia. However, *G. lucidum* polysaccharides and FYGL contain amino acids; therefore, it is very difficult to determine whether the hyperglycemic effects are exerted by polypeptides or by polysaccharides. Additional evidence is required to provide a comprehensive explanation of the mechanisms underlying the antidiabetic effects of *G. lucidum* polysaccharides and

FYGL. Despite previous research on the relationships between structure and activity in triterpenoids, the mechanisms involved in these relationships have yet to be elucidated. Most studies of LZ-8 have focused on immunomodulatory activities. An attempt to define what types of proteins or molecules are able to interact with LZ-8 could yield interesting results. Hence, directions for future research should focus on biologically active compounds to be explored in the mycelium.

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Chapter 9
Preventive and Therapeutic Effect of *Ganoderma* (Lingzhi) on Liver Injury

Zhiwei Qiu, Dandan Zhong, and Baoxue Yang

Abstract *Ganoderma lucidum* (*G. lucidum*, Lingzhi) has a wide range of hepatoprotective effects. Its bioactive substances include triterpenoids, polysaccharides, sterols, steroids, peptides, and other bioactive ingredients. Based on our research and other references, this chapter discusses the hepatoprotective effects of *G. lucidum* in different liver diseases, including hepatocellular carcinoma, nonalcoholic liver disease, alcoholic liver disease, hepatitis B, inflammation, fibrosis, and toxicant-induced liver injury. The liver protective mechanisms of *G. lucidum* vary from diseases to diseases. This chapter will summarize the hepatoprotective effects of *G. lucidum* on different liver injury and their clinical applications.

Keywords *Ganoderma* · Lingzhi · Polysaccharides · Triterpenes · Liver injury

Ganoderma lucidum (*G. lucidum*, Lingzhi) is a traditional Chinese medicine used to treat and prevent various diseases. It is widely used in Asian countries. The fungal family *Ganodermataceae* contains more than 200 species, which are mainly distributed in subtropical and tropical regions [1]. The bioactive substances of *G. lucidum* include polysaccharides, triterpenes, sterols, peptides, and so on. Pharmaceutical value of *G. lucidum*, such as anti-fatigue, antiaging, and hepatoprotective effects, has been recorded in traditional Chinese medicine books such as *Shen Nong's Herbal Classic* and *Compendium of Materia Medica* [2]. Modern researches also confirmed the antitumor, antiaging, immunity regulation, and other bioactivities of *G. lucidum*. Among them, hepatoprotective activities are particularly outstanding. As early as 1974, Lin et al. [3] reported that the extract of *G. lucidum* had potent hepatoprotective effects on liver injury. Administration of *Ganoderma* extract (10 g/kg; each milliliter is equivalent to 1 g fruiting body) for 8 days significantly

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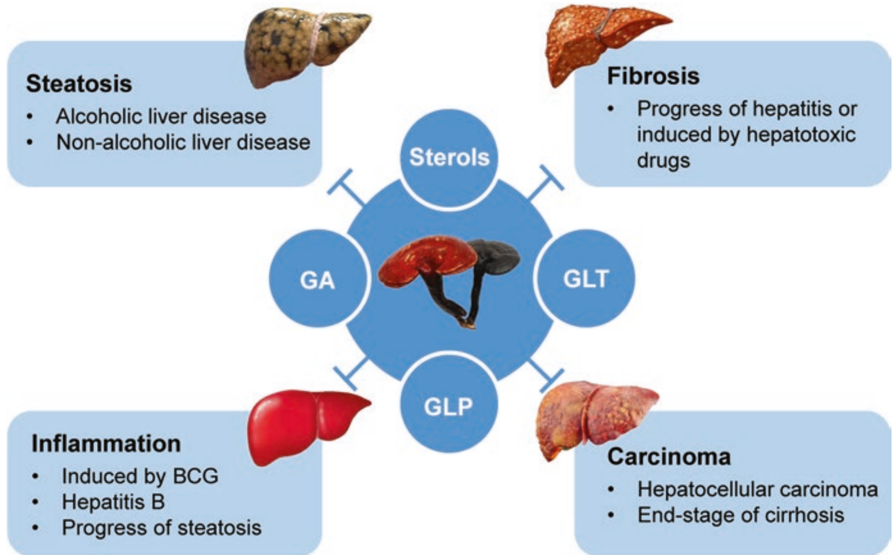

Fig. 9.1 The main bioactive components of *G. lucidum* and their preventive and therapeutic effects on liver injury. *GA Ganoderic* acid, *GLP G. lucidum* polysaccharides, *GLT* triterpenoid-rich extract of *G. lucidum*

ameliorated inflammation of the liver caused by carbon tetrachloride in mice. This is the earliest study on the therapeutic effect of *Ganoderma lucidum* on liver injury. In recent years, more and more studies have found that *Ganoderma* has multiple hepatoprotective effects on various liver injury, including hepatocellular carcinoma, nonalcoholic liver disease, alcoholic liver disease, hepatitis B, inflammation, fibrosis, and toxicant-induced liver injury (Fig. 9.1). Following, we review the researches in recent years on the hepatoprotective effects of *G. lucidum* on different liver diseases and its underlying mechanisms.

9.1 Antitumor Activity of *G. lucidum* on Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the sixth most common cancer globally and one of the most lethal cancers worldwide [4]. Treatments of HCC primarily consist of chemotherapy, surgical resection, and liver transplantation. In addition, ionizing radiation is a well-established and widely used therapeutic modality for patients with HCC [5]. However, the efficacy of chemoembolization or systemic therapies on HCC remains limited due to the toxicity and side effects. Therefore, novel and effective therapies are urgently required for HCC treatment.

G. lucidum is a widely used traditional Chinese medicine, which is beneficial for general health. The bioactive substances of *G. lucidum*, such as polysaccharide,

ganoderic acid and triterpene, have been proved to have antitumor activities, especially in the prevention of the recrudescence or metastasis of cancerous cells on HCC. It had been found that extracts of *G. lucidum* could remarkably mitigate the toxic and side effects of radiotherapy and chemotherapy in some patients [5, 6]. The therapeutic mechanisms of *G. lucidum* covers inhibition of tumor cell growth, promotion of tumor cell apoptosis, regulation of immune system, and inhibition of cancer cell migration [7–10]. The bioactive substances of *G. lucidum* with antitumor activity include the total extract of *G. lucidum*, polysaccharide, *ganoderic* acid, triterpene, and ergosterol peroxide.

9.1.1 The Antitumor Activity of Total Extract of G. lucidum on HCC

G. lucidum extract (GLE) is the total extract of *G. lucidum* [11]. It is a mixture of various bioactive components of *G. lucidum*, which has been widely confirmed to have anticomplement, anti-inflammatory, and antitumor activities, and plays a powerful therapeutic role in liver cancer [6, 10–12].

Cell cycle arrest effect is the main mechanism of the antiproliferative activities of GLE. In vitro study showed that GLE (40, 80, 120 μg/mL, in a dose-dependent manner) could decrease G1-phase progression through the cell cycle or G1/S phase transition and induced apoptosis through the alternation of mitochondrial transmembrane depolarization at the same time. Finally, the cell cycle of HepG2 was blocked with accelerated apoptosis, indicating that GLE have antitumoral proliferation effect through both apoptosis pathway and cell cycle arrest effect [12]. In vivo, GLE (8 g/kg) also showed a potent antitumor effect and significantly lower the tumor weight of tumor-burdened rats, which confirms the results of in vitro model [6].

Inhibition on the invasion of cancer cell is another mechanism of the antitumor effect of GLE. The invasion and metastasis of cancer cells involve degradation of environmental barriers, such as the extracellular matrix (ECM) and basement membrane, by various proteolytic enzymes, leading to enhanced mobility and metastasis [13]. The matrix metalloproteinase (MMP)-2 and MMP-9 are both enzymes capable of degrading type IV collagen, which is a major constituent of the basement [14]. In recent years, some in vitro and in vivo studies have demonstrated the relationship between MMP-9 expression and HCC invasion. Therefore, inhibitors against MMPs are effective for the prevention of tumor invasion and metastasis. In vitro study [10] showed that GLE (0.5, 1 mg/mL) could inhibit the phosphorylation of extracellular signal-regulated kinase (ERK1/2) and protein kinase B (PKB) in the cytosol as well as reducing activator protein-1 (AP-1) and nuclear factor-κB (NF-κB) levels in the nucleus of HepG2 cells. Finally, GLE significantly reduced the expression level of MMP-9 and inhibited cell invasion induced by phorbol-12-myristate-13-acetate (PMA) in a dose-dependent manner. Moreover, in a human tumor xenograft model [11], a dose response inhibition was observed in the average size, volume, and weight of tumors upon oral administration of GLE. The number of metastatic tumor-bearing mice, affected organs, and

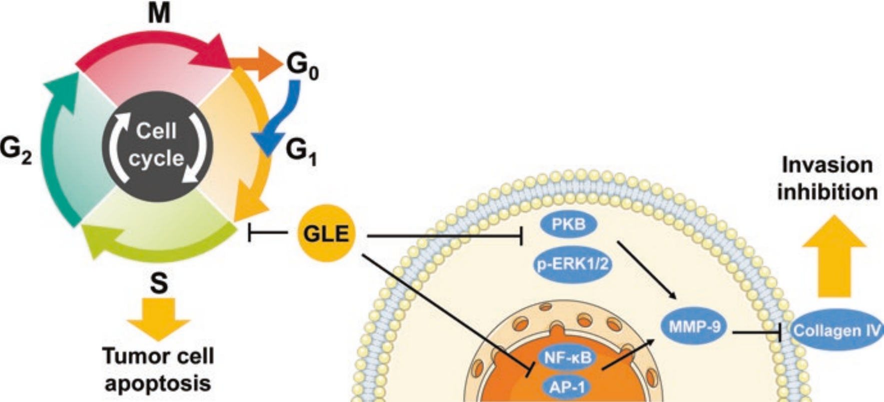

Fig. 9.2 The antitumor mechanism of total extracts of *G. lucidum* on HCC. *GLE G. lucidum* extract, *PKB* protein kinase B, *p-ERK1/2* phosphorylation of extracellular signal-regulated kinase, *AP-1* activator protein-1, *NF-κB* nuclear factor-κB, *MMP-9* matrix metalloproteinase-9, *collagen* type IV collagen

tumor foci as well as the MMP-2 and MMP-9 activities in serum of mice were significantly suppressed by GLE.

To sum up, GLE could block the cell cycle of hepatocarcinoma cells and induce apoptosis. Also, GLE suppressed the invasion of cancer cell through inhibiting the expression level of MMP-2 and MMP-9 and finally slowed the progression of HCC (Fig. 9.2).

9.1.2 The Antitumor Activity of G. lucidum Polysaccharides on HCC

G. lucidum polysaccharides (GLP) are main bioactive substances of *G. lucidum* with potent inhibitory effect on tumor [7]. Many researches have demonstrated that *G. lucidum* extract could both inhibit the tumor growth and invasion in vitro and in vivo [7, 15]. GLP could inhibit tumor growth by directly blocking the cell cycle of hepatocarcinoma cell or indirectly by regulating the immune system [7, 9].

One of the main antitumor mechanisms of GLP on HCC is the inhibition of tumor growth. GLP could inhibit the growth of HepG2 cell during earlier phase with lower dosage (500 μg/mL). For the other human hepatocarcinoma cell lines, such as BEL-7402 and Huh-7, the intracellular GLP presented an obvious positive dose- (500, 1000, 2000 μg/mL) and time-dependent (24, 48, 72 h) inhibition activity [16]. GLP may inhibit hepatocarcinoma cells directly through regulation of hepatocarcinoma genes. The research of Jie Shen et al. [8] showed that GLP could suppress HepG2 cells via regulating the hepatic miRNAs and immune-related miRNAs. After treated with GLP, the miRNAs including hepatic miRNAs (miR-3131, miR-10b, miR-23a, miR-92a, and miR-199a-3p) and non-hepatic miRNAs (miR-

1246, miR-20a, and miR-92b) are significantly upregulated, which devoted significant positive effect to the inhibition of GLP on HepG2. And the non-hepatic miR-4281 and miR574-5p that contributed unbeneficial effects to the cell suppressing are downregulated to the contrary. The results of in vivo experiments were consistent with those of in vitro experiments [17].

Meanwhile, GLP could also enhance the sensitivity of tumor cells to radiation. Yang Yu et al. found that GLP treatment (10, 100 μM) could enhance radiation-induced growth inhibition and apoptotic death of hepatocarcinoma cell [5]. GLP suppressed the activities of DNA repair-associated proteins including ataxia telangiectasia mutated (ATM) and DNA-dependent protein kinase (DNA-PK) in HepG2 cells under radiation condition at a molecular level. Furthermore, the addition of Akt inhibitor elevated the activities of DNA-PK and ATM and attenuated the GLP-induced HepG2 cell injury under the radiation condition. These findings suggest that GLP could enhance the radiation sensitivity of hepatocarcinoma cell via regulating the Akt signaling pathways, implying a potential therapeutic effect of GLP as a radiation sensitizer in HCC treatment.

Another important mechanism for antitumor of GLP is via regulating the function of immune system. Previous studies [9] had shown that GLP exhibited an effective antitumor capacity by increasing both humoral and cellular immune activities. After treatment with GLP (50, 200, 500 μg/mL in a dose-dependent manner), spleen-derived B lymphocytes from tumor-bearing mice were activated, proliferated, and produced large amounts of immunoglobulins. Bone marrow-derived macrophages from tumor-bearing mice also became activated after exposure to GLP, and they produced important immunomodulatory substances, such as IL-1β, TNF-α, and reactive nitrogen intermediates, like NO. GLP markedly increased phagocytosis of macrophages and raised the macrophage-mediated tumor cytotoxicity. Treatment of mice with GLP caused an inhibition of mouse sarcoma S180 tumor growth by 60% in vivo. It is reported [18] that GLP could also inhibit hepatocarcinoma cell by regulating the function of T cell. Aimei Li et al. [18] found that inhibition of miR-125b could obviously abolished the effect of GLP (50, 100, 200 mg/kg) on tumor growth, indicating that GLP may possess an antitumor activity via increasing the expression of miR-125b. The increase of miR-125b would induce the inhibition of Notch1 and FoxP3, which eliminated the suppression of T_{eff} proliferation by T_{reg} with an increase in IL-2 secretion and finally downregulated regulatory T cells accumulation and inhibited hepatocellular carcinoma growth. In vivo studies also showed that GLP could significantly suppress tumor growth in hepatoma-bearing mice associated with an increase of the ratio of T_{eff} to T_{reg}, which confirmed the above hypothesis (Fig. 9.3).

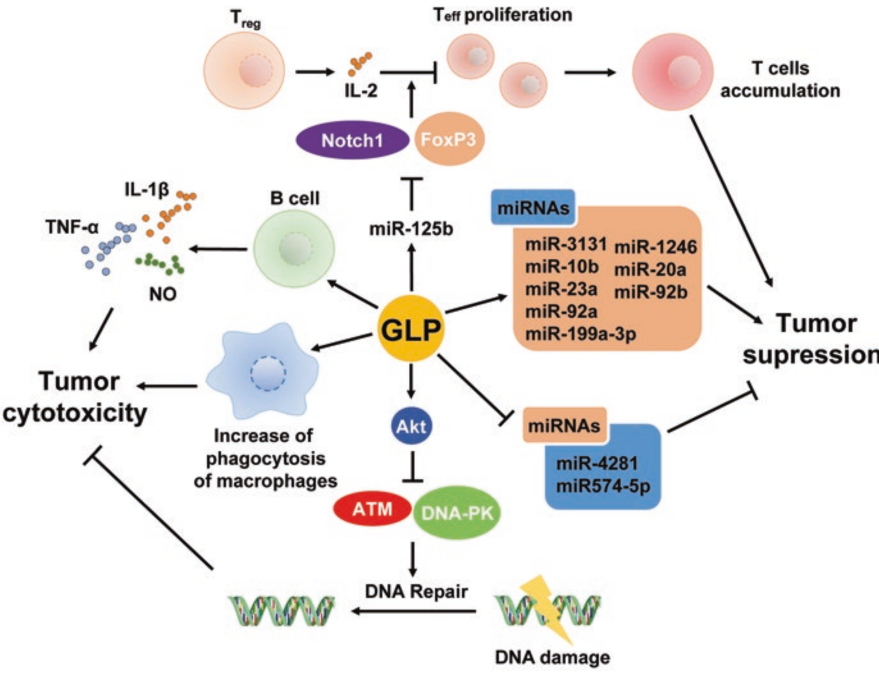

Fig. 9.3 The antitumor mechanisms of *G. lucidum* polysaccharides on HCC. *GLP G. lucidum* polysaccharides, *Akt* protein kinase B, *ATM* ataxia telangiectasia mutated, *DNA-PK* DNA-dependent protein kinase, T_{reg} regulatory T cell, T_{eff} effector T cells, *IL-2* interleukin-2, *IL-1β* interleukin-1β, *TNF-α* tumor necrosis factor-α, *NO* nitric oxide

9.1.3 The Antitumor Activity of Triterpenes on HCC

Triterpene is another major component of the bioactive ingredients of *G. lucidum*, which exhibits cytotoxic activity against tumor cells by inducing cell apoptosis and cell cycle arrest [19]. In detail, PKC, a known protein-regulating cell growth, is rapidly decreased in Huh-7 cells after treated with triterpene, while the JNK and p38 MAP kinases (p38 MAPK) are activated. These changes resulted in G2 phase arrest and tumor cell growth inhibition. Shwu-Bin Lin et al. [19] found that triterpenes (100 μg/ml) could inhibit the growth of Huh-7 cells, but not normal liver cells, suggesting a therapeutic potential of triterpenes on HCC (Fig. 9.4).

9.1.4 The Antitumor Activity of Ganoderic *Acid on HCC*

Ganoderic acid (GA), a lanostane-type triterpene isolated from *G. lucidum*, is another bioactive substance of the fruiting body and spores of *G. lucidum* [20]. It has been widely demonstrated to have antitumor activity on HCC [21]. In vitro

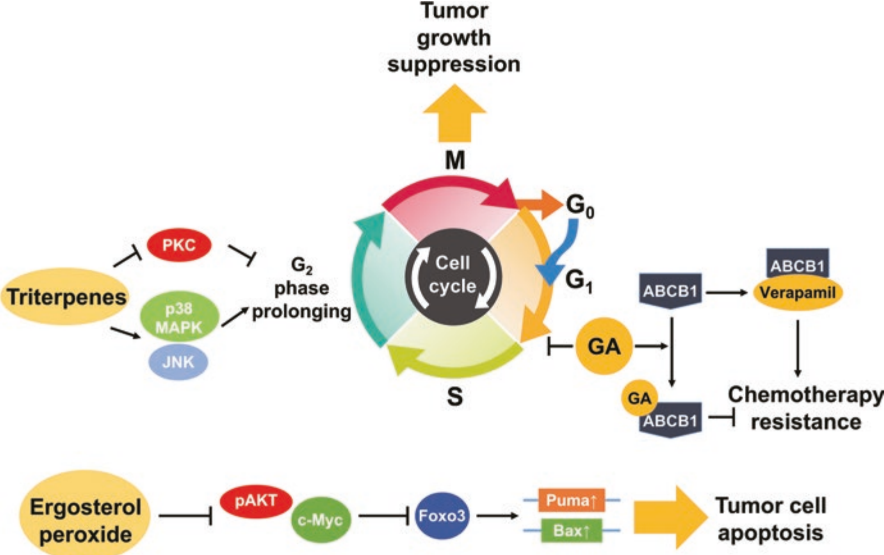

Fig. 9.4 The antitumor mechanisms of triterpenes, *Ganoderic* acid, and ergosterol peroxide on HCC. *PKC* protein kinase C, *p38 MAPK* p38 mitogen-activated protein kinase, *JNK* c-Jun N-terminal kinase, *GA Ganoderic* acid, *ABCB1* ATP binding cassette subfamily B member 1, *pAKT* phosphorylated protein kinase B, *c-Myc* cellular Myc, *Foxo3* Forkhead box O3, *Puma* p53 upregulated modulator of apoptosis, *Bax* the gene of bcl-2-like protein 4

studies have shown that GA (500 μg/ml) could inhibit the growth of BEL7402 cells while exerted little effect on a normal human liver cell line [20]. The inhibitory effect of GA on the proliferation of BEL-7402 cells might be attributed to the induction of apoptosis and cell cycle arrest effect. GA treatment resulted a marked increase in the percentage of cells at G1 phase, suggesting that the inhibitory effect of GA on hepatocarcinoma cell may be blocking the cell cycle at the G1 phase, thereby inhibiting their growth and promoting apoptosis.

Besides, GA could also reverse the multidrug resistance of chemotherapy. Chemotherapy is one of the most common therapeutic options for metastatic tumors and hematological malignancies [22]. However, drug resistance is the major obstacle for chemotherapy, especially mediated by ABCB1 [23]. GAB, a monomer of GA, can reverse multidrug resistance mediated by ABCB1. Dao-Lu Liu et al. found that GAB (10 μM) could notably increase the apoptosis HepG2/ADM cells but did not alter the expression level of ABCB1 and the activity of ABCB1 ATPase [24]. The positions of GAB binding to ABCB1 were different from the region of verapamil interacted with ABCB1, indicating that GAB can enhance the cytotoxicity of chemotherapeutics toward ABCB1-mediated MDR cancer cells via inhibition of the transport function of ABCB1. These findings provide evidence that GAB has the potential to be developed into an ABCB1-mediated multidrug resistance reversal agent.

9.1.5 The Antitumor Activity of Ergosterol Peroxide on HCC

Ergosterol peroxide is an oil fraction isolated from the *Ganoderma* spores with powerful hepatocellular toxicity, which could stimulate death of HepG2 cells via antiangiogenesis or cytotoxicity. Xiangmin Li et al. [25] found that ergosterol peroxide (23 μM, 10 μg/ml) could induce cell death and inhibit cell migration, cell cycle progression, and colony growth of human hepatocellular carcinoma cells. Treatment with ergosterol peroxide significantly inhibited the expression level of pAKT and c-Myc, which is the upstream signal proteins of Foxo3. The downregulation of pAKT and c-Myc promoted the expression of Foxo3 mRNA and protein, which in turn activated the downstream apoptosis, promoting genes Puma and Bax to initiate cancer cell apoptosis pathways and finally leading to the death of tumor cells. Therefore, ergosterol peroxide could promote the tumor cell apoptosis via Foxo3 pathway, making it a potential therapeutic for HCC (Fig. 9.4).

9.2 Hepatoprotective Effects of *G. lucidum* on Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD) is an increasing health concern worldwide [26]. The clinical pathophysiology of NAFLD involves a progressive spectrum of steatosis, steatohepatitis, cirrhosis, and increased risk of hepatic carcinoma. More and more evidences indicate that NAFLD is a multisystem disease, affecting extrahepatic organs and regulatory pathways, increasing the risk of type 2 diabetes mellitus, cardiovascular diseases, and chronic kidney disease [27]. However, the pathogenesis of NAFLD remains unclear. Treatments for NAFLD mainly rely on lifestyle intervention such as weight loss, lacking of effective clinical therapy and medicine [28]. In recent years, researchers found that the extracts of *G. lucidum* have significant therapeutic effect on NAFLD, indicating that it might be developed as a therapeutic for NAFLD.

Previous study has shown that *G. lucidum* extract (GLE) can ameliorate nonalcoholic steatosis in the liver by upregulating energy-metabolizing enzymes [29]. C57BL/6 mice fed with normal diet (ND) or high-fat diet (HFD) were administered GLE (50 mg/kg) extract or vehicle for 16 weeks. HFD feeding increased serum alanine aminotransferase level and hepatic lipid droplet, which were significantly attenuated by GLE. GLE inhibited the increase in epididymal and perirenal adipose tissue weights and serum cholesterol and LDL levels in HFD-fed mice. Fasting blood glucose levels were elevated in HFD-fed mice compared to ND-fed mice, and glucose and insulin sensitivities were deteriorated. These changes were markedly improved by GLE. Meanwhile, GLE restored the reduction of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) phosphorylation in the liver of HFD-fed mice and increased AMPK and ACC phosphorylation in HepG2 and 3T3-L1 cells. GLE could also induce the expression of GLUT4 protein in

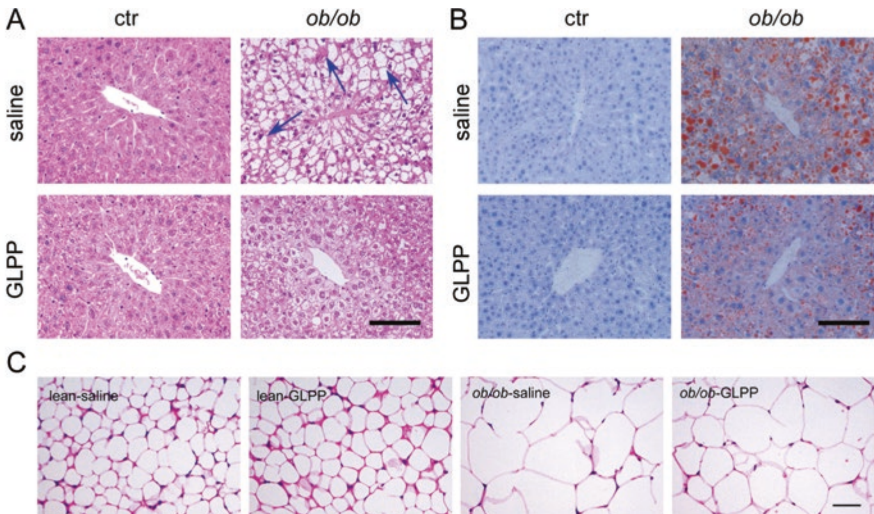

Fig. 9.5 GLPP treatment significantly reduced the lipid droplets accumulation in the liver and viscera of NAFLD mice. (a, b) H&E and oil red O staining of liver tissue section. Arrows refer to the lipid droplets on the liver. Scale bar, 100 μm. (c) H&E staining of mesentery fat. Scale bar, 100 μm

3T3-L1 cells and finally attenuated lipid accumulation induced by free fatty acid in HepG2 cells [30].

Another bioactive substances of *G. lucidum*, *G. lucidum* polysaccharide peptide (GLPP), also shows the hepatoprotective effects on NAFLD. Dandan Zhong et al. [31] reported that in the NAFLD model of *ob/ob* mice, the increase of liver size, liver weight, liver triglyceride, and total cholesterol was reduced by GLPP (100 mg/kg/day for 4 weeks). Meanwhile, the dyslipidemia, liver steatosis, and liver dysfunction demonstrated as the accumulated lipid droplets and ballooning degeneration of liver cells (Fig. 9.5a, b), and the increase of blood alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the *ob/ob* mice was also alleviated by GLPP. Besides, adipose tissue accumulation in the viscera is closely associated with NAFLD. GLPP administrated for a month significantly reduced the weight of mesentery fat without significant influence on weight of other organs and epididymal fat (Fig. 9.5c).

On the other hand, NAFLD is accompanied by insulin resistance. Glucose tolerance of *ob/ob* mice was significantly improved after GLPP treatment. The p-AKT2/AKT which plays key roles in regulating insulin resistance decreased in *ob/ob* mice while GLPP reversed the change. At the same time, GLPP reversed the downregulation of IRS2 (phosphorylation of IRS2) and AKT2 and upregulation of phosphorylation of GSK3β in the liver of *ob/ob* mice, suggesting that GLPP ameliorates the impaired insulin signaling pathway in the liver. [31]

The dyslipidemia of ApoC3 transgenic mice, a genetic detection model automatically forming hypertriglyceridemia and mild steatosis, was improved by

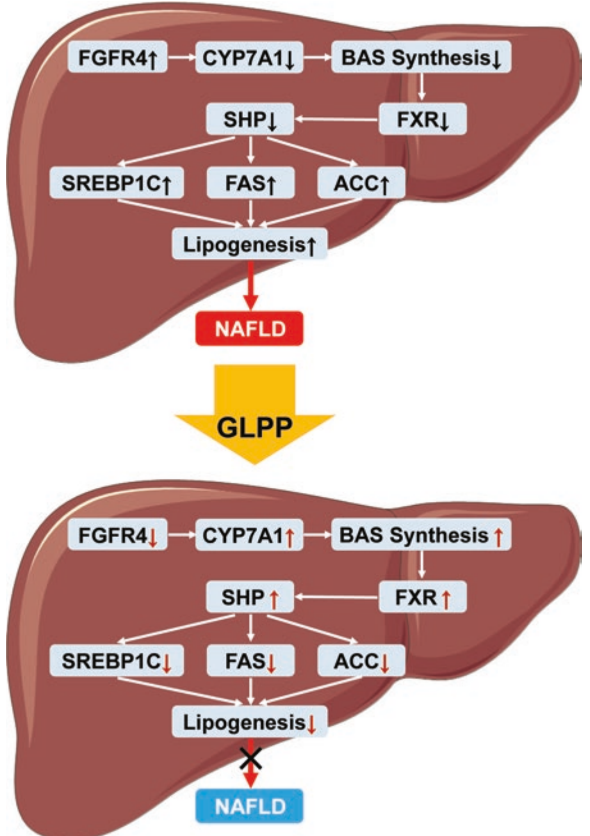

Fig. 9.6 Mechanisms of GLPP in the protection and treatment of NAFLD. *GLPP G. lucidum* polysaccharide peptide, *FGFR4* fibroblast growth factor receptor 4, *CYP7A1* cholesterol 7 alpha-hydroxylase, *BAS* synthesis, bile acid synthesis, *FXR* farnesoid X receptor, *SHP* small heterodimer partner, *SREBP1C* sterol regulatory element-binding proteins-1c, *FAS* fatty acid synthase, *ACC* acetyl-CoA carboxylase, *NAFLD* nonalcoholic fatty liver disease

GLPP. GLPP could alleviate the abnormal liver function via decreasing the levels of ALT and AST in ApoC3 transgenic mice. The inhibition of p-AKT2/AKT2, the downregulation of p-IRS2 and p-AKT2, and the upregulation of p-GSK3β in ApoC3 mice were reversed after GLPP treatment. Results suggested that GLPP may ameliorate the liver insulin resistance via modifying the impaired axis of IRS2-AKT2-GSK3β. Detection of key enzymes of glycerophospholipid metabolism, fatty acid metabolism, and primary bile acid biosynthesis pathways revealed that GLPP reversed low expression of CYP7A1, CYP8B1, FXR, and SHP and high expression of FGFR4 in *ob/ob* mice and ApoC3 mice. Besides, GLPP inhibited fatty acid synthesis by reducing the expression of SREBP1c, FAS, and ACC via a FXR-SHP-dependent mechanism and finally reduced the accumulation of lipid droplets and the content of TG in liver cell. In vitro experiments were in accordance with these results, which suggested that GLPP could alleviate hepatosteatosis via modulating bile acid metabolism dependent on FXR-SHP/FGF [31] (Fig. 9.6).

G. lucidum and its extracts have potent hepatoprotective effects on NAFLD, suggesting that it may be a potential therapy for NAFLD. However, currently, studies on the therapeutic effects of *G. lucidum* on NAFLD are limited to the total alcohol extract

of *G. lucidum* (GLE) or the polysaccharide mixture of *G. lucidum* (GLP). Nevertheless, the active ingredients of *G. lucidum* also include *Ganoderma* triterpenes and its monomer such as *Ganoderma* acid A and *Ganoderma* acid B. Therefore, studying the therapeutic effect of monomers of *G. lucidum* extract on NAFLD is not only important to elucidate the pathogenesis of NAFLD but also provides great help for finding drug targets for NAFLD, and it relies on the development of future research.

9.3 Hepatoprotective Effects of *G. lucidum* on Alcoholic Liver Disease

Alcoholic liver disease (ALD) is a global healthcare problem. It spans a spectrum of liver pathology, ranging from steatosis and alcoholic steatohepatitis to cirrhosis [32]. Various host factors including genetics modify the risk of ALD. Although metabolic alterations are responsible for ALD, epigenetic changes, oxidative stress, and inflammation contribute to ALD by affecting primarily hepatocytes but also hepatic stellate cells (HSCs) [33]. Currently, there are still no effective pharmacological or nutritional therapy for patients with ALD except cessation of drinking. And liver transplantation remains the lifesaving strategy for patients with end-stage alcoholic liver disease [32]. Recently, researcher found that the extracts of *G. lucidum*, including polysaccharide, triterpenoids, and pharmacopuncture, have decent therapeutic effect on ALD.

G. lucidum polysaccharides (GLP) are the main bioactive substance of *G. lucidum*. The bioactivities of GLP mainly rely on the anti-inflammatory and antioxidant effects [34]. Many studies had shown hepatoprotective effects of GLP on ALD. GLP (0.5125, 1.02, 2.04 g/kg, in a dose-dependent manner) showed potent antioxidant activities and declined lipid accumulation in ethanol-induced mice. GLP significantly lowered the liver injury biomarkers (AST, ALT, and ALP), triglyceride (TG), and cholesterol (TC) in plasma and liver. Treatment with GLP calmed the inflammation element expression, including iNOS, COX2, TNF-α NF-κB, and IL-6. These findings suggested that GLP inhibited ethanol-induced steatohepatitis via reducing lipid accumulation and liver inflammation, so it might be a potent therapeutic for ALD [34].

G. lucidum triterpenoids also show a significant hepatoprotective effects on alcohol-induced liver injury. Treatment with *G. lucidum* triterpenoids (50 mg/kg) significantly increases the level of antioxidant enzymes, SOD and GSH-px, and downregulated lipid peroxidation in the ALD mice [4]. The expression of CYP2E1, an important role in the conversion of ethanol to acetaldehyde/acetate, was sharply upregulated in the *G. lucidum* triterpenoids-treated mice, which accelerates the metabolism of alcohol. Meanwhile, *G. lucidum* triterpenoids suppressed apoptotic cell death and immune inflammatory response. In conclusion, *G. lucidum* triterpenoids played a hepatoprotective role in ALD via inhibiting alcohol-induced oxidative stress, indicating that it could be a therapeutic for ALD but needing clinical test.

G. lucidum pharmacopuncture is a mixture extracted from *G. lucidum*. Some researchers have reported that treatment with *G. lucidum* pharmacopuncture (10%) significantly reduced the histological changes due to acute liver injury induced by alcohol and significantly reduced the increase of the alanine aminotransferase (ALT) enzyme, and also ameliorated the superoxide dismutase (SOD) and the catalase (CAT) activities in the same time [35], suggesting that *G. lucidum* pharmacopuncture treatment is effective in protecting against ethanol-induced acute hepatic injury by modulating the activities of ethanol-metabolizing enzymes and attenuating oxidative stress.

The extracts of *G. lucidum* have shown effective therapeutic for ALD, respectively. The mechanisms mainly attribute to its inhibition on oxidative stress and radical scavenging capacity. *G. lucidum* also plays an important role in suppressing the expression of inflammation element such as iNOS, COX2, TNF-α NF-κB, and IL-6. However, current researches on the therapeutic effect of *G. lucidum* on ALD mainly focus on the mixture extracts of *G. lucidum*. Therefore, isolating the bioactive monomers of *G. lucidum* and assessing their therapeutic effect on ALD are very important to elucidate the specific mechanism of hepatoprotective effects of *G. lucidum* on ALD and find drug targets, which need to be further studied.

9.4 Anti-hepatitis B Activity of *G. lucidum*

Hepatitis B is an infectious disease caused by the hepatitis B virus (HBV), which leads to both acute and chronic infections. Although most people with infection of HBV have no symptom, cirrhosis and liver cancer may eventually develop, seriously affecting people's health [36]. *Ganoderic* acid (GA), a group of bioactive substances of *G. lucidum*, has been confirmed to have an inhibitory effect on replication of HBV [36]. GA (30 mg/kg/day, for 7 day) inhibited the replication of hepatitis B virus in HepG2215 cells at a dose of 8 μg/ml over 8 days. Production of HBV surface antigen (HBsAg) and HBV e antigen (HBeAg) was suppressed by GA which express only 20% and 44% of controls without GA, respectively. This finding suggested that GA had potential to be an anti-hepatitis B medicine, though further investigations on the mechanism of GA actions were necessary.

9.5 Antifibrotic Activity of *G. lucidum*

Hepatic fibrosis is an outcome of chronic liver diseases. It is one of the major public health problems related to life-threatening complications of portal hypertension, liver failure, and finally increased incidence of hepatocellular carcinoma. The activation and proliferation of hepatic stellate cells (HSCs) with accumulation of extracellular matrix (ECM), including α-smooth muscle actin (α-SMA) and type I collagen, during hepatic fibrogenesis is a key feature of liver fibrosis. The

platelet-derived growth factor (PDGF)-BB homodimer is a potent ligand for PDGF receptors (PDGFRs) to stimulate HSC growth and proliferation. The suppression of HSC activation and proliferation and induction of apoptosis in activated HSCs have been proposed as therapeutic strategies for preventing and treating hepatic fibrosis [37]. Guei-Jane Wang et al. [37] found that the triterpenoid-rich extract of *G. lucidum* (GLT) showed significantly antiproliferative effect on HSC-T6 (rat HSC) cell line stimulated with platelet-derived growth factor (PDGF)-BB. Treatment with GLT (25 μg/mL) both inhibited cell proliferation and induced cell apoptosis in HSC-T6 cells. Meanwhile, the cyclins D1 and D2 and the phosphorylation of the PDGFβR were inhibited, accompanied by the enhancement of JNK phosphorylation. Thereby, the expression of α-SMA was inhibited. These findings suggested that GLT inhibited PDGF-BB-activated HSC proliferation possibly through blocking PDGFβR phosphorylation, thereby indicating its efficacy for preventing and treating hepatic fibrosis.

Other researchers reported that the antifibrotic activity of *G. lucidum* may also come from the upregulation of collagenase. Treatment with GLE (1.0 g/kg) after the induction of liver fibrosis reduced the mRNA expression of collagen (α1)(I), smooth muscle α-actin, and tissue inhibitor of metalloproteinase 1 and metalloproteinase-13 [38], thereby decreasing the hepatic hydroxyproline content and improving liver histology. And the thioacetamide (TAA)-induced decrease in total collagenase activity was reversed by GLE treatment, which increased removal of the deposited collagen.

In addition, some researchers argue that free radicals and lipid peroxides play an important role in liver fibrosis [39, 40]. Considering that *G. lucidum* has a strong ability to remove free radicals, its hepatoprotective activity in hepatic fibrosis may also come from its ability of free radicals scavenging. GLE treatment (1600 mg/kg) significantly improved the live fibrosis caused by CCl_4 accompanied by increase of plasma transaminases, hepatic malondialdehyde (MDA), and hydroxyproline (HP) contents and spleen weight and decrease of plasma albumin A/G ratio and hepatic protein level [39]. Treatment with GLE also decreased the expression of TGF-β1 and changed the expression of MAT1A and MAT2A. Besides, Sang-Chul Kwon et al. [40] found that the fermentation filtrate of *G. lucidum* (FGL) also had the same pharmacological activity on carbon tetrachloride (CCl_4)-induced hepatic fibrosis.

Collectively, extracts of *G. lucidum* play an antifibrotic role in liver fibrosis through various mechanisms. On one hand, they could inhibit HSC proliferation as well as upregulate the expression of collagenase, which suppresses the sedimentation of collagen; on the other hand, the antioxidant activity of *G. lucidum* also played an important role in the hepatoprotective effect. The combination of these two mechanisms effectively alleviated the progression of liver fibrosis, indicating the potential of *G. lucidum* as an antifibrotic medicine.

9.6 Hepatoprotective Effects of *G. lucidum* on Obstructive Jaundice-Induced Oxidative Stress

Obstructive jaundice (OJ) develops after occlusion of the common bile duct as a result of several diseases such as choledocholithiasis, cholangiocarcinoma, or bile duct strictures [41]. Direct hyperbilirubinemia, which occurs secondary to OJ, causes liver dysfunction, gastrointestinal barrier dysfunction, immune dysfunction, coagulation dysfunction, lack of detoxification, and diminished wound healing, which does great harm to human health. Oxidative stress caused by OJ promotes the production of free radical, bringing injury to various viscera including liver [41]. Previous studies have shown that *G. lucidum* is an antioxidant with potent free radicals scavenging ability. Therefore, *G. lucidum* has the potential to improve the liver injury caused by OJ. Seval Aydin et al. [42] found that treatment with *G. lucidum* polysaccharide (GLP) (250, 500 mg/kg/day) ameliorated the abnormality of DNA damage and oxidative stress parameters caused by OJ in the common bile duct-ligated rat's plasma and liver including bilirubin, MDA, 8-OHdG, protein carbonyl, Cu-Zn SOD, thiol, and GSH. The data indicated that GLP had antioxidant activity in direct hyperbilirubinemic conditions and might protect DNA structure and liver tissue by reducing oxidative damage in OJ. However, the specific mechanism is unclear, which needs to be further studied.

9.7 Anti-inflammatory and Hepatoprotective Effects of *Ganoderma* on Liver Injury Induced by Hepatotoxic Drugs

The human body continuously and unavoidably exposes to a series of hepatotoxic substances, which usually exist in daily diet and medicine we take. These hepatotoxic substances are prone to cause hepatocyte damage, leading to oxidative stress and inflammation, progressing to liver fibrosis and cirrhosis, and eventually developing into hepatocellular carcinoma. In recent years, studies have shown the potent abilities of *G. lucidum* and its extracts in scavenging free radicals, which significantly inhibit the development of oxidative stress and inflammation. Therefore, it is feasible to use *G. lucidum* as a treatment for liver injury induced by hepatotoxic substances, such as carbon tetrachloride, BCG, benzo(α)pyren, α-amanitin, tert-butyl hydroperoxide, and so on.

9.7.1 Hepatoprotective Effects of Ganoderma on Carbon Tetrachloride-Induced Liver Injury

Carbon tetrachloride (CCl_4) is one of the most potent hepatotoxins, which is widely used in scientific research to evaluate hepatoprotective agents. Liver injuries induced by CCl_4 cause mitochondrial stress and disruption of membrane, resulting in

hepatocyte apoptosis, inflammation, and fibrosis [43]. A number of animal studies have shown the protective actions against acute liver injury caused by CCl_4 of water or ethanol extracts of *G. lucidum*, including *G. lucidum* extract (GLE) and *G. lucidum* polysaccharides (GLP).

GLE is a total extract of *G. lucidum*, which show antioxidant, anti-inflammatory, and mitochondria protection activities on the CCl_4-induced liver injury [40]. Wen-Chuan Lin et al. [39] found that oral administration of GLE (1600 mg/kg) significantly decreased the expression of TGF-β1 and changed the expression of MAT1A and MAT2A, thereby reducing CCl_4-induced hepatic fibrosis in rats. Panicker Sudheeshet et al. [43] showed that GLE enhanced the hepatic mitochondrial antioxidant status, preventing the deterioration of tricarboxylic acid cycle (TCA) enzyme activities and respiratory chain complexes I–IV. Treatment with GLE (100, 250 mg/kg) elevated the activities of SGPT, SGOT, and ALP and significantly increased the activities of mitochondrial enzymes downregulated by the CCl_4 challenge. The mitochondrial reactive oxygen species level was enhanced and mitochondrial membrane potential was declined significantly, which protected the liver mitochondria. Similarly, the hepatoprotective effect of GLP on CCl_4-induced liver injury mainly relies on its antioxidant and anti-inflammatory activities. Xiao-Jun Yang et al. [44] found that GLP alleviated the liver cells injury induced by CCl_4 through the measurements of ALT and AST activities both in vivo and in vitro. GLP treatment (500 mg/kg) significantly upregulated the expression of SOD, thereby eliminating the free radicals induced by CCl_4 and inhibiting the tension of liver injury. On the other hand, treatment with GLP also downregulated the expression of TNF-α induced by CCl_4, suggesting that GLP may also inhibit the progress of hepatitis through TNF-α pathway. These studies suggest that extracts of *G. lucidum*, GLE and GLP, may be developed into potential drugs that can effectively suppress liver inflammation and fibrosis.

9.7.2 Hepatoprotective Effects of Ganoderma on BCG-Induced Liver Injury

Bacillus Calmette-Guérin (BCG) is an important vaccine used to prevent tuberculosis (TB), especially meningeal TB and disseminated TB disease in children [45]. However, BCG vaccine could cause several adverse effects. According to previous studies, *Mycobacterium bovis* BCG infection has been proven to induce immune hepatic injury in rodent animal [46]. BCG could induce the release of hepatic endogenous cytokines, such as TNF-α, IFN-γ, and IL-1β in BCG-infected rats [47]. Under BCG-stimulated condition, augment of the liver weight and increase of the serum/supernatant ALT level were observed, as well as granuloma-forming and inflammatory cells soakage were observed by microscopic analysis within liver tissues [47]. Moreover, NO production was also increased by BCG stimuli in the culture supernatant, and a lot of iNOS-positive staining was observed in BCG-prestimulated hepatic sections [48], indicating that NO participates in immune liver injury induced by *Mycobacterium bovis* BCG infection.

G. lucidum had shown antioxidant and anti-inflammatory effects in previous studies, and it also played an important role in NO production, which had the potential to be a therapeutic for BCG-induced liver injury [49]. Guo-Liang Zhang et al. [48] reported that the immune liver injury induced by BCG infection could be markedly mitigated by GLP treatment. Application of GLP (100 mg/kg) significantly mitigated hepatic tumefaction, decreased ALT enzyme release and NO production in serum/supernatant, and improved the pathological changes of chronic and acute inflammation induced by BCG stimuli in mice. Moreover, GLP could also inhibit the expression of iNOS protein in BCG-immune hepatic damage model, indicating that the mechanisms of protective roles may be due to influence NO production. On the other hand, Xin Wang et al. [50] found that administration of GLP (50, 200 mg/kg, in vivo; 50, 100, 400, 800 μg/ml) reversed the decrease of P450 total content in microsomes induced by BCG, suggesting that the inhibitory effects of GLP on P450 oxidative metabolism might participate in the hepatoprotective mechanism. However, a more detailed mechanism still needs to be clarified, and clinical applicability of GLP remains to be established.

9.7.3 Hepatoprotective Effects of Ganoderma on Benzoapyrene-Induced Liver Injury

With the advent and population of barbecue and fried food, benzoapyrene (BaP) become the most common hepatotoxic substance we intake in daily life [51]. BaP has been reported to induce gene mutations, chromosomal aberrations, and other types of genotoxic effects in vitro and in vivo [52]. Although the precise mechanism of genotoxic effects caused by BaP is not clear, there is evidence that BaP causes generation of reactive oxygen metabolites, inhibits the activity of antioxidant enzymes, suppresses immune responses, and induces inflammation in tissue [53]. Many studies had shown that several radical scavengers and antioxidants exhibited protective effects against BaP-induced oxidative stress [54]; *G. lucidum* is one of them.

B. Lakshmi et al. [55] reported that administration of GLE (500 mg/kg) markedly inhibited mutagenicity induced by BaP. GLE prevented the increase of SGOT, SGPT, and ALP activities consequent to BaP challenge and enhanced the levels of reduced glutathione (GSH) and activities of glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT). The extract also profoundly inhibited lipid peroxidation induced by BaP.

Ganoderma applanatum terpenes (GAT), extract of *G. lucidum*, also showed a hepatoprotective effects on BaP-induced liver injury [56]. Application of GAT (100 mg/kg/day) significantly lowered levels of ALT and AST in blood and the liver histological injury in BaP-treated mice. In the liver, GAT markedly lowered the levels of ROS and MDA and lowered the GSH/GSSG ratio, which is accompanied by the inhibition of Cu/Zn-SOD, CAT, GPx, and GST activities. Thereby, GAT

significantly inhibited inflammation by pressing the expression of IL-1 and COX-2 and inhibiting NF-κB translocation in the liver of BaP-treated mice.

9.7.4 Hepatoprotective Effects of Ganoderma on α-Amanitin-Induced Liver Injury

Accidental consumption of poisonous mushrooms accounts for a large proportion of public health emergencies related to food poisoning [57]. Most fatal mushroom poisonings are caused by some species of the genus *Amanita* among which α-amanitin (α-AMA) has been demonstrated to be the primary lethal constituent. α-AMA inhibits RNA polymerase II and therefore blocks the synthesis of proteins, causing cell death, eventually leading to liver function failure [58]. Treatment of amatoxin poisoning includes toxin elimination, supportive care, and liver transplantation, lacking of efficient and specific antidotes [59]. Interestingly, clinical utility of *G. lucidum* as a remedy for *Amanita* poisoning had been reported and showed obvious clinical efficacy with a significant decrease in mortality [60].

Xin Wu et al. [61] reported that α-AMA induced a significant elevation in blood ALT and AST activities and provoked a significant reduction of superoxide dismutase (SOD) and catalase (CAT) activities and a significant increment of malondialdehyde (MDA) content in the livers of mice. And GLE treatment (500 mg/kg/day) significantly decreased blood ALT and AST levels and increased SOD and CAT activities, while decreasing MDA content in the liver compared with the α-AMA control group, which indicated that the hepatoprotective effects of GLE on α-AMA-induced liver injury may partially be related to the antioxidant properties. However, the research on the detoxification effects of *G. lucidum* on α-AMA-induced liver injury is limited to the application stage. The mechanism has not been elucidated, which requires further studies to be carried out.

9.7.5 Hepatoprotective Effects of Ganoderma on Tert-Butyl Hydroperoxide-Induced Liver Injury

Tert-butyl hydroperoxide (t-BHP) is usually used as an induction to induce inflammation in the liver [62]. According to precious studies, both ganodermanontriol and *Ganoderma* triterpenoids show anti-inflammation activities on t-BHP-induced liver injury.

Do Thi Ha et al. [63] and Li Bin et al. [64] reported that ganodermanontriol could induce HO-1 expression via the activation of Nrf-2 nuclear translocation and the subsequent transcription of the HO-1 gene in vitro and in vivo. Moreover, ganodermanondiol increased cellular glutathione levels and the expression of the glutamine-

cysteine ligase gene in a dose-dependent manner (40 μM, the best). Furthermore, ganodermanondiol exposure enhanced the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and its upstream kinase activators, LKB1 and Ca^{2+}/calmodulin-dependent protein kinase-II (CaMKII).

Jian-Guo Wu et al. [62] had reported the potent cytoprotective effect on oxidative damage induced by t-BHP in human hepatic HepG2 cells. Compared to the control groups, treatment with *Ganoderma* triterpenoids (50, 100, and 200 μg/ml) significantly increased the relative cell viability by 4.66%, 7.78%, and 13.46%, respectively, and reduced the level of ALT by 11.44%, 33.41%, and 51.24%; AST by 10.05%, 15.63%, and 33.64%; and LDH by 16.03%, 23.4%, and 24.07% in culture medium, respectively. *Ganoderma* triterpenoids could remarkably decrease the level of MDA and increase the content of GSH and SOD in HepG2 cells.

9.7.6 Hepatoprotective Effects of Ganoderma on Liver Injury Induced by Other Hepatotoxic Substances

D-Galactosamine (D-GalN) is a hepatotoxic agent, which has been found to induce liver damage that closely resembles human viral hepatitis [65]. Increased production of reactive oxygen species has been reported in primary culture of rat hepatocytes damage induced by D-GalN [66]. *G. lucidum* polysaccharides (GLP) are strong antioxidants and possess potent free radicals scavenging activities. It has the hepatoprotective potential in D-GalN-induced liver injury. Treatment with GLP (60, 120, 180 mg/kg, in a dose-dependent manner) could alleviate the increase of AST, ALT, and MDA induced by D-GalN and restore SOD and GSH to normal levels at the same time [67]. Moreover, GLP could also alleviate the structural abnormality of hepatocyte induced by D-GalN. These findings suggest the therapeutic effect of GLP on D-GalN-induced liver injury. Lin et al. [68] reported that the total triterpenoids isolated from *G. lucidum* (GT) also have powerful protective effects against liver damage induced by D-gal. Admonition of GT (80 mg/kg) significantly inhibits the increase of serum ALT, liver TG, and MDA and the decrease of SOD activity and liver GSH content caused by D-GalN. However, further studies on the hepatoprotective mechanisms of GLP and GT are necessary to assess the pharmacological values of *Ganoderma* as hepatoprotective agents (Fig. 9.7).

A few studies have reported that the terpenes of *Ganoderma resinaceum*, a species of *Ganoderma*, showed hepatoprotective activities on liver injury induced by H_2O_2. Xing-Rong Peng et al. [69] assessed the effects of various terpenes of *Ganoderma resinaceum* on H_2O_2-induced liver injury in HepG2 cells and found that ganoderesin B (15.00 μM), ganoderol B (33.20 μM), and lucidone A (82.10 μM) had significant hepatoprotective activities due to their remarkable in vitro inhibitory activities against the increase of ALT and AST levels in HepG2 cells induced by H_2O_2. Meanwhile, ganoderol B notably activated PXR-mediated CYP3A4 expression, suggesting that PXR-mediated CYP3A4 expression might be a potential molecular mechanism of ganoderol B (Fig. 9.7).

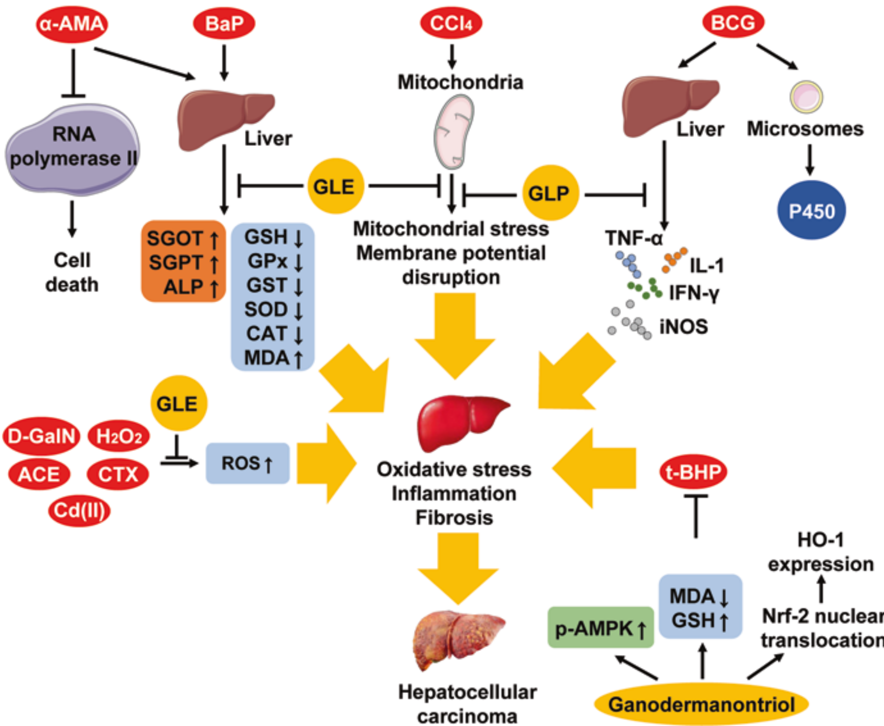

Fig. 9.7 The anti-inflammatory and hepatoprotective effects of *Ganoderma* on liver injury induced by hepatotoxic drugs and the underlying mechanisms. *α-AMA* α-amanitin, *BaP* benzoapyrene, *CCl₄* carbon tetrachloride, *BCG* Calmette-Guérin, *D-GalN* D-galactosamine, H_2O_2 hydrogen peroxide, *ACE* acetaminophen, *CTX* cyclophosphamide, *Cd(II)* cadmium, *t-BHP* tert-butyl hydroperoxide, *GLE G. lucidum* extract; *GLP G. lucidum* polysaccharides, *P450* cytochrome P450, *SGOT* serum glutamic oxaloacetic transaminase, *SGPT* serum glutamic pyruvic transaminase, *ALP* alkaline phosphatase, *GSH* glutathione, *GPx* glutathione peroxidase, *GST* glutathione S transferase, *SOD* superoxide dismutase, *CAT* catalase, *MDA* malondialdehyde, *TNF-α* tumor necrosis factor-α, *IL-1* interleukin-1, *INF-γ* interferon-γ, *iNOS* inducible nitric oxide synthase, *ROS* reactive oxygen species, *p-AMPK* phosphorylated mitogen-activated protein kinase, *HO-1* heme oxygenase-1, *Nrf-2* nuclear factor-like 2

Acetaminophen (ACE) is an analgesic drug and metabolized by the cytochrome P450 system, which leads to the formation of n-acetyl-p-benzoquinoneimine (NAPQI) [70, 71]. NAPQI reacts rapidly with glutathione, which consequently exacerbates oxidative stress in conjunction with mitochondrial dysfunction and finally causes depletion of GSH level in the liver. The GSH depletion, especially occurring in acute hepatotoxicity, affects liver functions and leads to massive hepatocyte necrosis, liver failure, or death [71]. *Ganoderma amboinense* showed the hepatoprotective effect in ACE-induced liver injury. Cheng-chin Hsu et al. [72] reported that pre-intake of *Ganoderma amboinense* (1%, 2%) dose-dependently protected liver against the subsequent ACE-induced elevation of ALT and AST.

Ganoderma ambonense also reversed ACE-caused GSH depletion, MDA and ROS increase, and GPX and catalase activity reduction (Fig. 9.7).

Cyclophosphamide (CTX) is another hepatotoxic substance that could cause significant hepatocyte apoptosis. Combination of *Cordyceps sinensis* polysaccharides (CSP) (150 mg/kg/day, for the first 3 days) and *Ganoderma atrum* polysaccharides (PSG) (180 mg/kg/day, for the next 7 days) could increase hepatic glutathione peroxidase and glutathione content depleted by CTX as well as prevented mitochondria-dependent apoptosis with regulation on Bcl-2 family proteins (Bad, Bax, and Bcl-2) through activation of peroxisome proliferator-activated receptor α (PPARα) [73]. This combination of hepatoprotective effect of PSG and anti-inflammatory effect of CSP provides us with an effective treatment scheme for drug-induced liver injury (Fig. 9.7).

Cadmium [Cd(II)] is a kind of environmental pollutant, which is extremely toxic to human body [74]. It can lead to the dysfunction of multiple organs, especially the liver. The mortality rate of acute Cd(II) poisoning is very high, but chelators have limited therapeutic effect on Cd(II) poisoning [75]. Fortunately, *G. lucidum* has been proved to have hepatoprotective effect in Cd(II) poisoning. Hai Jin et al. [76] reported that *G. lucidum* showed potent hepatoprotective activity on Cd(II)-induced hepatotoxicity in mice. Treatment with *G. lucidum* spore (1.0 g/kg/day, for 7 days) induced hepatic metallothionein-1 mRNA and the expression metallothionein protein. The metallothionein protein sequestered Cd(II) in the cytosol; decreased Cd(II) accumulation in hepatic nuclei, mitochondria, and microsomes; and finally protected hepatocyte under Cd(II) pressure. Meanwhile, Cd(II)-induced oxidative stress was decreased by *G. lucidum* spore, which effect is due, at least in part, to the induction of hepatic metallothionein to achieve beneficial effects (Fig. 9.7).

9.8 Hepatoprotective Effects of *G. lucidum* on Radiation-Induced Liver Injury

Ionizing radiations are an important source of oxidative stress, causing damage to cellular molecules either by direct transfer of energy or through the generation of oxygen-derived free radicals [77]. Radiotherapy is a common and effective tool for cancer treatment, but the radiosensitivity of normal tissues, especially those adjacent to the tumors, limits its therapeutic potential. Hence, protection of normal tissues against radiation-induced cellular injury is of immense importance in radiotherapy. Pillai TG et al. [78] reported that the aqueous extract isolated from *G. lucidum* showed effective radioprotective properties. T. P. Smina et al. [77] found that total triterpenes were highly effective in reducing the levels of lipid peroxidation and protein oxidation to near normal values both in vivo (100 mg/kg) and in vitro (100 μg/ml). Moreover, administration of total triterpenes extracted from *G. lucidum*, prior to γ-radiation exposure, significantly decreased the DNA strand breaks, which reveals the potential therapeutic use of *Ganoderma* total triterpenes

as a natural radioprotector to prevent hazardous effects of accidental radiation exposures. However, the hepatoprotective effect of *G. lucidum* triterpenes on radiation-induced liver injury has not been thoroughly clarified, and further investigations and clinical trials are necessary to be carried out.

9.9 Hepatoprotective Effect of *Ganoderma* on Exhaustive Exercise-Induced Liver Injury

Moderate exercise could be beneficial to heart and lung function, improve athletic performance, and reduce the incidence of chronic diseases from the preventive medicine point of view [79]. But intense or excessive exercise could cause muscle and tissue injury due to excessive free radicals produced from the increase in muscle oxygen consumption during exercise [80]. *Ganoderma tsugae* has the ability to scavenge free radicals and can be used as a drug to treat exhaustive exercise-induced liver injury. Chi-Chang Huang et al. [81] reported that *Ganoderma tsugae* intake (daily oral dose of 0.1875, 0.9375, and 1.875 g/kg, in a dose-dependent effect) decreased the blood glucose and FFA levels of exhaustive exercise rats, which associated with the decrease of lipid peroxidation and protection against exhaustive exercise-induced apoptosis and mitochondrial DNA deletion. These findings indicated that *Ganoderma tsugae* played a hepatoprotective role on exhaustive exercise-induced liver injury and could elevate exercise performance. However, which monomers play the major role in the treatment of liver injury caused by exhaustive exercise and how they play their therapeutic effect still need to be elucidated.

9.10 Hepatoprotective Effect of *Ganoderma* on Liver Injury Caused by Diabetes Mellitus

Diabetes mellitus is a metabolic disease characterized by hyperglycemia. It is one of the top three chronic diseases that endanger human health [82]. The liver plays an important role in glucose homeostasis such as glycolysis, gluconeogenesis, and glycogen metabolism, which is seriously affected during diabetes mellitus [83]. The liver injury caused by diabetes mellitus may be due to obesity and elevated fasting glucose [84]. Previous study had shown the hypoglycemic and hypolipidemic effects of polysaccharide isolated from *Ganoderma atrum* (PSG-1) [85], indicating that *Ganoderma* may play a hepatoprotective effect on liver injury caused by diabetes. Ke-Xue Zhu et al. [86] found that PSG-1 treatment (200 mg/kg and 400 mg/kg) decreased the activities of AST and ALT while increasing hepatic glycogen levels in type 2 diabetic rats. PSG-1 significantly upregulates the mRNA expression level of peroxisome proliferator-activated receptor-γ (PPAR-γ), glucose transporter-4 (GLUT4), phosphoinositide 3-kinase (PI3K), and phosphorylated-Akt (p-Akt) in

the liver of diabetic rats and increases the activities of antioxidant enzymes including SOD, GPx, and CAT. Moreover, the concentrations of short-chain fatty acids (SCFA) were significantly higher in the liver, serum, and feces of diabetic rats after treating with PSG-1 for 4 weeks. These results indicated that the improvement of PSG-1 on liver function in diabetes may be due to its antioxidant effects, SCFA excretion in the colon from PSG-1, and regulation of hepatic glucose uptake by inducing GLUT4 translocation through PI3K/Akt signaling pathways, suggesting that PSG-1 has great potential to be a hepatoprotective medicine in diabetes, but the clinical effect needs to be further studied.

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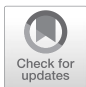
Chapter 10
Preventive and Therapeutic Effect of *Ganoderma* (Lingzhi) on Renal Diseases and Clinical Applications



Xiaoqiang Geng, Dandan Zhong, Limin Su, and Baoxue Yang

Abstract The mechanisms of kidney diseases, such as acute kidney injury (AKI) and chronic kidney disease (CKD), have been intensively studied. Nonetheless, the morbidity and mortality of AKI and CKD increased in recent years. Recently, natural products have been increasingly recognized as an alternative source for treating renal diseases on account of the conventional experience and the multi-target characteristics. *Ganoderma lucidum* (*G. lucidum*, Lingzhi) has been used for centuries as nutraceuticals and alternative medicine to improve health and to treat numerous diseases. Benefiting from various biological activities, such as anti-oxidation, anti-inflammation, anti-tumor growth and metastasis, etc., *G. lucidum* has been proved to exhibit significant role in preventing and treating various kidney diseases. In this chapter, we review certain researches and provide comprehensive insights into the renoprotective effects of *G. lucidum*.

Keywords *Ganoderma* · Lingzhi · Polysaccharides · Triterpenes · Kidney disease

Ganoderma lucidum (*G. lucidum*) is a well-known medicinal mushroom. Triterpenoids and polysaccharides are the major active components of *G. lucidum* and are well-known for their numerous pharmacological activities. The medicinal importance of the *G. lucidum* lies in the prevention and treatment of various diseases, such as cancer [1, 2], hypertension [3–5], asthma [6], liver disorders [7–9], hypercholesterolemia [10], obesity [11], and cerebral ischemia reperfusion injury [12]. Especially, the efficacy on renal disease of *G. lucidum* and its extracts have been intensively studied in the past 20 years [13]. This chapter mainly reviews the usefulness and molecular mechanisms of *G. lucidum* in kidney diseases based on basic and clinical researches.

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10.1 Renal Diseases

The kidney is a regulatory organ that helps to maintain constancy of the internal environment of body. The lay view of renal function is that the kidneys remove waste and potentially harmful end products of metabolism, such as urea, uric acid, sulfates, and phosphates. They accomplish physiological functions through ultrafiltration of plasma at the glomerulus, selective reabsorption of water and solutes, and selective tubular secretion of solutes. In recent years, renal diseases have gradually aroused attention on account of their booming prevalence worldwide and lack of effective therapies attributing to the intricate pathogenesis. Among the renal diseases, acute kidney injury (AKI) and chronic kidney disease (CKD) are the predominant types. Recent multiple epidemiologic, clinical, and basic researches have suggested that AKI and CKD are interconnected [14, 15]. Although the latest understanding brings new perspectives to the diagnosis and monitoring of kidney diseases, there is still no effective approach in preventing and treating AKI as well as CKD.

Acute kidney injury (AKI) is a complex syndrome defined as a kidney disease with rapid loss of renal function with high morbidity and mortality, often manifested with persistent oliguria and abruptly increased blood creatinine concentration, with major complications including volume overload, electrolyte disorders, uremic complications, and drug toxicity [16]. It is accepted that the definition and staging of AKI are mainly based on the risk, injury, failure, loss, end-stage kidney disease (RIFLE) criteria, and the acute kidney injury network (AKIN) criteria [17]. AKI is pretty common in hospital patients and especially in seriously ill patients. According to numerous epidemiological surveys, AKI occurs in approximately 20% of hospitalized patients especially after surgery. Specially, some AKI patients require kidney replacement therapy and have high mortality rate [18]. AKI patients have increased risk of subsequent chronic kidney disease. The primary causes of AKI include volume depletion, hypotension, ischemia reperfusion injury, sepsis, and nephrotoxicity [19]. Notably, chronic kidney disease is also an important risk factor in AKI development, and AKI predisposes patients to CKD in turn [16].

Chronic kidney disease (CKD) has emerged as a major cause of ever-increasing morbidity and mortality. The progression of CKD is characterized by continuously advancing and irreversible loss of kidney function, caused by loss of renal architecture and individual nephrons [20]. CKD arises from many heterogeneous disease pathways that alter the function and structure of the kidney irreversibly, over months or years [21]. Unfortunately, there is currently no effective therapy that can completely halt or reverse the progression of CKD [22].

There are abundant epidemiological studies showing that the incidence of CKD in the general population reaches to 13% in some countries [23, 24]. According to the 2017 Global Burden of Disease (GBD) study, global absolute CKD incidence increased by 28.2% among females and 25.4% among males from 2007 to 2017 [25]. Notably, a forecasting analysis pointed that the number of global YLL (the years of life lost) due to CKD will increase to 52.5 million in 2040 and deaths will rise from 1.2 million in 2016 to 3.1 million in 2040 [26]. Considering the rising

prevalence and growing heavy burden of CKD worldwide, all these studies highlight the need of effective practices focusing on prevention, treatment, and management of CKD.

In recent years, researchers have paid more attention to the renoprotective activities of natural products and their extractions [27]. Many lines of researches have elucidated the therapeutic effects of *G. lucidum* and its extractions in various AKI and CKD pathogenesis, including renal ischemia reperfusion injury [28], autosomal dominant polycystic kidney disease [29], cisplatin-induced renal injury [30], diabetic nephropathy [31, 32], Adriamycin-induced nephropathy [33], chronic proteinuric renal diseases [34], renal proximal tubular cell oxidative damage and fibrotic process [35, 36], etc. Apart from basic researches, certain clinical researches have also proved the potent anti-renal disease bioactivities of *G. lucidum* [37, 38].

10.2 Effect of *G. lucidum* on Renal Ischemia Reperfusion Injury

Ischemia reperfusion injury is defined as the tissue or organ damage caused when blood supply returns to tissue after a period of ischemia or lack of oxygen. Renal ischemia reperfusion injury (RIRI) as one type of AKI, characterized by an abrupt decrease in the glomerular filtration rate, is a common surgical complication leading to pretty high mortality, CKD, and end-stage renal disease [39]. Mechanically, both ischemia and reperfusion lead to cell injury and even death. In the process of ischemia, renal cell would suffer from apoptosis and necrosis. On the other hand, reperfusion causes additional cell injury which has been attributed to calcium overload, neutrophil infiltration, and the generation of ROS [40, 41].

G. lucidum polysaccharide peptides (GLPPs) are isolated from boiling water extract of the fruiting body of *G. lucidum*. GLPP has molecular weights of approximately 5×10^5 with a polysaccharide to peptide ratio of approximately 95%/5%. Previous researches have proved that GLPP is a major pharmacological component of *G. lucidum* extractive and has various bioactivities such as anti-tumor, antioxidant, and radical scavenging features [42, 43]. All these studies suggest that GLPP may exhibit protective effect in the pathophysiological mechanisms of RIRI.

In 2005, Qiu et al. investigated the protective effect of *Ganoderma* polysaccharide oral liquid on RIRI in rats [44]. They found that oral administration of *Ganoderma* polysaccharide (150 mg/kg and 300 mg/kg) for 7 days significantly rescued decreased SOD and increased MDA concentration in blood and kidney tissue after 24 h reperfusion. Besides, HE staining revealed that IR led to severe morphological changes including swelled and damaged tubular epithelial cells and highly dilated and congested interstitial vessels especially in the cortical medulla junction. Treatment with *Ganoderma* polysaccharide oral liquid significantly relieved such histological abnormality. The underlying mechanism was related to the bioactivities of *Ganoderma* polysaccharide in alleviating lipid peroxidation, scavenging free radical, therefore improving ischemia and hypoxia condition.

In 2015, our group comprehensively proved that GLPP had renoprotective ability in preventing renal ischemia reperfusion injury using an in vivo RIRI mouse model and an in vitro hypoxia/reoxygenation model and tunicamycin-stimulated NRK-52E cell model [28]. Results showed that occlusion of renal pedicle for 35 min and reperfusion for 24 h significantly increased the BUN and blood creatinine, as well as injured proximal tubular. However, the intraperitoneally administration of GLPP (100 mg/kg) daily for 7 days before ischemia and reperfusion significantly improved destroyed kidney function and attenuated proximal tubular damage structurally compared with vehicle control (Fig. 10.1). Further investigation indicated that post-IR treatment of GLPP could not significantly lower the IR-increased levels of BUN and blood creatinine and did not attenuate the morphological changes induced by IR.

Since IR could mediate oxidative stress that regulates the activation of mitochondrial and ER stress and further results in abnormal apoptosis, necrosis, and other serious consequences, we detected the related markers, such as myeloperoxidase (MPO), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT),

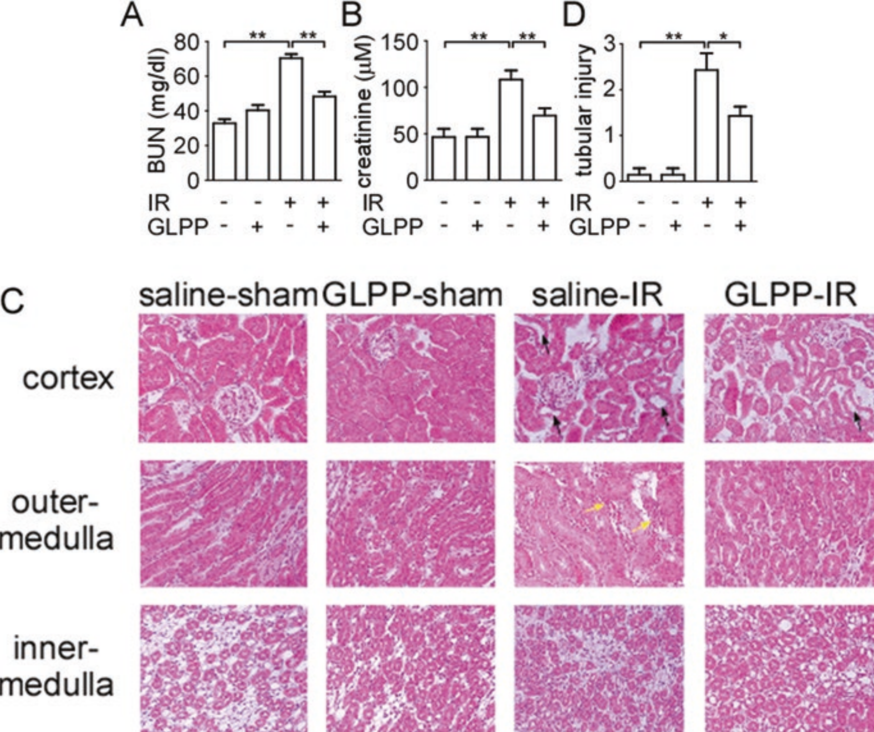

Fig. 10.1 GLPP protected kidneys against RIRI. C57BL/6 J male mice were intraperitoneally administered with vehicle or GLPP (100 mg/kg) daily for 7 days before surgery. Blood and kidney samples were collected for renal function tests and histological examination after reperfusion for 24 h. (**a**) BUN. (**b**) Blood creatinine. (**c**) Representative images of kidney tissue with H&E staining (magnification 400×). (**d**) Quantification of tubular injury. $^{*}P < 0.05$, $^{**}P < 0.01$ (quoted from [28])

reduced glutathione (GSH), and glutathione peroxidase (GSH-Px) in kidneys. Results showed that GLPP significantly reduced the abnormally elevated levels of MPO and MDA and rescued the decreased levels of SOD, CAT, GSH, and GSH-Px (Fig. 10.2a–f). Additionally, GLPP also inhibited the translocation of p47phox from cytosol to membrane (Fig. 10.2g).

All these results indicate that GLPP exerts mediating effect on renal oxidative stress and lipid peroxidation after IR. In order to uncover the underlying mechanisms, we focused on the apoptosis-related signaling pathways. Firstly, TUNEL assay and Western blot analysis confirmed that IR obviously caused renal cell apoptosis and GLPP reduced IR-induced TUNEL-positive cells by 21.75% and the ratio of p-p53/p53 and cleaved caspase-3/caspase-3 (Fig. 10.3), indicating that GLPP could protect kidneys by inhibiting IR-induced apoptosis.

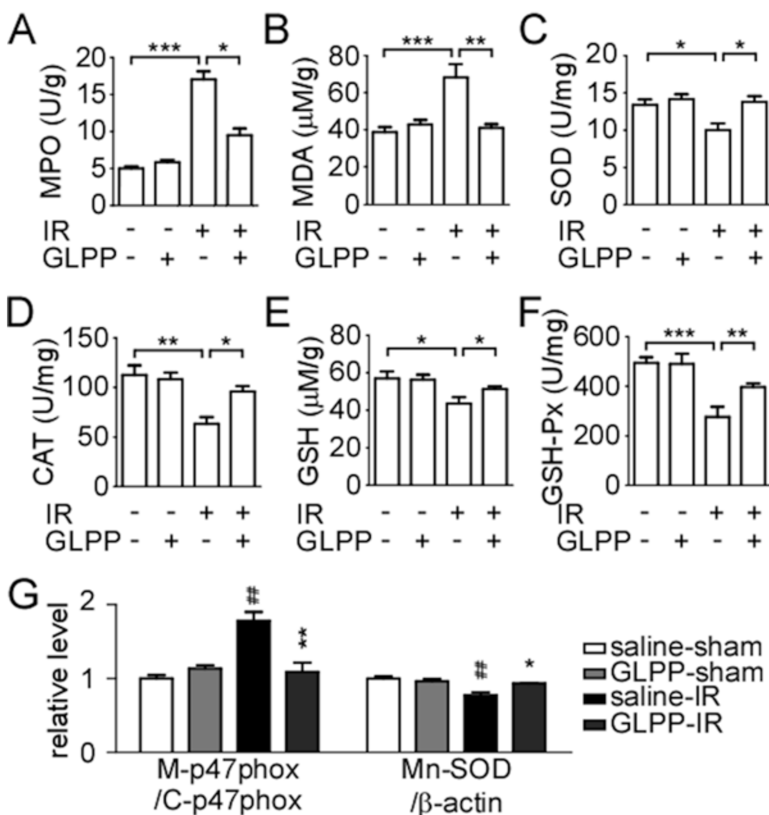

Fig. 10.2 GLPP prevented renal oxidative stress and lipid peroxidation after IR. Kidney tissues were homogenized for evaluating the levels of different enzymes. (**a**) MPO activity. (**b**) MDA concentration. (**c**) SOD activity. (**d**) CAT activity. (**e**) GSH concentration. (**f**) GSH-Px activity. (**g**) Quantification of key enzymes involved in oxidative stress and related protein. C-p47phox, cytosol p47phox; M-p47phox, membrane p47phox; T-p47phox, total p47phox. Data are presented as the mean ± SEM (n = 8 ~ 10). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. $^{\#\#}P < 0.01$ (quoted from [28])

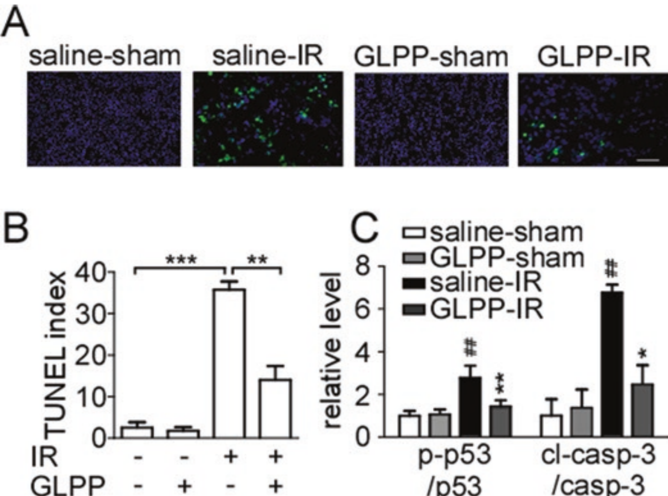

Fig. 10.3 GLPP attenuated cell apoptosis in the kidney after IR. (**a**) TUNEL staining (green fluorescence, magnification 200×) (**b**) TUNEL positive index. (**c**) Quantification of apoptosis-related protein. The data were normalized by the intensity of β-actin and related to the value of the sham. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. $^{\#\#}P < 0.01$ (quoted from [28])

In order to analyze mitochondrial-related apoptosis level, we measured the related parameters and found that GLPP significantly reduced the ratio of Bax/Bcl-2 caused by IR (Fig. 10.4a) and inhibited the release of cytochrome C from mitochondria to the cytosol, verifying that GLPP could inhibit the mitochondria-dependent apoptosis induced by IR (Fig. 10.4a). Considering that accumulated ROS also induced ER stress in IR model, we detected the level of related proteins and proved that GLPP treatment reduced the increased expression of ER stress biomarkers (GRP78, CHOP, and caspase-12) and inhibited the activation of JNK, indicating that GLPP inhibited IR-induced apoptosis partially by alleviating ER stress (Fig. 10.4b).

Subsequently, in order to assess the cytotoxicity of GLPP and to confirm the GLPP renoprotective mechanisms, they adopted hypoxia/reoxygenation (H/R) in vitro study. At concentrations from 1.1 μg/ml to 810 μg/ml, GLPP showed no obvious cytotoxicity on NRK-52E cells using CCK-8 assay and improved cell viability which was decreased after hypoxia for 12 h followed by reoxygenation for 1 h. Consistent with the in vivo results, GLPP was identified to have the effect on anti-cellular oxidative stress induced by H/R mainly through inhibiting the activation of NADPH oxidase and increasing antioxidant activity and finally reducing the accumulation of ROS in a dose-dependent manner (1, 5, and 25 μg/ml). Apart from antioxidative stress, pretreatment with GLPP reversed the upregulated ratios of p-p53/p53 and cleaved capsase-3/caspase-3 in a dose-dependent manner, which was confirmed by TUNEL analysis. All these results proved the protective effect of GLPP in inhibiting H/R-induced apoptosis in renal tubular cells.

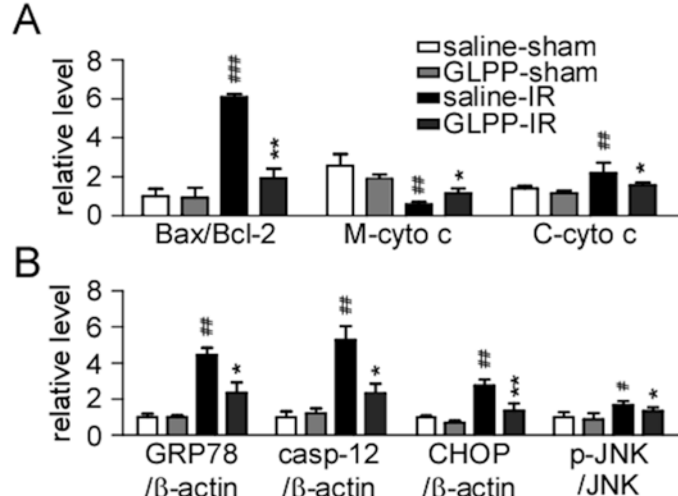

Fig. 10.4 GLPP attenuated mitochondrial and ER stress after IR. (**a**) Quantification of mitochondrial function-related proteins. (**b**) Quantification of ER stress-related proteins. M-cyto c, mitochondrial cytochrome c; C-cyto c, cytosolic cytochrome c. $^{*}P < 0.05$, $^{**}P < 0.01$. $^{\#}P < 0.001$, $^{\#\#}P < 0.01$ (quoted from [28])

Further study of the protective mechanism of GLPP in H/R-induced NRK-52E cells was carried out by fluorescent, lipophilic, and JC-1 (cationic probe) staining. It was found GLPP significantly diminished H/R-induced mitochondrial dysfunction because of the attenuated $\Delta\Psi$m dissipation, decreased Bax and increased Bcl-2, and reduced cytochrome C in cytosol. On the other hand, we explored the effect of GLPP on H/R-induced ER stress and related apoptosis by Western blot. Results showed that increased expression of GRP78, caspase-12, and CHOP induced by H/R were all reversed by GLPP. We adopted tunicamycin (TM) stimulation to create a special ER stress model and verified that GLPP relieved ER stress-induced apoptosis partially through the JNK signaling pathway.

These data suggest GLPP significantly rescued renal dysfunction and morphological damage in RIRI and reversed the imbalance of redox status and reduced ROS level. Further study demonstrated that GLPP dramatically inhibited mitochondrial- and ER stress-dependent apoptosis, which identified the beneficial effects of GLPP on IR caused AKI for the first time (Fig. 10.5).

10.3 Effect of *G. lucidum* on Autosomal Dominant Polycystic Kidney Disease

Autosomal dominant polycystic kidney disease (ADPKD) is a common monogenetic disease with a high prevalence of 1/1000 to 1/400 worldwide, characterized by the progressive development of renal cysts [45]. Progressive growth and

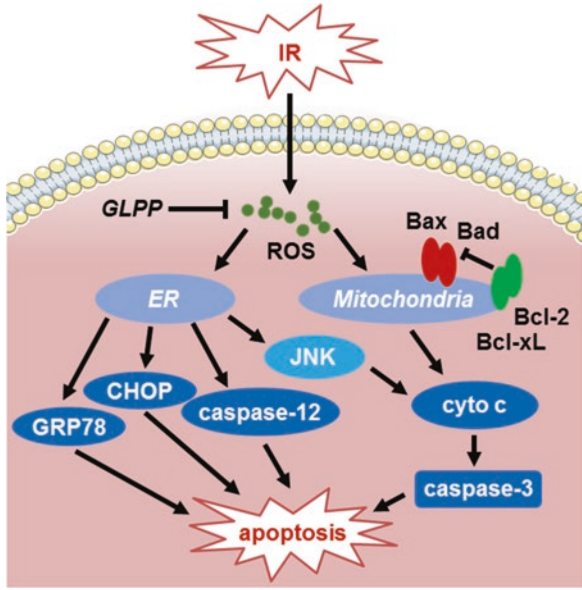

Fig. 10.5 Schematic diagram of the signal pathways involved in IR-induced apoptosis

enlargement of kidney cysts over time and subsequent destruction and replacement of the normal kidney parenchyma lead to renal dysfunction. According to certain researches, more than 50% of ADPKD patients would progress to end-stage renal disease. However, there are very limited drugs effectively used in preventing or reversing PKD progression except hemodialysis or kidney transplantation [46]. There have been many researches of underlying mechanisms of the pathogenesis of ADPKD, such as sustained proliferative signaling, suppressed cell differentiation, upregulated cellular energy, and resistance to cell death. And these characteristics share typical commonalities with solid tumors; previous studies indicated that some anticancer drugs also possess therapeutic benefit for ADPKD treatment. For example, curcumin and ginkgolide B were both shown to have anti-ADPKD activities by inhibiting related intracellular signaling pathways [47, 48].

In consideration of the similarities of ADPKD with tumor, and the reported anti-tumor effects of *Ganoderma triterpenes* (GT), in 2017, our group investigated the effect of GT on ADPKD using MDCK cyst model, MDCK tubulogenesis model, embryonic kidney cyst model, and two ADPKD mouse models [29]. Firstly, in vitro study showed that consecutive 7-day treatment of GT significantly inhibited FSK-stimulated MDCK cyst enlargement in a dose-dependent manner (6.25, 25, and 100 μg/ml) and MDCK cysts regrew to a large size after washing out GT on day 8, suggesting that such inhibitory effect of GT on MDCK cyst enlargement was reversible (Fig. 10.6a). In an embryonic kidney cyst model, 100 μM 8-bromoadenosine 3′, 5′-cyclic monophosphate (8-Br-cAMP) was used to stimulate cysts to develop and rapidly expand in cultured embryonic kidneys. In accordance with the results in MDCK cyst model, GT significantly inhibited renal cyst development in a dose-dependent manner (6.25, 25, and 100 μg/ml) (Fig. 10.6b). Washing out GT on day

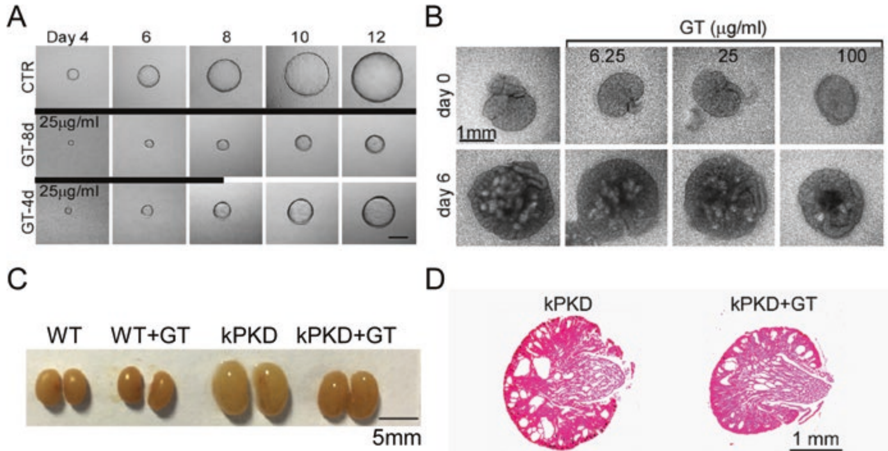

Fig. 10.6 *Ganoderma* triterpenes (GT) inhibit renal cyst development in vitro and in vivo. (**a**) MDCK cyst growth in collagen gel. Thick black lines indicate the culture time with GT. Bar = 200 mm. GT-4 d, GT washout from day 8, treated with GT only for 4 days; GT-8d, treated with GT for 8 days from day 5 to day 12. (**b**) Embryonic kidneys exposed to different GT concentrations in the presence of 100 mM 8-Br-cAMP. Bar = 1 mm. (**c**) Kidneys (bar = 5 mm) of wild-type (WT) mice and PKD mice aged postnatal 4 days treated with vehicle or GT for 4 days. (**d**) Hematoxylin- and eosin-stained pictures of kidneys in vehicle or GT-treated PKD mice. Bar = 1 mm. (quoted from [29])

4 led to the reformation and enlargement again of renal cysts, also suggesting the reversible inhibition of renal cysts by GT.

We further investigated the inhibitory activity of GT on renal cysts in vivo. Using ADPKD mouse models, we thoroughly identified and confirmed the anti-ADPKD activity of GT in vivo. Results showed that, in ADPKD mice, 100 mg/kg per day treatment from postnatal day 1 until day 4 significantly reduced the size of the cysts, kidney mass, fractional cyst area, as well as kidney index without affecting body weight and the kidney of wide-type mice (Fig. 10.6c, d).

Since the one of the key pathogenesis of ADPKD is the deficiency of tubule formation and extension, we detected the tubulogenesis of MDCK cells and cysts incubated with or without GT. Results showed that GT significantly induced the formation of more and longer tubules in a dose-dependent manner (6.25, 25, and 100 μg/ml), suggesting that GT could prevent cystogenesis by promoting tubule formation, extension, and epithelial cell differentiation.

To explore the underlying mechanisms of GT inhibiting renal cyst development, several key signaling pathways involved in ADPKD were detected using in vitro model by Western blot. After serum starvation, MDCK cells were treated with 10 mM FSK for 15 minutes that significantly elevated the phosphorylated ERK level, a critical downstream molecule of the Ras/MAPK signaling pathway. It was found that GT inhibited cyst development mainly through downregulating Ras/MAPK signaling pathway via reducing H-ras, B-raf, p-MEK, p-ERK, Egr-1, and c-fos levels and increasing Raf-1 expression in a dose-dependent manner (6.25, 25, and 100 μg/ml).

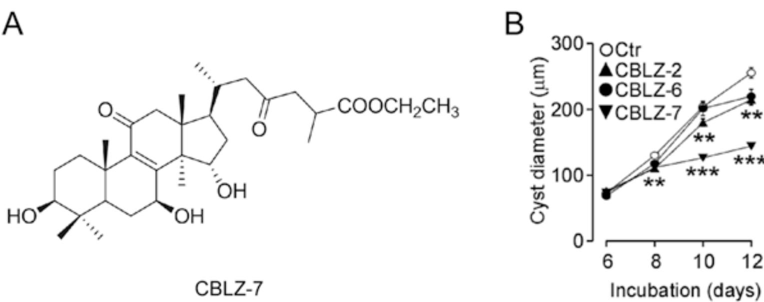

Fig. 10.7 Monomer CBLZ-7 inhibits Madin-Darby canine kidney (MDCK) cyst formation and enlargement. (**a**) Structural formula of CBLZ-7. (**b**) Cyst diameters were measured and shown as growth curves when cysts were treated without (Ctr) or with 12.5 μM CBLZ-2, CBLZ-6, or CBLZ-7 from day 7 to 12 in the presence of 10 μM FSK. Data are presented as means ± SEM. n = 30. *P < 0.05, ***P* < 0.01, ****P* < 0.001 vs control (Ctr). (quoted from [29])

Further study confirmed the effect of GT on the Ras/MAPK signaling pathway in kidneys of ADPKD mouse models. Results showed that GT treatment significantly downregulated the Ras/MAPK signaling pathway by reducing B-raf, p-ERK, and c-fos in ADPKD kidneys without detectable other effect on normal kidneys. To identify the related upstream of Ras/MAPK signaling pathway, we evaluated intracellular cAMP levels in cultured MDCK cells incubated with FSK in the presence or absence of GT and found 25 and 100 μg/ml GT significantly decreased cAMP levels in 10 mM FSK-treated MDCK cells.

Since crude GT is a mixture extractive, 15 monomers were isolated from GT, and their anticystic effect was tested using the MDCK cyst assay. It was identified that monomer CBLZ-7 (ethyl ganoderate C2) obviously inhibited cyst enlargement and had no effect in the total numbers of colonies (Fig. 10.7). Therefore, CBLZ-7 was selected as the most potent inhibitor of cyst development in vitro. Consisting with the in vivo study, CBLZ-7 downregulated the Ras/MAPK signaling pathway stimulated by FSK in MDCK cells in a dose-dependent manner (3.125, 12.5, and 50 μg/ml). On account of the limited availability of purified CBLZ-7, it was unable to examine its effect in ADPKD animal models, which will be investigated in the near future. GT have potential to be developed as novel therapeutic agents for treating ADPKD (Fig. 10.8).

10.4 Effect of *G. lucidum* on Cisplatin-Induced Nephrotoxicity

Cisplatin is a widely used antineoplastic agent in the treatment of many types of tumor such as bladder cancer, lung cancer, sarcomas, bone cancer, and lymphomas [49]. But one of the severe side effects of cisplatin chemotherapy is the toxicity profile, including nephrotoxicity characterized as tubular dysfunction. Such nephrotoxicity has most commonly limited the effective use of this drug. In 1999, Gui et al.

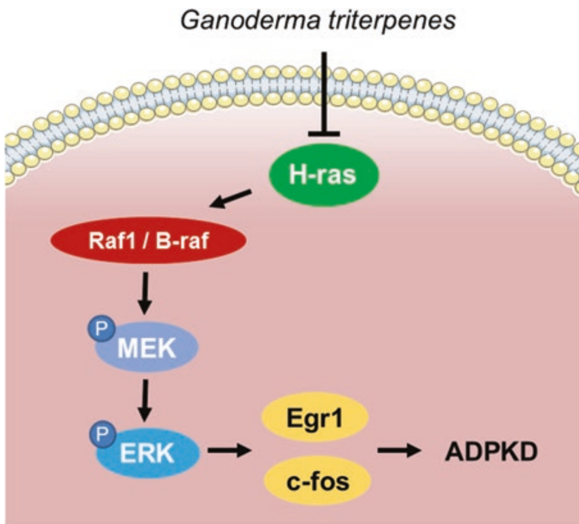

Fig. 10.8 Schematic diagram of the signal pathways involved in GT retarding renal cyst development in ADPKD

firstly identified the anti-cisplatin-induced nephrotoxicity activity of *G. lucidum* injection in Wistar rats [50]. They found that 5-day treatment with *G. lucidum* injection (5, 10, 20 ml/kg) significantly decreased the MDA and increased the SOD level in blood and kidney cortex. Functionally, *G. lucidum* administration reduced BUN and blood creatinine and significantly lowered them compared with model group. Histological detection also confirmed that *G. lucidum* injection relieved cisplatin-induced renal cortical tubular cell swell and damage and lowered the ALP level, suggesting the effect of *G. lucidum* on decreasing cisplatin-induced nephrotoxicity.

In 2011, Pillai et al. used cisplatin-induced nephrotoxicity mouse model to identify the renoprotective activity of total terpenes isolated from *G. lucidum*. They found that a single dose of cisplatin led to the inhibition of renal antioxidant enzyme activity, decrease of renal GSH levels, and increase in serum urea, creatinine, and ALP levels. Treatment with *G. lucidum* terpenes (50 and 100 mg/kg) ameliorated tubular injury and significantly rescued antioxidant activity by elevating GSH, SOD, CAT, and GPX and by decreasing MDA, exerting renal protection against cisplatin-induced nephrotoxicity [30]. The experimental results indicate that *G. lucidum* terpenes could exert antioxidant activity and possess the renal protection effect in cisplatin-induced nephrotoxicity and potential use in adjuvant therapy of cancer (Fig. 10.9).

10.5 Effect of *G. lucidum* on Diabetic Nephropathy

Diabetes mellitus, characterized by hyperglycemia and long-term complications, is the most common endocrine disorder in the world. Among those complications, diabetic nephropathy (DN) is a major complication of diabetes and a leading cause of

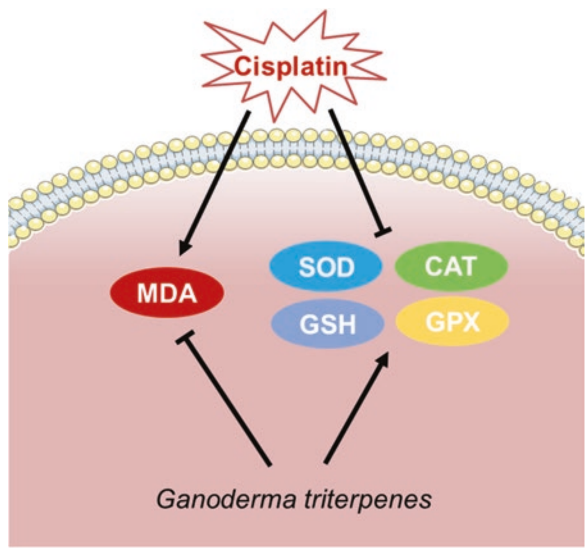

Fig. 10.9 Schematic diagram of the mechanism involved in *Ganoderma triterpenes* treating cisplatin-induced nephrotoxicity

end-stage renal failure throughout much of the world. As a deterioration of renal function and glomerular structure, DN is the major cause of morbidity [51]. So, it is pretty valuable to develop antidiabetic drugs with renal protection potential. Recently, *G. lucidum* and its extractions have become increasingly recognized as an alternative source for inhibiting DN. In 2014, Pan et al. investigated the effects of a novel proteoglycan (FYGL) isolated from *G. lucidum* fruiting bodies in protecting renal function and morphology in diabetic mice [32]. The study showed that FYGL dose-dependently (75, 250, and 450 mg/kg) decreased the blood glucose and slowed the age-dependent insulin decline. Specially, 8-week administration of FYGL ameliorated the DN by three possible pathways: (1) FYGL directly eliminated ROS and suppressed lipid peroxidation, therefore protecting oxidative stress of renal cell; (2) FYGL indirectly scavenged the radicals via activating antioxidant enzyme systems to improve oxidative injury in kidney tissues; and (3) FYGL chelated with metal ion by forming cross-bridge between carboxyl groups in galacturonic acid, decreasing ROS generation. Further study showed FYGL significantly rescued the reduced blood LDL-c levels and the increased HDL-c levels caused by profound alterations in lipid and lipoprotein profiles in db/db mice. The data indicate that FYGL isolated from *G. lucidum* fruiting bodies has the antidiabetic nephropathy advantages (Fig. 10.10).

Using streptozotocin-induced type 1 diabetic mouse model, Lin et al. identified that *G. lucidum* polysaccharides (GL-PS) (125 and 250 mg/kg) can prevent or delay the progression of diabetic renal complications [31]. They identified that GL-PS mainly decreased blood glucose and triglyceride levels in diabetic model mice, suggesting the metabolic modulation of GL-PS. Consistent with other studies, the effect of GL-PS on the oxidative stress in the development of diabetic nephropathy was confirmed. GL-PS could decrease the level of MDA and increase the level of SOD and effectively improve the metabolic imbalance in DN. Further, GL-PS dose-

Fig. 10.10 Schematic diagram of the mechanism involved in FYGL treating diabetic nephropathy

dependently (125 and 250 mg/kg) reduced the TGF-β expression that was abnormally elevated and played a crucial role in the development of pathologic lesions of diabetic nephropathy. Thus, GL-PS can protect diabetic nephropathy through the amelioration of metabolic disorders, oxidative stress, and renal dysfunction associated with renal lesions. In 2008, Li et al. adopted streptozotocin-induced diabetic rats to study the antidiabetic nephropathy effect of GL-PS [52]. Specially, 8-week administration of STZ administration induced typical of diabetic nephropathy including the decreased MMP-2 and TIMP-2. Interestingly, GL-PS significantly rescued the expression of MMP-2 and TIMP-2 and decreased the accumulation of extracellular matrix in a dose-dependent manner (100, 200, and 400 mg/kg), suggesting the renoprotective effect of GL-PS mainly depends on the rebalance of MMP-2/TIMP-2.

10.6 Effect of *G. lucidum* on Adriamycin-Induced Nephropathy

Adriamycin-induced nephropathy is a widely acknowledged CKD mouse model, characterized by initial podocyte injury and albumin urine and subsequent renal inflammation and fibrosis. In the pathogenesis of Adriamycin-induced nephropathy, increasing evidence suggests that oxidative stress including excess ROS generation associated with renal injury and the nuclear factor erythroid 2-related factor2 (Nrf2) has been recognized as the major role in cellular defense on account of its ability to upregulate the expression of antioxidant genes [53]. Based on previous in vitro study of the antifibrotic activity of meroterpenoid possessing an unusual 5/5/6/6 ring system isolated from *G. lucidum*, Yan et al. found that lingzhilactones B (25 mg/kg)

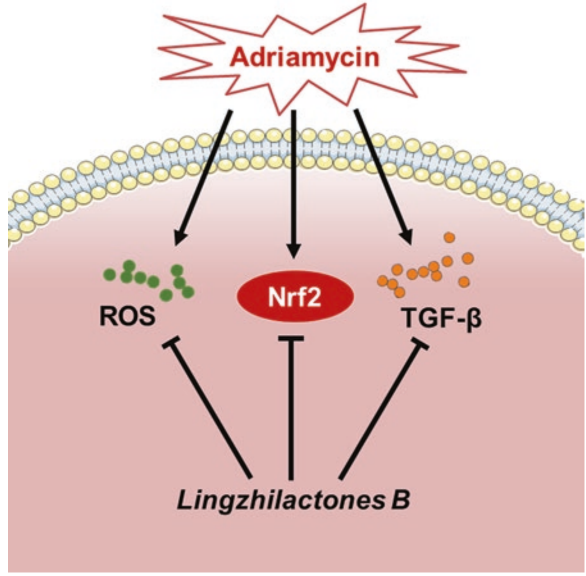

Fig. 10.11 Schematic diagram of the mechanism involved in lingzhilactones B treating Adriamycin-induced nephropathy

could protect Adriamycin-induced nephropathy by dose-dependently inhibiting TGF-β signaling and the generation of ROS and activating the Nrf2 in vivo [33]. Furthermore, lingzhilactones B reduced urinary albumin levels and abrogated myofibroblastic activation in this CKD model. These findings provide a promising structure template for anti-CKD drug design (Fig. 10.11).

10.7 Effect of *G. lucidum* on Albumin-Induced Human Proximal Tubular Epithelial Cell Damage

Apart from these in vivo studies, many in vitro researches also proposed the anti-chronic kidney disease potential of *G. lucidum.* The components of *G. lucidum* have a wide range of pharmacological activities including suppressing inflammation and scavenging free radicals. In many forms of chronic renal diseases, the severity of tubulointerstitial injury correlating with the amount of proteinuria is a major determinant of the degree and rate of progression of diseases. Several researches indicated that excess proteinuria-induced interstitial injury was mainly attributable to concomitant presence of tubulointerstitial inflammation. Lai et al. studied the effect of *G. lucidum* polysaccharides (4, 8, and 16 μg/ml) in anti-albumin-induced human proximal tubular epithelial cell oxidative damage and apoptosis and release of chemokines in vitro, suggesting the *G. lucidum* polysaccharides can be developed into a novel anti-inflammation drug targeting further downstream of proteinuria pathological processes [34] (Fig. 10.12).

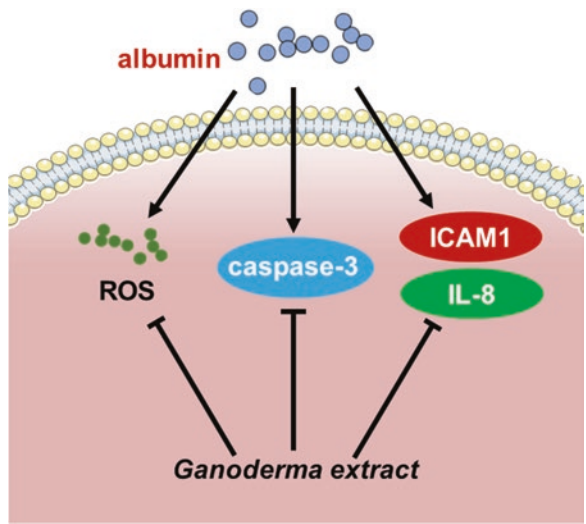

Fig. 10.12 Schematic diagram of the mechanism involved in *Ganoderma* extract treating albumin-induced human proximal tubular epithelial cell damage

10.8 Effect of *G. lucidum* on Oxidative Damage and Fibrotic Process of Renal Proximal Tubular Cells

In 2013, Yan et al. found that both (+)-Lingzhiol and (−)-Lingzhiol isolated from *G. lucidum* could significantly inhibit high-glucose-induced generation of ROS, collagen IV, fibronectin, and IL-6 and induce the transcription of Nrf2 that upregulated the expression of antioxidant genes to protect cells from oxidative damage in a dose-dependent manner (1, 3, 10, and 30 μM) [35]. Further study showed that phosphorylation of Smad3 of rat renal proximal tubular cells (NRK-52E) under the stimulation of TGF-β1 was significantly inhibited by (−)-Lingzhiol, indicating the selective inhibition effect on p-Smad3. Considering the pivotal role of TGF-β/Smads pathway in CKD and renal fibrosis, (−)-Lingzhiol can be developed into natural selective p-Smad3 inhibitor in treating chronic kidney disease.

In 2014, Dou et al. isolated two meroterpenoids with novel polycyclic skeletons from *Ganoderma cochlear* and identified their renoprotective bioactivity using TGF-β1-stimulated NRK52E cells [36]. The results showed that both racemic (+)- and (−)-cochlearols exerted renoprotectivity by inhibiting the expression of renal fibrosis related markers such as collagen I, fibronectin, and α-SMA in a dose-dependent manner (5, 10, and 20 μM). Moreover, these two compounds inhibited the phosphorylated Smad2 and Smad3, suggesting their antifibrotic action depended on TGF-β/Smads pathway. Compared with cochlearols A and (+)-cochlearols B, (−)-cochlearols B displayed apparent inhibitory effect on TGF-β1 induced activation of p-Smad2 and p-Smad3, disrupting the fibrotic kidney related TGF-β/Smads signaling (Fig. 10.13). The current studies provided potential role of (−)-cochlearols B in the therapy of renal fibrosis.

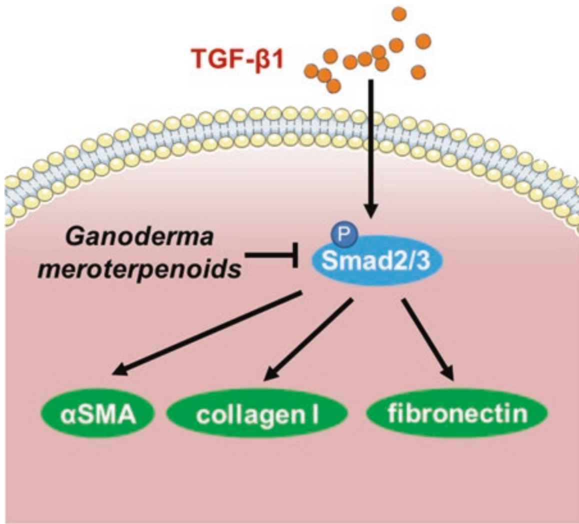

Fig. 10.13 Schematic diagram of the mechanism involved in *Ganoderma* meroterpenoids treating fibrotic process of renal proximal tubular cells

10.9 Clinical Applications

There are limited clinical research and reports on the usefulness of *G. lucidum* in treating renal diseases. In 2003, Xiao et al. reported their clinical observation on the effect of *G. lucidum* decoction (GLD) in treating *Russula subnigricans* poisoning (RSP) patients. In 14 patients of RSP treated with GLD (100 g of *Ganoderma lucidum* decocted with water to 600 ml), they found that RSP-induced kidney injury and GLD markedly lowered urine N-acetyl-D-glucosaminidase, red blood cell, and protein, demonstrating the renoprotective efficacy of *G. lucidum* compared with 11 patients who received conventional therapy [37]. In 2004, Narisa et al. conducted a clinical study in 14 nephrotic patients with focal segmental glomerulosclerosis (FSGS); results showed that treatment with *G. lucidum* successfully ameliorated injured kidney by inhibiting endothelial cell cytotoxicity, restoring immunocirculatory balance, and suppressing proteinuria [38]. Nevertheless, there are few reports on renoprotective effect of *G. lucidum* in human subjects. Further clinical researches are recommended in the future.

10.10 Conclusion and Perspectives

Comprehensive researches and overwhelming evidences have proved that *G. lucidum* contains a wide variety of bioactive components that have potent renoprotective effects and promote health. In this chapter, we reviewed researches and showed abundant evidences that triterpenoids and polysaccharides isolated from *G. lucidum* possess potent activities on anti-renal diseases. Based on these studies, we

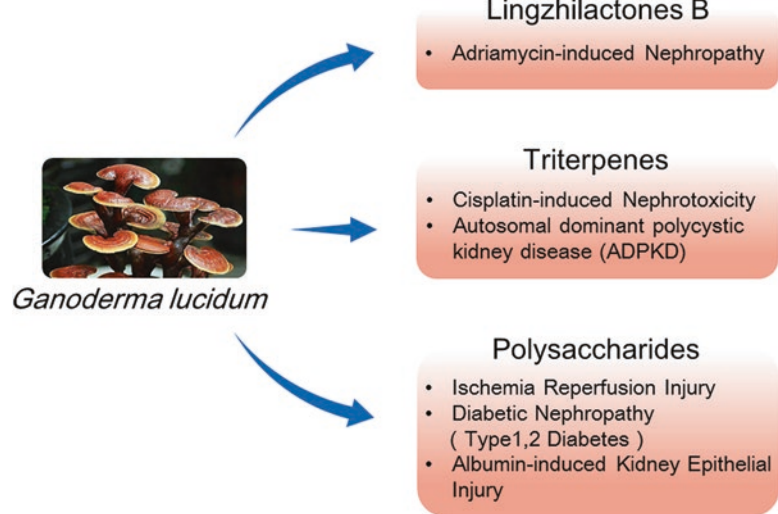

Fig. 10.14 Summary of anti-renal diseases effects of bioactive components of *G. lucidum*

can clearly conclude that the components of *G. lucidum* exhibited renoprotective effects mainly through antioxidant, anti-apoptosis, anti-proliferation signaling pathways such as Ras/MAPK and anti-TGF-β/Smad signaling pathways.

However, the studies were performed mostly in animals or in cell models; well-designed and more reliable clinical researches are needed to assess and clarify the authentic biological activities of *G. lucidum* in humans. Since the approaches to attenuate or reverse kidney diseases are limited, further investigations of *G. lucidum* could facilitate the development of new therapeutic strategies in treating various kidney diseases (Fig. 10.14).

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Chapter 11
Anti-osteoporosis Effect of *Ganoderma* (Lingzhi) by Inhibition of Osteoclastogenesis

Yajun Yang and Baoxue Yang

Abstract The anti-osteoporosis effect of *Ganoderma lucidum* (*G. lucidum*, Lingzhi) is closely associated with inhibition of osteoclastogenesis in the charge of osteoclasts. This article reviewed the anti-osteoporosis effect of *Ganoderma* and its active components, including a kind of triterpenoids, a polysaccharide and a protein named Ling Zhi-8 (LZ-8). Triterpenoids are a kind of active compounds as candidates for the treatment of osteoporosis. Among these, ganoderic acids are currently considered as the most important and potential components, and their structure-activity relationship highlights the essential group to hamper osteoclast differentiation. The data confirmed that the active compounds isolated from triterpenoids and meroterpenoids could suppress bone resorption of osteoclast via RANKL/RANK pathway and/or its downstream signaling transduction related to ERK, JNK and p38 MAPKs, contributing to inhibition of the level of c-Fos and NFATc1, two key target genes of osteoclast for osteoclastogenesis. However, the comprehensive mechanism remains to be elucidated in the future.

Keywords *Ganoderma* · Lingzhi · Ganoderic acids · Osteoclastogenesis · Osteoporosis

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration in bone tissue, leading to enhanced bone fragility and increased fracture risk [1]. It is a silent disease, affecting about 200 million people worldwide and responsible for 8.9 million fractures annually all over the world [2]. Osteoporotic fractures are the most devastating consequence of this disease, contributing to significant burden of healthcare cost, morbidity and mortality [3].

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Annual costs of osteoporosis among persons aged 65 and older were estimated at $6.1 billion only in the United States [4]. According to the mechanisms of bone metabolism, the anti-osteoporotic strategies are classified into three types clinically: anti-resorptive therapies, anabolic treatment and bone mineralization drugs [5].

Ganoderma lucidum (*G. lucidum*) is commonly used in traditional Chinese medicine. It is recorded by Compendium of Materia Medica that *G. lucidum* can strengthen the bones and muscles and disinhibit the joints [6]. In the past, the development of anti-osteoporosis formulas was mainly pursued by scientists in Asian countries, including China, Japan and Korea [7–9]. *G. lucidum* can be used mainly to study the inhibitory effect on bone loss induced by ovariectomy [10], and its mechanism involved in inhibition of bone resorption of osteoclast regulated by RANKL/RANK pathway [11]. This article reviewed the anti-osteoporosis effect of *Ganoderma* and the underlying mechanisms.

11.1 Anti-osteoporosis Effect of *G. lucidum* by Inhibiting Function of Osteoclasts

Adult male albino rats were fed with diet supplementation contained of a fine powder ground from dried spore of *G. lucidum* (10~50 $g{\cdot}kg^{-1}$). Anti-osteoporotic activity of *G. lucidum* could be stimulated either by binding with estrogen receptors which reveal responses at the cellular and molecular levels or by improving the serum mineral content associated with bone health such as calcium, iron and phosphorus [12]. Ethanol extract from *G. lucidum* could prevent ovariectomy-induced bone loss and downregulate the blood serum osteocalcin level, the same as the action of 17β-estradiol (an endogenous estrogen). Bone loss triggered by estrogen deficiency can also be hampered by ethanol extract from *G. lucidum* [12]. Further, ethanol extracts of *G. lucidum* clearly suppressed osteoclastogenesis from the RAW 264 cell D-clone induced by RANKL and TNF-α. These results suggested the direct action of *G. lucidum* on osteoclast precursors to suppress osteoclastogenesis [10]. Other evidence showed that decoction extract of *Ganoderma sinense* fruiting body exerted a good stimulatory effect on fracture healing, including the formation of callus, disappearance of fracture line, restoration of marrow cavity and almost cure of the fracture. The beneficial effect on fracture was linked to its regulation to Ca homeostasis, phosphorus level and alkaline phosphatase content, contributing to increase of cartilage connection [13]. In brief, these evidences may provide some clues to development and research of anti-osteoporosis agents from *Ganoderma*.

11.2 Anti-osteoporosis Effect of Ganoderic Acids, the Underlying Mechanism, and the Structure-Activity Relationships

A significant kind of components of *G. lucidum* are triterpenoids, from which over 120 different compounds have been isolated up to now [14]. Among triterpenoids, ganoderic acid DM has been found firstly to show inhibitory activity against osteoclastic differentiation [10, 15]. In brief, both 0.03 and 0.3% ethanol extracts of *G. lucidum* diet could prevent against the ovariectomy-induced bone loss in 11-week-old female SD rats [10]. From the separation of the ethanol extracts of *G. lucidum* by a silica gel column guided with a blocking effect of the formation of osteoclast like multinucleated cells from the RAW 264 cell D-clone, ganoderic acid DM was isolated as one of the effective active compounds, and it (12.5 μM) clearly blocked osteoclastogenesis by inhibiting the osteoclasts differentiation [15]. Further study revealed that ganoderic acid DM (20 μM) suppressed the expression of c-Fos and nuclear factor of activated T cells c1 (NFATc1), two crucial transcription factors for osteoclast formation, leading to inhibition of dendritic cell-specific transmembrane protein (DC-STAMP) expression and reducing transformation toward osteoclast fusion [10].

Later, ganoderic acid DM and other components with similar structure were analyzed comparatively by its structure-activity relationship methods. The data indicated that not all ganoderic acids depressed osteoclast differentiation (Fig. 11.1 and Table 11.1). Among them, ganoderic acid D (1), ganoderic acid G (3) and ganoderic acid I (4) showed neither inhibition of osteoclast differentiation nor cytotoxicity. In contrast, both ganoderic acid F (2) and ganoderic acid DM (5) showed the two activities [16]. The concentrations leading to 50% inhibition (IC_{50}) of differentiated cells and viable cells lost were established at 32 and 40 μM for ganoderic acid F (2) and at 30 and 65 μM for ganoderic acid DM (5). Interestingly, ganoderic acid F (2) and ganoderic acid DM (5), which showed inhibition of osteoclast differentiation, have a carbonyl in C7 (Table 11.2). In contrast, the other three ganoderic acids (1, 3, and 4), which showed no inhibitory effect on osteoclast differentiation, have a hydroxy in C7. Despite different functional groups at R3, R4, R5, and R7, ganod-

Fig. 11.1 Structures of ganoderic acids isolated from *G. lucidum*

Table 11.1 Ganoderic acids (1–4) isolated from *G. lucidum*

Compounds	R_1	R_2	R_3	R_4
Ganoderic acid D (1)	O	OH / H	H_2	H
Ganoderic acid F (2)	O	O	OAc / H	H
Ganoderic acid G (3)	OH / H	OH / H	OH / H	H
Ganoderic acid I (4)	OH / H	OH / H	H_2	OH

Table 11.2 IC_{50} value of each ganoderic acid for inhibition of differentiation and cytotoxicity

Compounds	IC_{50} (μM) Inhibition of differentiation	Cytotoxicity
Ganoderic acid D (1)	> 400	> 400
Ganoderic acid F (2)	32	40
Ganoderic acid G (3)	> 400	> 400
Ganoderic acid I (4)	> 400	> 400
Ganoderic acid DM (5)	30	65

eric acid F and ganoderic acid DM showed similar osteoclast differentiation IC_{50} values. These results suggested that a carbonyl in C7 is essential to elicit inhibition of osteoclast differentiation among ganoderic acids. The same tendency was also observed among ganoderic acids regarding cytotoxicity to osteoclasts. In summary, ganoderic acid F and ganoderic acid DM inhibited osteoclast differentiation, and a carbonyl in C7 is an essential group to elicit the inhibitory effect on osteoclast differentiation. Thus, the two compounds might be used as a novel therapeutic strategy for osteoporosis due to inhibiting osteoclast differentiation.

11.3 Anti-osteoporosis Effect of Other Components without Triterpenoid-like Structure and Mechanism

Recently, the anti-osteoporosis effect of Ganomycin I (GMI), a meroterpenoid isolated from Vietnamese mushroom *G. lucidum*, was reported. The study confirmed that GMI could attenuate RANKL-mediated osteoclastogenesis in mouse BMMs and RAW264.7 cells. Specifically, GMI (3 μM, 10 μM, and 30 μM) significantly

inhibited RANKL-induced osteoclast differentiation by decreasing the number of osteoclasts, osteoclast actin-ring formation and bone resorption in a dose-dependent manner without affecting cell viability. At molecular level, GMI inhibited the RANKL-induced phosphorylation of ERK, JNK, and p38 MAPKs, as well as the expression levels of c-Fos and NFATc1. In addition, GMI decreased expression levels of osteoclastogenesis-specific marker genes including c-Src, CtsK, TRAP, MMP-9, OSCAR and DC-STAMP in RANKL-stimulated BMMs. The evidences suggest that GMI can attenuate osteoclast formation by suppressing RANKL-mediated MAPKs and NFATc1 signaling pathways (Fig. 11.2) [17]. However, the detailed mechanism by which GMI suppresses RANKL-induced activation of MAPKs remains to be elucidated in vivo.

Polysaccharide is a significant kind of component of *G. lucidum*. A polysaccharide preparation (1 g·kg^{-1}, 5 g·kg^{-1} and 10 g·kg^{-1}) for subcutaneous injection extracted from Taishan red *G. lucidum* showed a protective effect on bone mass in ovariectomized rats [18]. However, the indices of bone size and bone mass were evaluated according to the subjective scores, and the data were insufficient to confirm anti-osteoporosis effect. Therefore, it should be further consolidated by more specific assay.

A protein named Ling Zhi-8 (LZ-8, Mr = 13,100) was purified from the mycelia of *Ganoderma tsugae* (a Chinese mushroom Songshan Lingzhi), and it has shown mitogenic activity in vitro and in vivo to have immunomodulating activity [19]. A

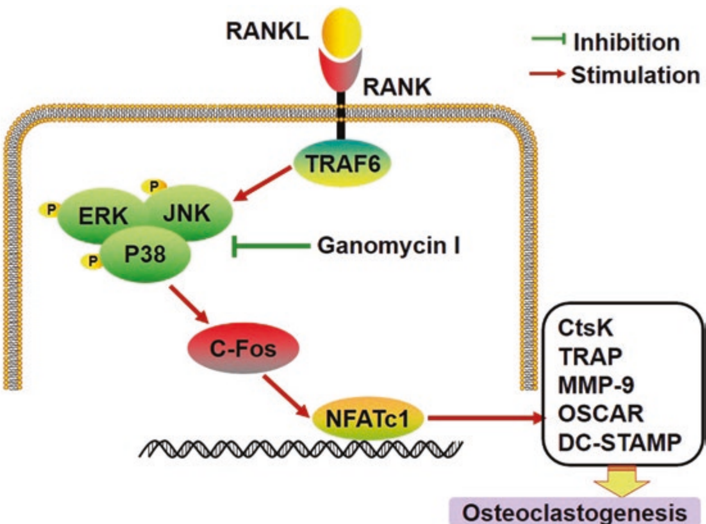

Fig. 11.2 GMI can attenuate osteoclast formation by suppressing RANKL-mediated NFATc1 signaling pathways**.** Note: *RANK* receptor activator of nuclear factor-κB, *RANKL* receptor activator of nuclear factor-κB ligand, *TARF6* TNF-receptor-associated factor 6, *ERK* extracellular signal-regulated kinase, *JNK* c-Jun N-terminal kinase, *NFATc1* nuclear factor of activated T cells cytoplasmic 1, *TRAP* tartrate-resistant acid phosphatase, *MMP-9* matrix metallopeptidase 9, *OSCAR* osteoclast-associated receptor, *DC-STAMP* dendritic cell-specific transmembrane protein

synthetic biodegradable polyurethane named Nasopore was prepared as the carrier for LZ-8 (0.1 mg) in a cylindrical shape (4 mm in diameter and 4 mm in height) of the nasal bone in the male New Zealand rabbits. The results showed that the biomaterial implants using LZ-8 could promoted osteogenetic capabilities through stimulation of the new bone volume in the rabbit sinus model [20]. Chronic inflammatory diseases harm bone growth next to orthopedic implants. Recombinant LZ-8 (rLZ-8, 1 μg/ml) treatment was found to induce the expansion of both murine and human CD4(+) T cells into FOXP3(+) regulatory T (Treg) cells through a CD45-mediated signaling pathway and the CD18-dependent induction of IL-2 [21]. A nano-protein extract from *G. lucidum* can greatly suppress the proliferation of macrophages, thus potentially inhibiting harmful acute and chronic inflammation, which may be the basis of its bone-protective effects [22]. To further investigate the osteogenetic potential of this immunoregulatory protein isolated from *Ganoderma*, rLZ-8 was cloned and expressed in *Pichia pastoris* to explore its anti-osteoporosis effect in a rat model of glucocorticoid-induced osteoporosis (GIO). The results showed that treating rats by intraperitoneal injection with the varied doses of rLZ-8 (28 $\mu g \cdot kg^{-1}$, 56 $\mu g \cdot kg^{-1}$ and 112 $\mu g \cdot kg^{-1}$) could exert a protective effect on the impaired bone trabecular structure and bone metabolic disorders triggered by glucocorticoid through recovering the balance between bone resorption and bone formation via regulation of the OPG/RANKL/RANK pathway [23]. Further investigations regarding the mechanism and application of this protein in osteogenesis are necessary.

In summary, *Ganoderma* is regarded as a vital medicinal and nutraceutical source as a result of the abundance of pharmacological active compounds obtained from fruit bodies, mycelium and spores. This review summed up the protective effects of *Ganoderma* on bone diseases, especially osteoporosis. This attempt highlighted the significance of anti-osteoporosis effect based on triterpenoids and other kinds of components it possesses. These pharmacological properties to intervene osteoporosis require further in vivo studies and clinical trials to confirm the efficacy and safety.

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Chapter 12
Antioxidative and Free Radical Scavenging Activity of *Ganoderma* (Lingzhi)

Zhibin Lin and Aoyi Deng

Abstract This article reviewed the antioxidative effect and free radical scavenging activity of *Ganoderma lucidum* (*G. lucidum*, Lingzhi). *G. lucidum* has apparent protective effects against the lipid peroxidation caused by a variety of factors in the brain, heart, pancreas, liver, gastrointestinal, kidney, and other vital organs. *G. lucidum* can significantly reduce the content of lipid peroxidation product such as malondialdehyde (MDA) and lipofuscin and can enhance the activities of antioxidative enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-P), and other antioxidative enzymes. In macrophages (mouse), islet cells (mouse), cerebral cortex cells (rat), pheochromocytoma cells (PC12 cells, rat), vascular endothelial cells (rat, human), and keratinocytes (human), *G. lucidum* has significant protective effect against the oxidant-induced oxidative damage. Antioxidative effect and free radical scavenging activity of *G. lucidum* on different animal models in vivo and in vitro may be related to its pharmacological mechanism of immunomodulating, antitumor, antihypertensive, hypoglycemic, brain-protective, liver-protective, cardiovascular-protective, renal-protective, and antiaging effects.

Keywords *Ganoderma lucidum* · Lingzhi · Antioxidative · Free radical scavenging · Mechanism

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12.1 Introduction

Ganoderma lucidum (Leyss. ex fr.) Karst. (Lingzhi) is a medicinal fungus with long history in China as a sovereign remedy. Lingzhi was highly ranked as an herbal medicine in *Shennong Ben Cao Jing* (*Shen Nong Materia Medica*) that was published in the second century B.C. Li Shi-Zhen, a well-known ancient Chinese physician, also described the efficacy and medical uses of Lingzhi in the world-renown classic *Ben Cao Gang Mu* (*Compendium of Materia Medica*) in the sixteenth century. Ancient Chinese medical scholars suggested that Lingzhi could strengthen body resistance and consolidate the constitution of patients, i.e., "Fuzheng Guben," which is one of the major principles in the therapeutics of traditional Chinese medicine (TCM).

In the *Pharmacopoeia of the P.R. of China* (2000, 2005, 2010, and 2015 edition, Part 1), both *Ganoderma lucidum* (Chi Zhi) and *Ganoderma sinensis*(Zi Zhi)are listed as Lingzhi. The use of Lingzhi as a drug or health food is on the rise in China. The main chemical constituents of *G. lucidum* consist of polysaccharides, triterpenes, nucleosides, steroids, alkaloids, protein, amino acids and peptides, inorganic elements and fatty acids, etc. Among these ingredients, polysaccharides, triterpenes, steroids, and small molecular proteins appear to be the major components with significant pharmacological effects. A great deal of experimental evidence has accumulated in the past decades, suggesting that *G. lucidum* polysaccharides have wide pharmacological activities, such as immunomodulating, antitumor, antiatherosclerosis, lipid-lowering, hypoglycemic, liver-protective, antioxidative and free radical scavenging and antiaging activities, etc. (Lin ZB 2015) [1].

The antioxidative effect and free radical scavenging activity of *G. lucidum* have been the subject of academic research. A great deal of experimental evidence has suggested that the pharmacological activities of *G. lucidum* are related to antioxidative and free radical scavenging activity.

Free radicals are active substances produced during the metabolism of cells. They can induce oxidation reactions, cause superoxidative degeneration of various unsaturated lipids in biofilms, and form lipid peroxides, causing changes in cell structure and function and therefore resulting in damage to tissue and organ. Under normal condition, the production and elimination of free radicals in the body are in dynamic equilibrium. However, if the free radicals are produced too much or the removal function are suppressed, a vast of free radicals will inevitably cause damage to the body: (1) damage to cell lipids and cell membranes; (2) damage to proteins and enzymes; and (3) destroying nucleic acids and chromosomes. The pathological processes of many diseases such as aging, tumors, cardiovascular and cerebrovascular diseases, inflammation, and autoimmune diseases are related to lipid peroxidation and excessive free radical production. The antioxidative and free radical scavenging effects by *G. lucidum* are related to its therapeutic mechanism of preventing and treating many diseases, such as chronic bronchitis, hypertension, hyperlipidemia, diabetes, hepatitis, tumor, and aging. The pathological mechanisms of all these diseases are associated with the reactive oxygen species (ROS) (Yang HL et al. 1998; Hessler R et al. 1979; Ceriello A et al. 2000; Mecocci P et al. 1993) [2–5].

12.2 Protective Effect of *Ganoderma lucidum* on Oxidative Damage of Macrophages

G. lucidum polysaccharide peptide (GLPP) is isolated from *G. lucidum* (leyss.ex fr.) karst with an average molecular weight of 5.13×10^5 and contained 16 kinds of amino acids as follows: Asp 8.49, Thr 3.58, Ser 3.93, Glu 5.81, Gly 3.50,Ala 3.84, Cys1.06, Val 2.68, Met 5.33, Iso-Leu 0.25, Leu 1.5, Phe 1.99, Lys 3.30, His 1.21, Arg 3.94, and Pro1.22 (mg/g). The polysaccharides peptide consisted of rhamnose, xylose, fructose, galactose, and glucose with molar ratio of 0.549:3.614:3.167:0.556:6.89 and linked together by β-glycosidic linkages (Lin SQ et al. 2003) [6]. Our primary studies demonstrated that GLPP decreased oxidation of low-density lipoprotein (LDL) and the relative electrophoretic mobility (REM) of oxidative product of LDL, reduced the content of malondialdehyde (MDA), and increased glutathione peroxidase (GSH-Px) enzyme activity in blood and heart of mice (You YH and Lin ZB 2003) [7].

Tertbutylhydroperoxide (tBOOH), as a membrane-permeant oxidant, has been extensively used as a model of oxidative injury in different cells in vitro. Result by light microscopy and electron microscopy showed that intraperitoneal injection of GLPP (50, 100, 200 mg/kg) significantly increased cell viability and significantly improved morphological changes of macrophages caused by oxidative damage of tBOOH, such as inhibition of macrophage membrane degeneration and necrosis. Protected cell membrane microvilli and mitochondria from tBOOH damage and restored mitochondrial membrane potential of macrophages reduced by free radical damage (You YH and Lin ZB 2002, 2005) [8, 9]. In addition, using alloxan and tBOOH as oxidants, mouse peritoneal macrophages were damaged in vitro and in vivo, and 2′,7′-dichlorofluorescein diethyl ester (DCHF-DA) was used as a fluorescent indicator. The fluorescence changes of macrophages and the dynamic changes of fluorescence were observed by confocal microscopy. The scavenging effects of GLPP on free radicals in mouse peritoneal macrophages were studied. The results showed that intravenous injection of alloxan (75 mg/kg) or in vitro addition of tBOOH (7.76×10^{-5} mol/L) can cause oxidative damage of mouse peritoneal macrophages and increase the fluorescence density of macrophages. Injecting GLPP into mice or using them to macrophages cultured in vitro can reduce the fluorescence density of macrophages and reduce the damage. Confocal microscopy time series scans show that GLPP can reduce the fluorescence density of mouse peritoneal macrophages at rest and reduce the peritoneal macrophages of mice exposed to PMA (50 nmol/L). Fluorescence density indicates that GLPP has antioxidation effect and has a scavenging effect on free radicals produced by oxidant-damaged mouse peritoneal macrophages (You YH and Lin ZB 2004) [10].

Li MC et al. (2000) used a confocal microscope to dynamically monitor the effect of *G. lucidum* polysaccharide (GLB_7) on the reactive oxygen species content of mouse peritoneal macrophages. It was found that the macrophage cultured in vitro was treated with 20 μg/mL GLB_7 and the fluorescence intensity of the fluorescent probe (DCHF-DA) was significantly decreased and the average decrease was (36 ± 6) % compared with the resting level. The macrophage cultured in vitro was stimulated

with 12-myristate-13-acetate phorbol ester (PMA), and the fluorescence intensity of DCHF-DA was increased (48 ± 7) %. At this time, GLB_7 (20 μg/mL) was added. The fluorescence intensity decreased significantly up to 19 ± 4%. The results showed that GLB_7 inhibited the production of reactive oxygen species in macrophages cultured in vitro and had the effect of scavenging reactive oxygen species [11].

12.3 Antioxidative and Free Radical Scavenging of *Ganoderma lucidum* with the Tumor Prophylaxis and Treatment

Pan K et al. (2013) observed the effect of *G. lucidum* polysaccharide (GLP) on the immunity and antioxidative activities of gastric cancer induced by N-methyl-N9-nitro-Nnitrosoguanidine (MNNG). The results showed that the levels of blood inflammatory cytokines IL-6 and TNF in rats with gastric cancer were significantly higher than those in normal control rats and blood IL-2, IL-4, and IL-10 levels were significantly decreased; MDA content in blood and gastric tissue was increased, while the content of GSH is reduced. GLP (400 mg/kg group was administered from the first day of MNNG-induced gastric cancer to 20 weeks; 800 mg/kg group was administered from the sixth week of MNNG-induced gastric cancer to the 20th week) could reverse the changes of cytokines in the above two groups of rats with gastric cancer, reduce the level of lipid peroxidation in blood and gastric tissue, and restore the abnormal levels of blood SOD, CAT, and GSH-Px in rats with gastric cancer to normal. This result indicates that *G. lucidum* polysaccharide has immunomodulatory effects and resistance to gastric cancer in rats. Therefore, antioxidation and scavenging free radicals of *G. lucidum* provide an experimental basis for adjuvant treatment of gastric cancer [12].

Deepalakshmi K et al. (2013) studied the antioxidant effects of *G. lucidum* extracts on 7,12-dimethylbenz (a) anthracene (DMBA)-induced rats with breast cancer in vitro and in vivo. The results showed that the ethanol extract of *G. lucidum* had obvious antioxidative and free radical scavenging effect in vitro and the half-inhibitory concentration (IC_{50}) of ABTS, DPPH, and hydroxyl radical scavenging was 29.15, 26.22, and 28.07 μg/mL, respectively. The in vivo levels of antioxidant enzyme, such as SOD, CAT, and GPx, were decreased in DMBA-induced animals. Moreover, pretreatment with *G. lucidum* (500 mg/kg) to DMBA-induced animals significantly ($P < 0.05$) increased the levels of SOD, CAT, and GSH-Px in plasma, mammary, and liver tissues compared to DMBA-induced animals. These findings suggested that *G. lucidum* extract could be considered as a potential source of natural antioxidants and can be used as an effective chemopreventive agent against mammary cancer [13].

Our research group also found that *G. lucidum* polysaccharide (Gl-PS) can significantly improve the intestinal mucosal oxidative stress damage induced by methotrexate (MTX). MTX, as a chemotherapy drug, can shorten and fuse the small

intestine villi, disappear crypt cell, and decrease goblet cell. Under the electron microscope, the microvilli of intestinal epithelial cells were disordered, shortened, and deleted, and the nuclear membrane and mitochondria were swollen. Compared with the normal control group, the MDA in the intestinal homogenate of the MTX model group was significantly increased, and the activity of total superoxide dismutase (T-SOD) was significantly decreased. After treatment with Gl-PS (50, 100, 200 mg/kg), the above morphological changes of the small intestine in mice were significantly reduced. The decreased T-SOD activity was significantly increased, while the increased MDA was significantly reduced. Gl-PS also increased blood IgA levels in mice with MTX injury (Chen LH et al. 2011) [14]. Further studies have also found that Gl-PS promotes proliferation and remodeling of IEC-6 cells in rat small intestine and the expression of ornithine decarboxylase (ODC) mRNA and c-Myc mRNA in IEC-6 cells simultaneously in the healing of small intestinal cells significantly increased, which may be associated with Gl-PS improving IEC-6 cell injury healing (Sun LX et al. 2011) [15].

12.4 Protective Effects of *Ganoderma lucidum* on Oxidative Damage of the Brain

Wang MF et al. (2004) studied to examine the effects of *Ganoderma* on aging, learning, and memory ability in senescence-accelerated mice (SAMP8). Six-month-old SAMP8 mice were divided into four groups: control and three experimental groups supplemented with 0.3, 0.6, or 1.8% *Ganoderma* for 12 weeks. Body weight, food intake, aging score, open-field test, and active shuttle avoidance tests were performed during the experiment. The activities of SOD, GSH-Px, glutathione reductase (GSH-Rd) in RBC, brain and liver, and the amyloid of brain were analyzed after sacrificed. The results showed that there was no significant difference in the body weight, food intake, and locomotion among four groups. The aging score of 0.6 and 1.8% *Ganoderma* groups were significantly lower than that of the control group ($P < 0.05$). The escape responses of 0.3, 0.6, and 1.8% *Ganoderma* groups at the fourth day were significantly better than control group in the female groups ($P < 0.05$), whereas there was no difference among the male groups. *Ganoderma* groups had significantly higher activities of SOD, GSH-Px, and GSH-Rd and lower brain amyloid when compared with the control group ($P < 0.05$). Result suggested that the *Ganoderma* may improve learning and memory ability and promote the activities of antioxidation [16].

Zhao HB et al. (2004) observed the effects of *Ganoderma* total sterol (GS) and its active ingredient GS_1 (Fig. 12.1) purified from fruiting bodies of *G. lucidum* in the middle cerebral artery occlusion (MCAO) ischemia-reperfusion (I/R) injury rats. It was found that GS (50 and 100 mg/kg) intragastrically administered to rats had protective effects on focal cerebral ischemia-reperfusion injury (I/R). GS can significantly reduce the neurobehavioral score of rats, reduce the volume of cerebral

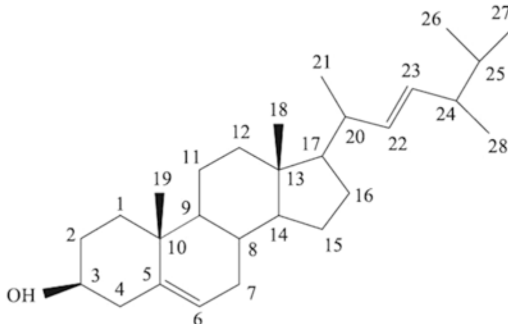

Fig. 12.1 The molecular structure of GS_1
GS_1 (24-methyl-cholest-5,22-dien-3-ol) were isolated from an ethanol extract of the fruiting body of *G. lucidum* (Leyss. ex fr.) karst. (Reproduced with permission from Ref. [17])

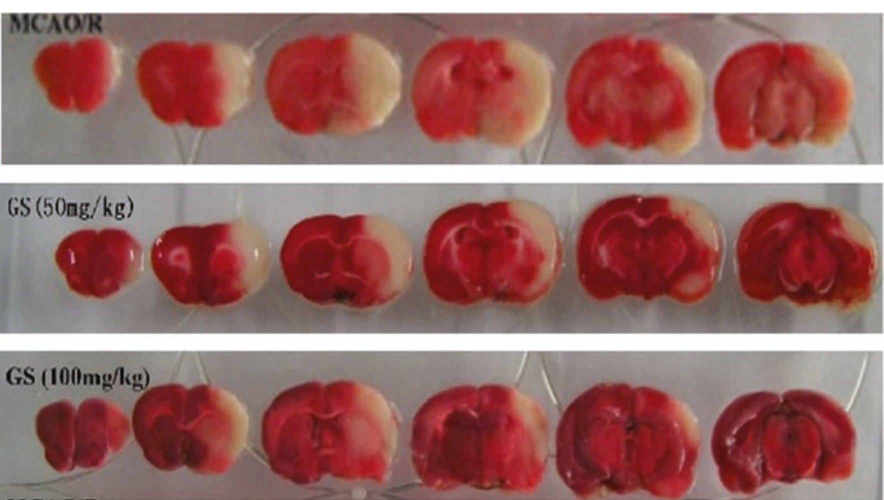

Fig. 12.2 Effect of GS on brain infarct volumes in rats subjected to MCAO/R
TTC staining displayed that brain tissue in the MCAO model group had white infarction involving the caudate putamen and cortex. Compared to the MCAO/R group (top), GS groups, the infarction volume decreased by 19.34 and 32.51 % in doses of 50 mg/kg (middle) and 100 mg/kg (bottom), respectively. (Reproduced with permission from Ref. [17])

infarction, reduce brain edema, improve the pathological changes of cortical brain tissue in I/R-damaged rats (Figs. 12.2 and 12.3), inhibit the formation of lipid peroxidation product MDA in brain tissue, and improve SOD (Table 12.1). The result suggests that the mechanism of protective action may be related to antioxidant damage [17]. Further studies on in vitro cultured rat cerebral cortical neurons with hypoxia/reoxygenation (H/R) injury model revealed that GS and GS_1 have significant protective effects on H/R damage in rat cerebral cortical neurons. GS (0.1, 1 μg/mL) and GS_1 (0.01, 0.1, 1 μg/mL) significantly increased rate of neuronal survival. GS significantly reduced MDA and increased total SOD and Mn-SOD levels. During H/R, GS also blocked the translocation of NF-κB into the nucleus of cortical neu-

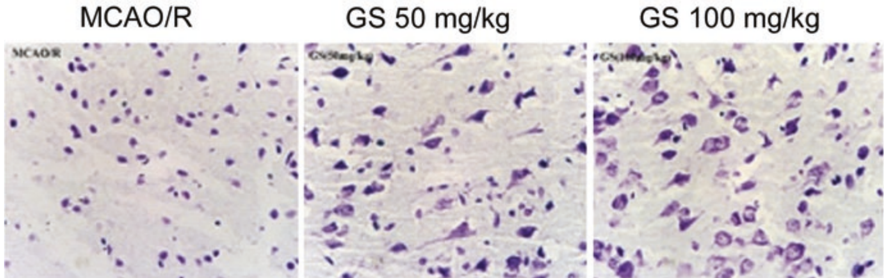

Fig. 12.3 Effect of GS on cortical histological evaluation in rats subjected to MCAO/R. In the model group (MCAO/R, up), different degrees of tissue cell damage a small amount of residual neurons, inattentive cells arrangement, and morphological irregularity; cells separated from intercellular substance, staining either empty or light, were observed. In 50 mg/kg (middle) and 100 mg/kg (down) groups, staining more uniform and deep staining, light shrinkage and pyknosis of nerve cells were observed. Some neurons are in the process of degeneration; part cell structure still exist, but the denaturation and pyknosis are not complete. (Reproduced with permission from Ref. [17])

Table 12.1 Effect of GS on MDA content and SOD activity in rat brain tissue [Ref. 17]

Group	MDA (nm ol/mg prot)	SOD (NU/mg prot)	Mn SOD (NU/mg prot)	Cu Zn SOD (NU/mg prot)
Sham	4.745±0.771	69.397±7.730	49.294±4.754	20.105±3.063
I/R	13.140±3.243**	60.962±7.786*	47.120±4.567	11.842±3.780 *
GS				
100 mg/kg	5.955±1.259$^{\Delta\Delta}$	73.450±8.069$^{\Delta}$	56.073±3.520$^{\Delta\Delta}$	17.379±4.788
50 mg/kg	7.583±2.032$^{\Delta\Delta}$	64. 16±8.167	48.420±3.346	15.895±5.158
SV	13.015±3.317	61.079±2.405	44.592±1.515	16.488±3.118

Mean ± SD; n = 10; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with sham group; $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$ compared with IR group

rons, i.e., inhibited its activation. Further using Western blot demonstrated that GS can inhibit IκB protein degradation in the cytoplasm of cortical neurons [18].

The effect of polysaccharide extract isolated from *G. lucidum*(Gl-PS)on rat cortical neuronal cultures exposed to hypoxia /reoxygenation (H/R) was also studied in vitro. Gl-PS(1, 10, 100 μg/ml)increased neuron viability following H/R as measured by the inhibition of MTT reduction. Gl-PS significantly reduced malondialdehyde content and reactive oxygen species production and increased the manganese superoxide dismutase (Mn-SOD) activity; furthermore, the translocation of nuclear factor-kappa B induced by H/R was blocked. These findings suggest that Gl-PS might be useful in treating H-/R-induced oxidative stress and Mn-SOD might play a critical role in the neuroprotective effect of Gl-PS against H/R injury [19].

Yang HM et al. (2009) observed the effects of *G. lucidum* polysaccharide peptide (GLPP) on the spatial learning and memory ability, the ultrastructure of hippocampus, SOD activity, and MDA content in hippocampus in Alzheimer-like rats that exposed to light (illuminance 400 Lux) for 30 days. Results showed that the latency

of the model group was significantly longer than that of normal control group ($P < 0.05$), and that of GLPP (250 mg/kg) group was significantly shorter than model group ($P < 0.05$). Compared with the normal control group, the SOD activity in the hippocampus was significantly decreased, and the MDA content was significantly increased in the model group ($P < 0.05$). The SOD activity was significantly higher, and the MDA content was significantly lower in the GLPP group than that of the model group in hippocampus ($P < 0.05$). The results of transmission electron microscopy showed that the mitochondrial axons and synapses in the hippocampus of the normal control group and the GLPP group were basically normal. The mitochondrial membrane structure of the hippocampus was destroyed, and the mitochondria were swollen, blurred, and disappeared in the model group. More results included rare neurofilaments, loss of synapses, decreased synaptic density, unclear synaptic cleft, and decreased synaptic vesicles. These finding suggested that GLPP could prevent the damage of rat hippocampal ultrastructure and reduce the spatial memory disorder in Alzheimer-like rats [20].

Zhang Y et al. (2007) observed effects of *Ganoderma lucidum* triterpenoids (GLT) on learning and memory function and antioxidative ability of aging model mice induced by D-galactose. GLT (0.35 and 1.4 g/kg, per day for 8 weeks) had been intragastrically administered to two therapy groups. All mice of different groups were tested with Morris water maze. Then the mice were killed and biochemically assayed of total antioxidative capacity (T-AOC), SOD, and MDA in the brain. Results showed the model mice had worse ability in learning and memory than control mice. The T-AOC activity and SOD activity in the brain decreased, and the MDA content increased in model rats in comparison with control. GLT significantly improved the changes mentioned above. It means that GLT improve the learning and memory dysfunction in aging model mice by modulation of the antioxidative ability [21].

Zhang Q et al. (2005) used H_2O_2-induced rat pheochromocytoma cells (PC12) as a model of oxidative stress-injured neurons. The cultured PC12 cells were randomly divided into normal control group in which PC12 cells were cultured in serum-free DMEM medium; H_2O_2 treatment group in which PC12 cells were treated with H_2O_2 at a final concentration of 200 μmol/L for 4 h; *G. lucidum* polysaccharide peptide group in which PC12 cells and 10 mg/L of *G. lucidum* polysaccharide peptide were pretreated for 24 h and finally added to a final concentration of 200 μmol/L H_2O_2 acted together for 4 h; and nerve growth factor group in which PC12 cells were pretreated with 0.1 mg/L nerve growth factor for 24 h and then added with final concentration of 200 μmol/L H_2O_2 for 4 h. MTT assay was used to detect cell viability, and Western blotting was used to detect the active fragment of Caspase-3 P20. The results showed that the cell viability of the H_2O_2-treated group (38.6±7.1%) was significantly lower than that of the control group (100±9.3%); the cell viability (83.4±10.2%) and (75.9±7.4%) of the *G. lucidum* polysaccharide peptide group and nerve growth factor group were significantly higher than that of the H_2O_2-treated group, but there was no significant difference between the two groups. These results suggest that *G. lucidum* polysaccharide peptide is similar to nerve growth factor and can significantly improve the cell survival rate of PC12 cells injured by H_2O_2 stress. At the same time, both *G. lucidum* polysaccharide peptide and nerve growth factor

inhibited the activity of caspase-3 and reduced the caspase-3 P20 fragment significantly. The results showed that *G. lucidum* polysaccharide peptide could protect PC12 cells from oxidative damage induced by H_2O_2 and inhibit the apoptosis of PC12 cells induced by H_2O_2 by inhibiting the activation of caspase-3 [22].

Li WJ et al. (2011) observed the protective effect of *Ganoderma atrum* polysaccharide (PSG-1) on the brain of D-galactosamine (D-gal) oxidative stress mice. The results showed that PSG-1 (50, 100, and 150 mg/kg) significantly reduced apoptosis in the mouse brain in a dose-dependent manner. PSG-1-evoked reduction of apoptosis was associated with the decrease of MDA and oxidized glutathione (GSSG) contents and the increase of SOD, CAT, GPx and GSH-Rd activities, and GSH contents. PSG-1 treatment was also found to attenuate ROS production and calcium accumulation. This finding suggests that PSG-1 has a potential to be used as a novel therapeutic agent for the protection of aging brain tissue against oxidative damage by modifying the redox system and maintaining calcium homeostasis [23].

Shao HX et al. (1996) reported antioxidative effect of *G. lucidum* and *G. lucidum* compound (*G. lucidum* fruiting body is the main component, supplemented by *Atractylodes macrocephala*, *Licorice*, *Poria cocos*, *Lycium barbarum*, etc.) in vivo experiment. Both water decoction of *G. lucidum* (1 g/kg, per day) and *G. lucidum* compound (1, 5 g/kg and 5 g/kg, per day) intragastric administration for 3 continuous weeks could significantly reduce the content of MDA in the myocardium, brain, and plasma in rats. The content of MDA in the myocardium, brain, and plasma with high-dose group of *G. lucidum* compound was significantly lower than that of *G. lucidum* group. The above doses of *G. lucidum* and *G. lucidum* compound can significantly increase SOD activity in the myocardium, brain, and blood. The effect of *G. lucidum* compound on increasing SOD activity in the myocardium, brain, and plasma was stronger than that of *G. lucidum*. *G. lucidum* and *G. lucidum* compound could also significantly reduce the lipofuscin content in rat brain tissue, but there was no significant difference between the two groups. The results showed that *G. lucidum* and *G. lucidum* compound could significantly inhibit the production of MDA and lipofuscin in the myocardium, brain, and plasma of and enhance the activity of SOD in rats, indicating that *G. lucidum* and its compound had obvious antioxidant effect [24].

12.5 Antioxidative and Free Radical Scavenging Effect of *Ganoderma lucidum* on Cardiovascular Protection

At present, serum pharmacological method is widely used to investigate traditional Chinese medicine. Serum pharmacology means to make in vitro experiment using an animal serum with drug when the animal has been taken this drug. The drug-treated serum may contain drug, metabolites of the drug, or endogenous active substances induced by drug in vivo; thus the serum with drug produces pharmacological effect. Zhang HM et al. used serum-pharmacological method to study the effect of sera containing Lugu lingzhi(containing 70% of *G. lucidum* extract and 20% *G. lucidum* spore powder)on the LDL oxidation, monocyte adhesion to the human

endothelial cells, and adhesion molecules expression induced by ox-LDL and AGE in rats. At the dose of 0.12, 0.24, and 0.72 g/kg, Lugu lingzhi were intragastrically administered by stomach tube, once a day for 10 days. The rats were killed 10 days later, and serums were taken and stored at −80 °C for use. In the experiment Lugu lingzhi-treated serum was added to human umbilical cord vascular endothelial cell culture media in vitro. Results revealed that serum treated with Lugu lingzhi (0.12, 0.24 and 0.72 g/kg) significantly inhibited LDL oxidation mediated by endothelial cells and decreased monocyte adhesion to endothelial cell (EC) induced by oxidative low-density lipoprotein (ox-LDL) and advanced glycation end products (AGE). Further experiments indicated that Lugu lingzhi-treated serum could markedly inhibit the expression of intercellular cell adhesion molecule-1 (ICAM-1) induced by ox-LDL and AGE. Lugu lingzhi-treated serum also significantly inhibits the expression of vascular cell adhesion molecule-1 (VCAM-1) induced by AGE. The inhibition of expression on adhesion molecule induced by ox-LDL and AGE might be contributed to the effects of Lugu lingzhi in preventing the development of vascular complications of diabetes and other cardiovascular disease [25, 26].

You YH and Lin ZB (2007) found that the protective effects of *G. lucidum* polysaccharide peptide (GLPP) on oxidative injury of umbilical vein endothelial cells (ECV304) were induced by t-butyl hydroperoxide (tBOOH) in vitro. The survival rate of cells was measured by MTT assay. The morphological change of cells and injury of mitochondria were examined under the light and electron microscope. The percentage of apoptosis of ECV304, labeled with Annexin V/PI, was measured by flow cytometry. Results showed that GLPP (12.5, 25, 50, 100 mg/L) could reduce oxidative injury induced by tBOOH in ECV304 cells. The survival rate of cells treated with GLPP was increased. The light microscopic examination showed that the injured cells were decreased in GLPP-treated groups. Under the electron microscope, it was found that GLPP (50 mg/L, incubated for 24 h) could protect the organelle such as mitochondria from oxidative injury and cells from apoptosis by tBOOH. The result of flow cytometry showed that the total percentage of apoptosis in control, GLPP, and injury-treated group was 2.24±0.43%, 24±6.4% ($P < 0.01$), and 82.1 ± 7.9% respectively. These results indicate that GLPP had protective effects on ECV304 from oxidative injury [27].

Yang LJ et al. (2011) further studied the effects of GLPP on oxidative damage of human umbilical vein endothelial cells (HUVECs) induced by tBOOH. Results showed that GLPP (6.125, 12.5, 25, 50, 100 mg/L) could inhibit the apoptosis of HUVECs by ROS. The survival rate of HUVECs was increased, and the percentage of cell apoptosis was decreased by GLPP. GLPP decreased the activation of caspase-3 of HUVECs upregulated by ROS. Electron microscope showed that the organelle such as mitochondria injury by ROS could be relieved by GLPP. It can be seen that GLPP can prevent oxidative injury of human umbilical vein endothelial cells [28].

Mao HH and You YH (2015) found protective effect of GLPP on cultured neonatal cardiomyocytes injured by hypoxia/reoxygenation (H/R).Results showed that the survival rate of cardiomyocytes in H/R group was markedly decreased compared with the control group. After treatment with GLPP 12.5, 25, 50, and 100 mg/L,the survival rate of cardiomyocytes was significantly increased compared with the H/R

group. Mitochondrial injury was observed in cardiomyocytes in H/R group but was relieved by GLPP.The total percentage of apoptosis in H/R group was significantly increased compared with control group, and GLPP treatment could markedly decrease the total percentage of apoptosis compared with H/R group.The intracellular [Ca^{2+}]i and ROS in the H/R group significantly increased compared with control group. GLPP treatment could significantly decrease intracellular [Ca^{2+}]i and ROS compared with H/R group (Fig. 12.3). GLPP may protect cultured neonatal rat cardiomyocytes from injury by H/R. The mechanism might be related to decreasing intracellular ROS and overload of [Ca^{2+}]i [29] (Fig. 12.4).

Chen WQ et al. (2005) observed the effect of *G. lucidum* polysaccharide on blood lipid and lipid peroxidation in hyperlipidemia rats. The results showed that *G. lucidum* polysaccharides (200, 400, and 800 mg/kg, per day for 30 days) could significantly decrease the serum contents of TC, TG, and LDL-C in the experimental hyperlipidemic rats and markedly increase the level of blood HDL-C, mean level of blood LPO in the experimental groups treated by *G. lucidum* polysaccharides at different doses were much lower than those in hyperlipidemia group, and the GSH-Px and SOD activities of blood in the *G. lucidum* polysaccharides group were much higher than those in the hyperlipidemia group. This finding suggests that *Ganoderma* can regulate lipid metabolism, enhance the antioxidation, and reduce the lipid peroxidation in the rats with hyperlipidemia [30].

Li WJ et al. (2011) studied the effect of *Ganoderma atrum*, one of fungi from *Ganoderma* species, polysaccharide (PSG-1) on myocardial cell injury induced by oxidative stress of hydrogen peroxide (H_2O_2) in cardiomyocyte of neonatal rats. The results showed that pretreatment with PSG-1 (25, 50, and 100 μg/ml) could decrease MDA content, ROS production, and LDH leakage and increase beating frequency, cell viability, SOD activity, and protein expression in cardiac myocytes. These results suggest that PSG-1 has protective effect on oxidative stress injury of cardiac myocytes [31]. Li WJ et al. (2010) also reported protective effect of *Ganoderma atrum* polysaccharide (PSG-1) on injury induced by high glucose (HG) and possible mechanisms involved in human umbilical vein cells (HUVECs). Results showed that compared to control group, cell viability and SOD activity were significantly decreased and ROS generation and MDA contents were significantly increased in HG group. Compared to HG group, PSG-1 increased cell viability and SOD activity and very significantly decreased ROS generation and MDA contents [32].

Sargowo D et al. (2018) observed the clinical effect of polysaccharide peptide (PsP) isolated from *G. lucidum* as potent antioxidant in high-risk and stable angina patients. The clinical trial was conducted to 37 high-risk and 34 stable angina patients, which were determined based on ESC Stable CAD Guidelines and Framingham risk score, with pre- and posttest design without control group. The parameters are SOD activity and MDA content and circulating endothelial cell (CEC) and endothelial progenitor cell (EPC) counts. The patients were given PsP 750 mg/day in three divided doses for 90 days. Paired *t*-test was performed for normally distributed data and Wilcoxon test for not normally distributed data and significant level of $P \leq 0.05$. The result showed that level of SOD in high-risk patients slightly increased from 3.12±0.70 U/mL to 3.62±4.26 U/mL, but not statis-

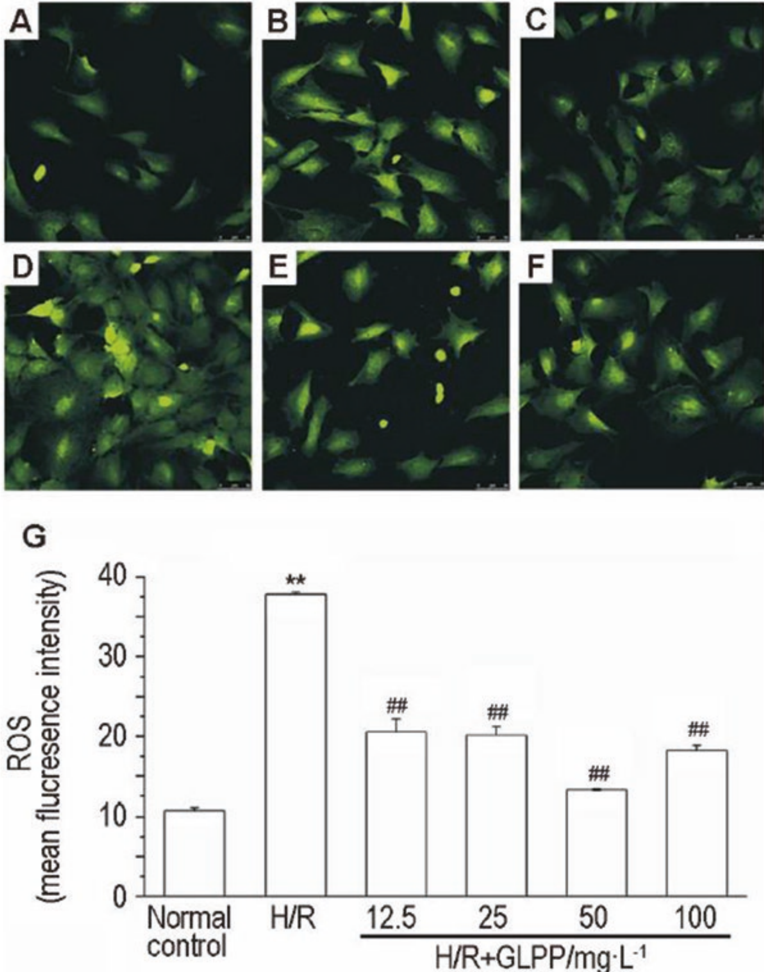

Fig. 12.4 Effect of GLPP on reactive oxygen species (ROS) level in rat cardiomyocytes injured by H/R detected with DCFH-DA staining by laser confocal microscopy (×40). (**a**) normal control; (**b**) H/R; (**c-f**) H/R+GLPP 12.5, 25, 50, and 100 mg/L, respectively; G, ROS quantified by mean fluorescence intensity of A–F.x±s, n=6.$**P < 0.01$, compared with control group; $\#\#P < 0.01$, compared with H/R group. (Reproduced with permission from Ref. [29])

tically significant, with $P = 0.22$. Level of SOD in stable angina group significantly increased from 3.41±0.46 U/ml to 5.79±4.19 U/ml with P = 0,001. MDA content significantly reduced from 114.13±24.56 U/mL to 36.84±28.39 U/mL and 95.63±21.27 U/mL to 44.84±50.95 U/mL, respectively, in both of high-risk and stable angina patients. CEC significantly reduced from 7.91±9.11 cells/ml to 1.76±1.56 cells/ml in stable angina patients and from 7.38±4.44 cells/ml to 2.23±3.05 cells/ml in high-risk patients. EPC count significantly reduced from 15.1±7.44 cells/ml to 6.14±5.30 cells/ml and 12.94±6.97 cells/ml to 6.10±3.95

cells/ml, respectively, in both high-risk and stable angina patient. It could be concluded that PsP is a potent antioxidant against pathogenesis of atherosclerosis in stable angina and high-risk patients [33].

12.6 Antioxidative and Free Radical Scavenging Effect of *Ganoderma lucidum* on Diabetes Prevention and Protection

A water extract of fruit bodies of *G. lucidum* significantly decreased plasma sugar level in mice. Fractionation of the extract by monitoring the hypoglycemic activity afforded two glycans, ganoderans A and B. These glycans elicited remarkable hypoglycemic actions in normal and alloxan-induced hyperglycemic mice (Hikino H et al. 1985) [34]. Ganoderan B increased the plasma insulin level in normal and glucose-loaded mice but elicited no effect on insulin binding to isolated adipocytes. Administration of ganoderan B elicited significant increases of the activities of hepatic glucokinase, phosphofructokinase, and glucose-6-phosphate dehydrogenase, decreased the hepatic glucose-6-phosphate and glycogen synthetase activities, and did not affect the activities of hexokinase and glycogen phosphorylase. Ganoderan B reduced the glycogen content in the liver but had no influence on total cholesterol and triglyceride levels in the plasma and liver (Hikino H et al. 1989) [35].

Alloxan is a prompt and potent inducer of diabetes in experimental animals because of its damaging effect on insulin-producing B cells. It has been generally accepted that alloxan-induced hyperglycemia is mainly due to its ability to induce oxygen free radicals, which damage the pancreas (Heikkila and Cohen 1974; Winterbourn and Munday 1989) [36, 37]. It has been reported that *G. lucidum* polysaccharides have the ability to scavenge the reactive oxygen species (Gui XF et al. 1996; You YH and Lin ZB 2002) [38, 39] and significantly inhibited iron-induced lipid peroxidation in rat brain homogenates and to inactivate hydroxyl radicals and superoxide anions (Lee JM et al. 1999) [40].

Our research group investigated the protective effect against alloxan-induced pancreatic islets damage by *G. lucidum* polysaccharides (*Gl*-PS) isolated from the fruiting body of *G lucidum*. In vitro, alloxan caused dose-dependent toxicity on the isolated pancreatic islets. Pretreatment of islets with *Gl*-PS (25, 100 μg/ml) for 12 h and (100 μg/ml) 24 h significantly reversed alloxan-induced islet viability loss. *Gl*-PS was also found to inhibit the free radical production induced by alloxan in the isolated pancreatic islets using confocal microscopy. *Gl*-PS (50, 100, and 200 mg/kg)dose dependently increased serum insulin and reduced serum glucose levels when pretreated intragastrically for 10 days in alloxan-induced diabetic mice. It was found that the pancreas homogenates had higher lipid peroxidation products in alloxan-treated mice than in the *Gl*-PS-treated animals. Aldehyde fuchsin staining revealed that alloxan caused nearly all the β cells disappearing from the pancreatic

islets, while *Gl*-PS partly protected the β cells from necrosis. Alloxan (60 mg/kg) induced NF-kB activation in the pancreas at 30 min after injection; pretreatment with *Gl*-PS inhibited alloxan-induced activation of NF-kB. These results suggest that *Gl*-PS was useful in protecting against alloxan-induced pancreatic islets damage in vitro and in vivo; one of the mechanisms is through its scavenging ability to protect the pancreatic islets from free radical damage induced by alloxan (Zhang HN et al. 2003) [41].

Diabetic nephropathy (DN) is the major cause of morbidity among diabetic patients. Thus, antidiabetic drugs with protection potential in the kidneys would have a higher therapeutic value. The effects of *G. lucidum* polysaccharides (GL-PS) on renal complication in streptozotocin-induced diabetic mice have been investigated by He CY et al. (2006). Results showed that treatment of GL-PS (125 and 250 mg/kg) for 8 weeks, increasing serum glucose and triglyceride levels in diabetic mice, could also be lowered by GL-PS. GL-PS was able to reduce the blood Cr and BUN levels and urinary albumin excretion (UAE) compared with diabetic model mice in a dose-dependent manner. Moreover, GLPS had the capacity to improve the renal morphometric changes and oxidative stress state of diabetic mice. The diabetic model mice had much higher MDA levels than normal mice. However, treatment of GL-PS at 250 mg/kg reduced the renal MDA level significantly. In addition, the renal SOD activity was notably lower in diabetic model mice than in normal mice but was significantly increased by GL-PS. Integral intensity of TGF-β1 in the renal cortex of diabetic mice was higher than that of nondiabetic control mice. Treatment with GL-PS dose dependently reduced the TGF-β1 expression. In summary, GL-PS can improve the metabolic abnormalities of diabetic mice and prevent or delay the progression of diabetic renal complications [42].

The effects of a novel proteoglycan, named FYGL, isolated from *G. lucidum* fruiting bodies, on the kidney function were investigated systematically by Pan D et al. (2014). FYGL (250 mg/kg) not only dose dependently reduced the blood glucose concentration (23.5%, $P < 0.05$), kidney/body weight ratio (23.6%, $P < 0.01$), serum creatinine (33.1%, $P < 0.01$), urea nitrogen (24.1%, $P < 0.01$), urea acid contents (35.9%, $P < 0.01$), and albuminuria (30.7%, $P < 0.01$) of diabetic nephropathy (DN) mice compared to the untreated DN mice but also increased the renal SOD (75.3%, $P < 0.01$), GSH-px (35.0%, $P < 0.01$), and CAT activities (58.5%, $P < 0.01$) compared to the untreated DN mice. The decreasing of renal MDA content (34.3%, $P < 0.01$) and 8-hydroxy-2′-deoxyguanosine expression (2.5-fold, $P < 0.01$) was also found in FYGL-treated DN mice compared to the untreated DN mice, along with an amelioration of renal morphologic abnormalities. Results indicate that FYGL confers protection against the renal functional and morphologic injuries by increasing activities of antioxidants and inhibiting accumulation of oxidation, suggesting a potential nutritional supplement for the prevention and therapy of DN [43].

Gl-PS dose dependently rescued the delay of wound closure in diabetic mice. 50 and 250 mg/kg/day of *Gl*-PS treatment significantly increased the mean perfusion rate around the wound in diabetic mice. In diabetic wound tissues, the protein level

of manganese superoxide dismutase (MnSOD) was unchanged, whereas MnSOD activity was inhibited, and its nitration was potentiated; *Gl*-PS administration suppressed MnSOD nitration and increased MnSOD and glutathione peroxidase (GPx) activities. Moreover, *Gl*-PS attenuated the redox enzyme p66Shc expression and phosphorylation dose dependently in diabetic mice skin. *Gl*-PS rescued the delayed wound healing and improved wound angiogenesis in STZ induced type 1 diabetic mice, at least in part, by suppression of cutaneous MnSOD nitration, p66Shc and mitochondrial oxidative stress(Tie L et al. 2012) [44].

Cheng PG et al. (2013) also demonstrated that the application of *G. lucidum* extract hydrophilic ointment (containing 25.1% polysaccharide, 0.45% ganoderic acid and 0.069% adenosine) on the wound could promote the wound healing of streptozotocin-induced type I diabetic rats and the antioxidant activity in serum increased significantly [45].

Mahargo W et al. (2015) evaluated antioxidative effect of polysaccharide peptide (PsP) extracted from *G. lucidum* on the process of atherogenesis in diabetes rats induced by high fat diet (HFD) plus streptozotocin. After 5 weeks of treatment with PsP (50, 150, and 300 mg/kg), level of MDA and H_2O_2 in diabetes rats was lower significantly, respectively, and PsP can significantly enhance the level of SOD. This result suggested that PsP can be useful alternative as antioxidant that attenuate oxidative stress to inhibit the process of atherogenesis in diabetes mice induced by HFD and streptozotocin [46].

Zhang H et al. (2018) studied to optimize the extraction process of amino acids isolated from *Ganoderma lucidum* (GLAA) and analyze the amino acid composition of GLAA, combined with preliminary activity evaluation. Results showed that the hydrochloric acid hydrolysis method for extraction of GLAA was optimized by response surface methodology (RSM) with Box Behnken design (BBD). The optimal extraction conditions were obtained: liquid-to-solid ratio 20:1 mL/g, extraction time 9.5 h, hydrochloric acid concentration 7.0 mol/L, and the yield of GLAA was 4.78±0.21%. The result of amino acid automatic analyzer showed that the content of total amino acids in GLAA was 2.94%, including 18 kinds of amino acids, and the most abundant amino acid was leucine (0.37 %). The results of activity tests in vitro showed that GLAA exhibited high alpha-glucosidase inhibitory rate with IC_{50} value of 380.62 μg/mL and a certain scavenging DPPH activity with IC_{50} of 484.54 μg/mL. GLAA possessed strong hypoglycemic and antioxidant activities [47].

HE YM et al. (2015) observed the effect of Lingzhi herb granule on glucose metabolism, insulin sensitivity, and oxidative stress of patients with type 2 diabetes. Seventy-five type 2 diabetes patients were randomly divided into Chinese herb group (receive conventional treatment and Lingzhi herb granule, n = 47) and control group (received conventional treatment and placebo granule, n = 28).After 8 weeks treatment, the pre- and post-treatment changes in blood glucose, glycated albumin, insulin, C-peptide, insulin resistance index (HOMA-IR), SOD, and MDA were compared. Results showed that after treatment, postprandial blood glucose, glycated albumin, HOMA-IR, SOD, and MDA had obviously decreased in Chinese herb group. Lingzhi herb granule also showed a greater reduction of postprandial blood glucose, HOMA-IR, and MDA compared with those of the control group.

This clinical study indicated that Lingzhi herb granule can effectively ameliorate the glucose metabolism, the insulin sensitivity, and the oxidative stress level of type 2 diabetes [48].

Hao HY et al. (2018) observed the influence of Fu Fang Ling Zhi Jian Shen Tang combined with *Tripterygium wilfordii* tablet on oxidative stress and endothelial function of patients with early diabetic nephropathy (DN). One hundred and twenty six of patients with early DN were divided into observation group and control group according to random number table method, 63 cases in each group.Besides receiving the basic intervention for controlling blood pressure and sugar, the control group received the *Tripterygium wilfordii* tablet and the observation group received the *Tripterygium wilfordii* tablet plus the Fu Fang Ling Zhi Jian Shen Tang (*Ganoderma lucidum*, *Ligusticum wallichii* each of 90 g, *Astragalus membranaceus*, *Cordyceps militaris* each of 60 g, water decoction, per day).In order to evaluate the curative efficacy of the medicine, the serum concentrations of creatinine (SCr), urea nitrogen (BUN), urinary albumin excretion rate (UAER), MDA, SOD, NO, von Willebrand factor (vWF) and endothelin-1 (ET-1), as well as the TCM syndrome scores and clinical effectiveness were examined in both groups. Results showed that after treatment, TCM syndrome scores were significantly declined in both groups compared with those before treatment, and the score in the observation group lessen much more than the control group. In the two groups, the serum concentrations of SCr, BUN, and UAER decreased significantly compared with those before treatment, and the changed magnitudes in the observation group were more obvious than the control. The total clinic effective rate of the observation group revealed a better than that of control group. The blood levels of MD, vWF, and ET-1 decreased significantly compared with their pretreatment owns, and the observation group had lower levels of MDA, vWF, and ET-1 than those of control group. After treatment, both groups obtained higher level of SOD and NO than their pretreatment, and the observation group appeared markedly higher levels in both substances than that of control group. The Fu Fang Ling Zhi Jian Shen Tang assisted with the tripterygium wilfordii tablet and effectively alleviated oxidative stress, repaired endothelial function, and improved renal function and clinical symptoms in the patients with early DN [49].

12.7 Antioxidative and Free Radical Scavenging Effect of *Ganoderma lucidum* on Liver Protection

Early research found that the ethanol extract isolated from *G. lucidum* and *G. sinense* could reduce liver injury induced by carbon tetrachloride (CCl_4) in mice (Department of Pharmacology, Beijing Medical College, 1974; Liu GT et al. 1979) [50, 51] and total triterpenoids isolated from *G. tsugae* could decrease alanine aminotransferase (ALT) activity of serum in CCl_4-intoxicated mice (Su CH et al. 1993) [52].

In our laboratory, two major components, GT and GT_2, were isolated from *G. lucidum*, and they were determined as total triterpenoids by high-performance liquid

chromatography (HPLC) and thin-layer chromatography (TLC). The effect of GT and GT_2 on three different experimental liver injury models induced by carbon tetrachloride (CCl_4), D-galactosamine (D-Gal), and Bacillus Calmette-Guerin (BCG) plus lipopolysaccharides (LPS) was studied in mice. The results indicated that both the activity of serum ALT and the content of liver triglycerides (TG) were increased significantly in CCl_4-damaged mice. GT (80 mg/kg) and GT_2 (10, 20, 40 mg/kg) successfully decreased ALT and TG. The histopathological results indicate that liver structures of the normal control group were basically normal. The liver of the CCl4-injured group was hurt severely and showed large necrotic changes. However, the histopathological changes of liver in the animals treated with GT and GT_2 were improved significantly. The results also demonstrate that GT given as 32.5, 65, and 130 mg/kg could antagonize the decrease of activity of SOD and GSH content of liver and inhibit the increase of liver MDA content in liver injured by CCl_4 (Wang MY et al. 2000a, 2002) [53, 54]. Further experiment reveals that the serum ALT and liver TG increased significantly in D-Gal liver injury mice. GT 80 mg/kg and GT_2 10, 20, and 40 mg/kg reduced ALT and TG significantly. GT 32.5, 65, and 130 mg/kg can antagonize the decrease of activity of SOD and GSH content of liver and inhibit the increase of liver MDA content in the D-Gal liver injury mice. In immunological liver injury model induced by BCG plus LPS, GT (80 mg/kg) and GT_2 (10, 20, 40 mg/kg) significantly inhibited the increase of blood ALT, NO, and liver TG levels and improved the liver injury in different degree. GT (0.5, 5, 50, 100 μg/ml) and GT_2 (0.5, 2, 10, and 50 μg/ml also decreased ALT and NO level in primary cultured hepatocytes in vitro (Wang MY and Lin ZB 2000b) [55].

Zhang GL et al. (2002) reported that immune hepatic injury was markedly induced by BCG or BCG plus inflammatory cytokines in BALB/c mice in vivo and in vitro. Under BCG-stimulated condition, augment of the liver weight and increase of the serum/supernatant ALT level were observed, and granuloma forming and inflammatory cell infiltration were observed by microscopic analysis within liver tissues. Moreover, NO production was also increased by BCG or/and CM stimuli in the culture supernatant, and a lot of iNOS-positive staining was observed in BCG prestimulated hepatic sections. Application of *G. lucidum* polysaccharide (GLP) significantly mitigated hepatic tumefaction, decreased ALT enzyme release and NO production in serum/supernatant, and improved the pathological changes of chronic and acute inflammation induced by BCG stimuli in mice. Moreover, the immunohistochemical result showed that GLP inhibited iNOS protein expression in BCG immune hepatic damage model [56].

Yang XJ et al. (2006) reported that proteoglycan GLPG extracted from *G. lucidum* mycelium (300, 600, 900 mg/kg) by gavage could improve the pathological changes of liver in CCl_4-induced liver injury mice, reduce the plasma TNF level, and increase the plasma SOD activity caused by CCl_4 poisoning. GLPG can also reduce the toxicity of CCl_4 to hepatocytes (L02) cultured in vitro and decrease the activity of ALT and aspartate aminotransferase (AST). The results showed that the hepatoprotective effect of GLPD in vivo and in vitro may be related to inhibition of TNF and scavenging activity of free radicals [57].

Chen TQ et al. (2018) proposed and successfully applied a novel and efficient technique-ultrasonic-circulating extraction (UCE) integrating superfine pulverization to extract and prepare antioxidant crude polysaccharides and other natural active substances from *G. lucidum*. This study evaluated the antioxidant and hepatoprotective activities and active ingredients in the powder from UCE (UCEP) through comparison with powder from hot water extraction (HWEP). The DPPH radical, ABTS system, superoxide anion, total antioxidant activity, and ferric-reducing antioxidant power assay. Results showed that the UCEP exhibited stronger in vitro antioxidant activity than the HWEP ($P < 0.01$). The hepatoprotective activity of the extracts was evaluated against CCl_4-induced hepatic oxidative damage in rats. Measurements of reduced GSH, SOD, and MDA in rat liver; measurements of ALT, AST, and lactate dehydrogenase (LDH) in rat blood; and Western blotting for antioxidant proteins of transforming growth factor-β_1 (TGF-β_1), heme-oxygenase 1, and GSH-Px showed that the UCEP had antioxidant activity in vivo either similar to or slightly stronger than the HWEP ($P < 0.1$). Further analysis of the active ingredients revealed that the UCEP and HWEP have similar mean yield and total triterpenoid content, but the former has significantly higher mean yield and total polysaccharide content than the latter ($P < 0.05$). Results suggest that the UCEP displays stronger antioxidant activities because of the larger amount of total polysaccharides; the UCEP may be able to be used as an antioxidant and liver protectant [58].

Li TQ et al. (2019) found that cadmium exposure caused accumulation of cadmium in liver tissue, inhibited antioxidant enzyme activity (SOD and GSH-Px), and increased MDA content, inflammatory cytokines (TNF-α, IL-1β, and IL-6), and heat shock protein (HSP 27, 40, 60, 70, and 90) mRNA levels and heat shock protein (HSP 60, 70, and 90) levels, with severe tissue damage and inflammatory infiltrates in chickens. *Ganoderma* triterpenoids not only reduced the accumulation of cadmium in the chicken liver but also significantly increased the activities of antioxidant enzymes as SOD and GSH-Px and reduced the content of MDA and mRNA expressions of inflammatory cytokines (TNF-α, IL-1β, and IL-6), and heat shock proteins (HSP 27, 40, 60, 70, and 90) and protein levels of heat shock proteins (HSP 60, 70, and 90) in chickens. Simultaneously, pathological tissue sections showed that the pathological damage of the liver tissue was significantly reduced. The results clearly demonstrated that *Ganoderma* triterpenoids can significantly reduce the accumulation of cadmium in the liver of chicken, thereby reducing oxidative stress and inflammation [59].

Gao SY et al. (2018) found that four meroterpenoids, applanatumols F (1), H (3), I (2), and lingzhiol (4), were isolated from the 95% EtOH extract of the fruiting bodies of *Ganoderma sinense*. Their structures were established on the basis of NMR spectroscopic analyses, optical rotatory dispersion data, ECD spectra, and X-ray crystallography. Compounds 1, 2, and 4 existed as racemic mixtures ((+) 1a, 2a, 4a; (−) 1b, 2b, 4b) while 3 as a single enantiomer. Basing on the separated enantiomers, they sought to explicit possible effects of compounds 1–4 on hydrogen peroxide (H_2O_2)-induced cell death and to determine their underlying molecular mechanisms in human normal liver LO2 cells. Among them, compound 2a treatment effectively protected LO2 cells against H_2O_2-induced cell damage and apopto-

sis. H_2O_2 exposure increased ROS, which was inhibited by 2a treatment. Mitochondrial membrane potential decrease, nuclear fragments, caspase-3 activation, and PARP cleavage were also arrested by 2a. Further increased levels of Nrf2, HO-1, phosphorylation Akt, and upregulation of antioxidant enzymes were detected in 2a-treated cells, indicating that the antioxidative effects of 2a might protect LO2 cells against oxidative damage via PI3K-/Akt-mediated activation of Nrf2/HO-1 pathway. In addition, compound 2a showed potential protective role of cardiomyocyte from ischemia/reperfusion injury, and pretreatment with 2a could decrease CK and LDH levels and increase GSH level [60].

Chiu HF et al. (2017) observed antioxidation and hepatoprotective efficacy of triterpenoids and polysaccharide-enriched *G. lucidum* (GL) in healthy volunteers. Forty-two healthy subjects (22 males and 20 females) were recruited and segregated into 2 groups as experimental or placebo and requested to intake GL or placebo capsule (225 mg; after lunch or dinner) for 6 consecutive months and vice versa with 1 month washout period in between. The anthropometric analysis and biochemical assays, as well as abdominal ultrasonic examination, were performed. Results showed that GL substantially improved the total antioxidant capacity (TEAC; 79.33~84.04), total thiols (0.19~0.28), and glutathione content (6~8.05) in plasma ($P < 0.05$) as well as significantly enhanced the activities of antioxidant enzymes as SOD, CAT, G-6-PDH, GPx, and GR ($P < 0.05$), whereas the levels of thiobarbituric acid reactive substances (TBARS; 3.37~2.47), 8-hydroxy-deoxyguanosine (8-OH-dG; 15.99~11.98), and hepatic marker enzymes (glutamic-oxaloacetic transaminase; GOT and glutamic-pyruvic transaminase; GPT) were concomitantly reduced (42 and 27%) on treatment with GL. Furthermore, the abdominal ultrasonic examination in GL subjects displayed a notable alteration on hepatic condition by reversing from mild fatty liver condition (initial) to normal condition. The outcome of intervention by GL demonstrated the antioxidation, anti-aging, and hepatoprotective nature of GL by effectively inhibiting oxidative stress [61].

12.8 Antioxidative and Free Radical Scavenging Effect of *Ganoderma lucidum* on Kidney Protection

Gui XF et al. (1996) observed the effects of *G. lucidum* injection on free radicals scavenging and renal cortex protecting in rats with cisplatin-induced kidney injury. The results showed that the levels of serum urea nitrogen (BUN) and creatinine (Cr) and MDA content of plasma and renal cortex increased significantly, while the activities of SOD decreased significantly in rats with renal injury induced by cisplatin. These results suggest that cisplatin can induce free radical production in vivo, accelerate lipid peroxidation in blood and renal cortex, lead to renal cortex injury, cause renal dysfunction, and cause urea nitrogen and creatinine retention in rats. Intraperitoneal injection of *G. lucidum* injection (0.2 g/mL) 20 mg/kg daily for 5

days had no significant effect on the abovementioned indexes of normal rats, but it could protect the kidney injury induced by cisplatin, reduce the increase of serum urea nitrogen and creatinine caused by cisplatin to normal levels, and significantly increase the activity of SOD in plasma and renal cortex. It could also decrease MDA content of plasma and renal cortex to normal level. The results suggest that *G. lucidum* injection could antagonize the renal injury induced by cisplatin and protect the function of renal cortex by scavenging free radicals and inhibiting lipid peroxidation [62].

Wang L et al. (2003) studied the preventive actions of *G. lucidum* polysaccharide (GLP) against kidney damage induced by cisplatin. Results showed that the contents of serum Cr and BUN of cisplatin group were significantly higher than that of normal control group. Activity of RBC SOD reduced, and the contents of serum MDA increased. Contents of MDA increased, and the activity of SOD declined in renocortical tissue. The contents of serum Cr and BUN of GLP-treated group were significantly lower than that of cisplatin group. The activity of RBC SOD increased, and the content of serum MDA declined. The content of MDA declined, and the activity of SOD increased in renocortical tissue. The pathological observation indicated that renal structure was significantly improved. GLP may reduce cisplatin nephrotoxicity, and its mechanism may be correlative with that GLP inhibited the blood and renocortical tissue lipid peroxidation increasing [63].

Zhong DD (2015) was to determine whether *G. lucidum* polysaccharide peptide (GLPP) could attenuate renal ischemia reperfusion injury (RIRI) in vivo mouse RIRI model and in vitro hypoxia/reoxygenation model by counteracting the oxidative stress [64]. For detailed experimental results, see Chap. 14.

12.9 Other Research Findings

Smina TP et al. (2011) reported antioxidant activity of total triterpene fraction isolated from *G. lucidum* in vitro and in vivo. Total triterpenes successfully scavenged DPPH(+), ABTS(+), and superoxide radicals, showed significant ferric reducing activity, and was highly effective in reducing the in vitro lipid peroxidation. Activities of the antioxidant enzymes as the SOD, CAT, GPx, and GSH in blood and the liver were increased, and level of MDA in the liver was significantly decreased by the oral administration of total triterpenes (10, 50, 100 mg/kg, daily for 30 days) to Swiss albino mice. The ability of total triterpenes to scavenge the free radicals and to enhance the body's antioxidant defense systems indicates its potential use as an antioxidant. An attempt was also done to gauge the toxicity of total triterpenes (100, 200, 500 mg/kg) using acute and 30-day subacute study models in Swiss albino mice. The results showed that *Ganoderma* triterpenes did not possess significant toxicity [65].

Smina TP et al. (2016) studied the radioprotective effect of total triterpenes isolated from *G. lucidum*. Results showed that total triterpenes were highly effective in reducing the levels of lipid peroxidation and protein oxidation to near-normal level

in both the liver and brain tissues. Total triterpenes, when administered in vivo, were also found to be successful increasing the activities of antioxidant enzyme such as SOD, CAT, and GPx and reducing reduced glutathione (TAD) level in the liver and brain of irradiated mice. Administration of total triterpenes, prior to radiation exposure, significantly decreased the DNA strand breaks. The present study thus revealed the potential therapeutic use of *Ganoderma* total triterpenes as an adjuvant in radiation therapy [66].

Wu SJ (2018) studied that the male Sprague-Dawley rats were randomly divided into four groups: one group received a high-fat diet (control group), and the three other groups received a high-fat diet containing 100, 300, and 500 mg/kg of *G. lucidum* polysaccharide (GLP). GLP administration reduced the body weight gain, food efficiency ratio, levels of plasma triacylglycerol (TG), plasma total cholesterol (TC) and low-density lipoprotein cholesterol and liver weight, TC and TG levels and malondialdehyde values; improved the levels of fecal fat, cholesterol, and plasma high-density lipoprotein cholesterol; and enhanced the activities of serum SOD and GSH-Px in rats compared with the control group. The appropriate dose of GLP was 300 mg/kg. Results indicate that GLP exhibits hypolipidemic and lipid antioxidant activities and may be used as a drug for hyperlipidemia treatment [67].

Chen JH et al. (2016) investigated the protective effects of polysaccharides extracted from *G. lucidum* (PGL) on bleomycin-induced pulmonary fibrosis in rats. This study demonstrated that treatment with PGL of 100 and 300 mg/kg for 28 days led to significant reduction in the pulmonary index, inflammatory cell infiltration, and collagen deposition in rats with bleomycin-induced pulmonary fibrosis, which was associated with increased levels of GSH, GSH-Px, CAT, and SOD and decreased contents of MDA and hydroxyproline in the lung. These results indicated that PGL played a positive protective role in the pulmonary fibrosis, and its possible mechanism was to improve lung antioxidant ability [68].

Zhao ZH et al. (2014) was designed to determine the effects of *G. lucidum* polysaccharides (GL-PS) on exhaustive exercise-induced oxidative stress in skeletal muscle tissues of mice. The mice were divided into four groups (three GL-PS administered groups and the control group). The control group was administered with distilled water, and GL-PS-administered groups were administered with GL-PS (50, 100 and 200 mg/kg body weight per day). After 28 days, the mice performed an exhaustive swimming exercise, along with the determination activities of SOD, GPX, CAT, and content of MDA in the skeletal muscle of mice. The results showed that GL-PS could increase abovementioned antioxidant enzyme activities and decrease the MDA levels in the skeletal muscle of mice. This study provides strong evidence that GL-PS supplementation possessed protective effects against exhaustive exercise-induced oxidative stress [69].

Xie SQ and Liao WQ (2006) explored the effects of *Ganoderma* polysaccharides against hydrogen peroxide (H_2O_2)-induced human keratinocytes oxidative stress in vitro. The activity of antioxidases as SOD and GSH-Px and the content of MDA of the cultured human keratinocytes were determined by biochemical assay. Results showed that *Ganoderm*a polysaccharides (400 μg/ml) reduced the content of MDA and enhanced the activity of SOD and GSH-Px in human keratinocytes treated with

H_2O_2 (50 μmol/L). The result indicates that *Ganoderma* polysaccharides have protective effects against H_2O_2-induced human keratinocyte oxidative stress [70].

Shi M et al. (2013) reported that four polysaccharides (GLP-I, GLP-II, GLP-III, and GLP-IV) were obtained from fermented soybean curd residue by *G. lucidum* and then purified using anion-exchange DEAE Sephadex A-50. Their structural characterization was conducted by Fourier transform infrared spectroscopy (FTIR), and their monosaccharide compositions were determined. The results demonstrated that the basic structural characterization of four polysaccharides were similar; however, monosaccharide compositions of four kinds of polysaccharides were significantly different. GLP-III and GLP-IV were composed of six kinds of monosaccharide. Nevertheless, GLP-II was composed of three kinds of monosaccharide. Moreover, their antioxidant activities were investigated on the basis of hydroxyl radical, reducing power, DPPH free radical, chelating activity, ABTS radical scavenging, and SOD-like activity. The results showed that four polysaccharides exhibited antioxidant activities in a concentration-dependent manner. Among four polysaccharides, GLP-III and GLP-IV exhibited the higher scavenging effects on hydroxyl radicals, ABTS radical, DPPH free radical, and stronger reducing power and SOD-like activity than GLP-I and GLP-II. In addition, treatment with 40 μg/mL of GLP showed significant stimulation to the macrophage proliferation and higher nitric oxide production. Overall, GLP from fermented SCR could have potential applications in the medical and food industries [71].

Liang et al. (2018) determined the effects of GLP on lipid metabolism, oxidative stress, and apoptosis in mice fed with a high-fat diet (HD). GLP administration (200 and 400 mg/kg bw) significantly lowered the body weight, liver, heart, and white adipose tissue indexes, serum lipid accumulation, and serum and small intestine oxidative stress in mice fed with a HD. Moreover, GLP inhibited HD-induced apoptosis by decreasing the Bax/Bcl-2 ratio and suppressing caspase-3 activation in splenic lymphocytes. These findings indicate that GLP can exert hypolipidemic, antioxidant, and antiapoptotic effects in HD-induced obese mice [72].

G. lucidum polysaccharides as one of water-soluble polysaccharides has attracted much attention because of its bioactivities, especially antioxidant activity. Deproteinization is an essential step in the purification process of polysaccharides. Zeng XT et al. (2019) used three classic deproteinization methods, including neutral protease method, TCA precipitation, and $CaCl_2$ salting out, to be evaluated for crude *Ganoderma lucidum* polysaccharides (GLP) processing. The methods had ability to remove proteins (71.50%~87.36%), and meanwhile polysaccharide loss (8.35%~11.39%) was observed. Structure analysis indicated that these deproteinization methods had no significant effect ($p > 0.05$) on molecular weight of polysaccharides but led to varying degrees of glycoside bond losses (1.14%–64.05%). Moreover, the antioxidant activities of deproteinized polysaccharides were measured in vitro by hydroxyl radical, reducing power, 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) free radical, and ferric-reducing antioxidant power tests. Purified GLP by enzymolysis maintained the strongest antioxidant activities with the retention rate of over 47.40%, and its deproteinization efficiency and polysaccharide loss ratio

were 74.03 and 11.39%, respectively. In view of relatively high purity and antioxidant activity, enzymolysis was a suitable deproteinization method for GLP production [73].

12.10 Conclusion

Today, the antioxidative and free radical scavenging effect is one of the hotspots for *G. lucidum* research. In this chapter, the authors focus on the protective effects of *G. lucidum* on oxidative stress damage in various animal disease models, but also consideration is given to the study of cell molecular level from the perspective of screening or in-depth study of the mechanism of its action. Some studies on the antioxidative and free radical scavenging effects of *G. lucidum* by redox reaction in vitro are far-fetched from the whole level. Therefore, these articles were less included at the time of writing this chapter.

A large number of studies have proved that *G. lucidum* has multi-target pharmacological effects, which may be the reason why *G. lucidum* can be used to prevent and treat many diseases. However, this is not the only possibility. The antioxidative and free radical scavenging effect of *G. lucidum* in various organs, tissues, and disease states may be another reason that *G. lucidum* can be used in different diseases.

The clinical studies of *G. lucidum* on the antioxidative and scavenging free radical effect are still less, which deserve further exploration.

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Chapter 13
Anti-aging Effect of *Ganoderma* (Lingzhi) with Health and Fitness

Yan Pan and Zhibin Lin

Abstract Although *Ganoderma* (Lingzhi in Chinese) has been used as an elixir for thousands of years, its anti-aging effects still need to be clarified. Aging is related to immunoregulation, oxidation stress, and free radical product. Till now, *Ganoderma* exert life span elongation activities by inhibiting ROS production, lipid peroxidation, and advanced oxidation protein products; increasing production of mitochondrial electron transport complexes, SOD, CAT, GSH and GSH-Px, DPPH, and ABTS radical scavenger activities; and having immunomodulatory and antioxidant activity by increasing radical scavenging activity and ferric reducing antioxidant power. *Ganoderma's* anti-aging effect on human remains a mystery, and its potential mechanisms underlying anti-aging effect for its clinical application still need to be elucidated.

Keywords *Ganoderma* · Lingzhi · Anti-aging · Immune · Oxidation · Free radical

13.1 Introduction

Aging is one of the risk factors for various chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative diseases. With aging, a gradual decline in biochemical and physiological functions of most organs happens; then some age-associated disorders arise [1].

Ganoderma lucidum (*G. lucidum*: Lingzhi in Chinese) is a traditional medicine in China that prevents and treats various diseases. Lingzhi was classified as a drug of "high grade," that is, a herb of medicinal value and without toxicity in the *Shen Nong*'s *Materia Medica* (*Shen Nong Ben Cao Jing*), which was published in the second-century B.C. Li Shi-Zhen, a well-known ancient Chinese medicine scientist, also described the efficacy and medical uses of Lingzhi in the world-renown classic

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Compendium of Materia Medica (Ben Cao Gang Mu) in the sixteenth century. Ancient Chinese medical scholars held the view that *G. lucidum* could strengthen body resistance and consolidate the constitution of patients, that is, "Fuzheng Guben," which is one of the major principles in the therapeutics of traditional Chinese medicine [2].

A great deal of works have been carried out on therapeutic potential of *G. lucidum*. The basidiocarp, mycelia, and spores of *G. lucidum* contain approximately 400 different bioactive compounds, which mainly include polysaccharides, triterpenoids, nucleotides, sterols, steroids, fatty acids, proteins/peptides, and trace elements which have been reported to have a number of pharmacological effects including immunomodulation, anti-atherosclerotic, anti-inflammatory, analgesic, chemopreventive, antitumor, chemo- and radioprotective, sleep promoting, antibacterial, antiviral (including anti-HIV), hypolipidemic, anti-fibrotic, hepatoprotective, antidiabetic, anti-androgenic, anti-angiogenic, anti-herpetic, antioxidative and radical scavenging, anti-aging, hypoglycemic, estrogenic activity, and anti-ulcer properties [3].

G. lucidum has been worshipped as a kind of herbal medicine the emperors of the great Chinese and Japanese dynasties drank with their special teas and mushroom concoctions to achieve greater vitality and longer life. *G. lucidum* has also become popular because of its promising properties that might extend life span while increasing vigor and vitality [4].

13.2 *Ganoderma* Improves Immune Function with Aging

Immune function decreases when the aging happens. Thymus degeneration, T lymphocyte function, and the ability to produce cytokines decrease with age, which is the main reason for the low immune function of the elderly. The function of B lymphocyte regulated by bone marrow and its ability to secrete immunoglobulin also decreased. Immunological dysfunction causes increased susceptibility of the aged population to bacterial and virus infections, which are commonly seen in the elderly [5].

The plaque-forming cell (PFC) response is a specific method to examine the effect of medicine on the animal's humoral immune function. Xia D et al. (1989) observed that sheep red blood cell (SRBC)-induced PFC response in 14-month-old mice was significantly lower than that in 3-month-old mice. Three kinds of *G. lucidum* polysaccharide BN_3A, BN_3B, and BN_3C were injected intraperitoneally for five consecutive days, and the reduced PFC response of 14-month-old mice was significantly restored (Table 13.1). BN_3A, BN_3B, and BN_3C significantly increased lymphocyte proliferation induced by concanavalin A (ConA) and IL-2 production in the aged mice [6].

Lei LS and Lin ZB (1993) found that the mixed lymphocyte response (MLR) to alloantigen, automatic proliferation, and IL-2 production of splenocytes in 24-month-old mice was significantly lower than that of 3-month-old mice.

Table 13.1 Effect of BN3A, BN3B, and BN3C on the PFC response to SRBC in 14-month-old mice [Ref. 6]

Age(month)	Group	Dose (mg/kg i.p.)	PFC/10^6
3	Control	–	2061 ± 309
14	Control	–	864 ± 386^{+++}
14	BN3A	5 × 5	1957 ± 392***
14	BN3B	5 × 5	1792 ± 577**
14	BN3C	5 × 5	1405 ± 336*

Note: Mean ± SD, n = 6, $^{+++}P < 0.001$ vs. the control of 3-month-old mice; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the control of 14-month-old mice

Table 13.2 Effect of GL-B(i.p. administration) on DNA polymerase α activity of splenocytes in aged mice [Ref. 7]

Groups	Age (months)	Dose (mg/kg)	DNA polymerase alpha activity (U/1 × 10^{10} cells)
Young control	3	Saline×4	16.29 ± 3.18
Old control	24	Saline×4	9.23 ± 2.42^{++}
Old+GL-B	24	25 × 4	13.30 ± 2.99*
Old+GL-B	24	50 × 4	14.62 ± 3.62*

Note: Mean ± SD; $^{++}P < 0.01$ vs. young control; $*P < 0.05$ vs. old control

G. lucidum polysaccharides (GL-B, 50, 100, and 200 μg/ml) can wondrously restore these cellular immune factors to normal level in 3-month-old mice. The activity of DNA polymerase α in spleen cells of 24-month-old mice was 35.6–43.3%, which was lower than that of 3-month-old mice. Intraperitoneally injecting GL-B (25 and 50 mg/kg) once a day for 4 days can significantly enhance the activity of DNA polymerase α by 44.0 and 58.4%, respectively, in the spleen cells of the 24-month-old mice and make it normal (Table 13.2) [7].

Li WJ et al. (2012) investigated whether oxidative stress and immune dysfunction could be attenuated by *Ganoderma atrum* polysaccharide (PSG-1) in D-galactose (D-gal)-induced aging mice. They found that PSG-1 at the dosages of 50, 100, and 150 mg/kg could significantly decrease lipid peroxidation in the liver, brain, and spleen but concomitantly increased the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-px) compared with the D-gal group. Elevation of glutathione contents and attenuation of glutathione disulfide contents were also found in PSG-1-treated animals. Furthermore, they found that PSG-1 treatment increased basal lymphocyte proliferation as well as T cell and B cell proliferation and enhanced IL-2 production. In brief, PSG-1 had potential as a novel agent to promote health and improve aging-associated pathologies, at least in part, via modification of the redox system and improvement of immune function [8].

Tao SX and Ye CS (1993) observed the effect of *G. lucidum* on cellular immune function in 30 elderly volunteers. After oral administration of *G. lucidum* powder (1.5 g, three times a day, for 30 days), the activities of IL-2, IFN-γ, and NK cells increased in 30 healthy volunteers, reaching the peak on day 20 after taking the medicine (Table 13.3). The results showed that *G. lucidum* could improve the immune function of the elderly people [9].

Table 13.3 Effect of *G. lucidum* on cellular immune function in 30 elderly volunteers [Ref. 9]

	IL-2 (U/ml)	IFN (U/ml)	NK (%)
Before treatment	134.1 ± 39.4	8.3 ± 3.9	40.1 ± 10.3
10 days after treatment	150.7 ± 41.3**	10.6 ± 4.3*	48.7 ± 9.6**
20 days after treatment	159.2 ± 39.4**	11.5 ± 5.2**	50.7 ± 8.4**
Discontinued 10 days	157.8 ± 41.9**	12 .1 ± 5.9**	50.1 ± 9.3**

Note: Mean ± SD, *$P < 0.05$, **$P < 0.01$ vs. before treatment

13.3 Anti-aging Effect of *Ganoderma* by Antioxidative Stress Mechanism

As we all know, the oxidative stress and free radical accumulation theories stand out the most when it comes to aging [5]. The antioxidant function deteriorates in aging. The balance between radical oxygen species production and elimination was broken, leading to oxidative cellular damage [10]. The accumulation of free radicals and attenuation of respiratory chain enzyme complex activity in the brain cause damage to cerebral mitochondria [11], wherein their dysfunction can induce the onset of some neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and others [5].

The role of oxidation and free radicals could damage lipids and cell membranes, damage proteins and enzymes, and damage nucleic acids and chromosomes in aging. Lipid peroxidation and excessive free radical production can lead to cell, tissue, and organ injury.

According to the free radical and the oxidative stress theories of aging, the disruption of the delicate balance between generation of reactive oxygen species (ROS) and antioxidant scavenging systems with increasing age could lead to a shift toward oxidative cellular damage [12]. During aging, antioxidant functions decline in almost all mammals with concomitant increase in oxidative damage to biomolecules [10]. Though aging affects all types of nucleated cells, tissues with few or no cellular division will be theoretically more susceptible to accumulative damage induced by ROS. Many of the significant age-related changes are exhibited in post-mitotic tissues such as the brain, heart, and skeletal muscle [13].

Sudheesh NP et al. (2010) found that the extractions of *G. lucidum* (EGL) could protect the heart, liver, and brain against aging in mice mainly through its antioxidant effect. Administration of EGL significantly ($P<0.05$) elevated the levels of GSH as well as activities of MnSOD, GPx, and GST and decreased significantly ($P < 0.05$) the levels of lipid peroxidation, AOPP, and ROS [1]. The research suggests that *G. lucidum* administration could improve the age-related decline of antioxidant status which was partly ascribed to free radical scavenging activity.

Cherian E et al. (2009) studied the effects of both EGL and *Trichopus zeylanicus* for their free radical scavenging property and for their effects on liver mitochondrial antioxidant activity in 15-month-old mice. Both extracts were administrated orally to aged BALB/c mice at doses of 50 and 250 mg/kg body weight for 15 days. SOD and CAT activity and levels of reduced glutathione (GSH) and lipid peroxidation as

equivalents of methane dicarboxylic aldehyde (MDA) formed were determined. Groups of young mice and aged mice were taken as controls. *G. lucidum* extracts increased antioxidant status in liver mitochondria of aged mice compared with the aged control. The GSH, SOD and CAT were increased in EGL-treated group compared with the control group. EGL possessed significant 2,2-diphenyl-1-picrylhydrazil (DPPH), 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities and ferric-reducing antioxidant power (FRAP). And the DPPH, ABTS, and FRAP activities were higher in *G. lucidum* extract than in *Trichopus zeylanicus*. *G. lucidum* extract also showed superoxide and hydroxyl radical scavenging activities [14].

Ajith TA et al. (2009) reported that effect of ethanol extract of *G. lucidum* on the activities of mitochondrial dehydrogenases; complex I and II of electron transport chain in the aged rat. Aged male Wistar rats were administered with ethanol extract of *G. lucidum* (50 and 250 mg/kg, p.o.) once daily for 15 days. Similarly, DL-a-lipoic acid (100 mg/kg, p.o.)-administered group was used as positive control. Results of the study demonstrated that the extract of *G. lucidum* (50 and 250 mg/kg) significantly enhanced the activities of pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (α-KGDH), succinate dehydrogenase (SDH), and complex I and II in the mitochondria of rat brain compared to that of the aged control animals. The level of MDA was significantly lowered in the *G. lucidum*-treated group compared with aged control. The activity exhibited by the extract of *G. lucidum* in the present study can be partially correlated to its antioxidant activity. The results of the study indicated that the extract of *G. lucidum* may be effective to improve the function of mitochondria in aged rat brain, suggesting its possible therapeutic application against aging-associated neurodegenerative diseases [15].

Sudheesh NP et al. (2010) evaluated the effect of *G lucidum* on the antioxidant status in the mitochondria of the heart and brain of aged mice. Administration of *G. lucidum* ethanoic extract (50 and 250 mg/kg), once daily for 15 days, significantly ($P < 0.05$) elevated the levels of GSH as well as activities of manganese superoxide dismutase (MnSOD), glutathione peroxidase (GSH-Px), and glutathione-S-transferase (GST) and decreased significantly ($P < 0.05$) the levels of lipid peroxidation, advanced oxidation protein products (AOPP), and ROS in the heart and brain mitochondria of aged mice compared with that of aged and young control animals. As we all know, postmitotic cells such as brain and heart cells are particularly vulnerable to oxidative damages during aging. *G. lucidum* administration could improve the age-related decline of antioxidant status which was partly ascribed to free radical scavenging activity [1].

YU Q et al. (2009) studied the anti-aging effect of PSG-1 on aging mice induced by D-gal. The results showed that in comparison with the aging model group, PSG-1 (50, 100, 150 mg/kg, once a day, for 10 weeks) significantly increased the activities of SOD, GSH-P_X, and CAT in liver (Table 13.4) and brain tissue (Table 13.5) and decreased the MDA content. In addition, the loss of body weight and the liver atrophy in PSG-1-treated groups markedly were counteracted. It suggests that PSG-1 has anti-aging effects on aging model mice, and the mechanism may be related to its inhibitory effect on free radicals, the enhancement of activity of antioxidases,

Table 13.4 Effects of *G. atrum* polysaccharide on activities of SOD, GSH-PX, and CAT and MDA content in liver tissue of D-gal-induced aging mice [Ref.16]

Groups	SOD (U/mg pro)	MDA (nmol/mg pro)	GSH-PX (U/mg pro)	CAT (U/g pro)
Control	116.1 ± 6.1	1.18 ± 0.87	126.2 ± 2.98	484.77 ± 41.61
Aged model	74.55 ± 3.95▲	2.48 ± 0.31▲▲	77.38 ± 0.57▲▲	336.81 ± 26.15▲
PSG-1 (50 mg/kg)	84.275 ± 4.0⋆	2.19 ± 0.18⋆	86.31 ± 1.93⋆	390.23 ± 12.79
PSG-1 (100 mg/kg)	95.025 ± 1.05⋆	1.67 ± 0.44⋆⋆	97.99 ± 3.42⋆	423.88 ± 7.98⋆
PSG-1 (150 mg/kg)	117.325 ± 10.8⋆	1.37 ± 0.26⋆⋆	107.95 ± 1.09⋆⋆	445.64 ± 9.47⋆
Vitamin C	104.1 ± 0.55	1.79 ± 0.17⋆	100.62 ± 1.99⋆⋆	445.45 ± 7.57⋆

Note: mean ± *SD*; ▲*P* < 0.05, ▲▲*P* < 0.01 vs control; ⋆*P* < 0.05, ⋆⋆*P* < 0.01 vs. aged control

Table 13.5 Effects of *G. atrum* polysaccharide on activities of SOD, GSH-PX, and CAT and MDA content in brain tissue of D-gal-induced aging mice [Ref. 16]

Groups	SOD (U/mg pro)	MDA (nmol/mg pro)	GSH-PX (U/mg pro)	CAT (U/g pro)
Control	263.36±1.44	2.35 ± 0.30	157.88 ± 6.16	37.30±0.77
Aged model	185.87±4.01▲▲	26.36± 0.37▲▲	109.20± 2.94▲▲	16.50±0.81▲▲
PSG-1 (50 mg/kg)	203.35±3.95⋆	24.08 ± 0.68⋆	122.67 ± 2.32⋆	17.57±0.90⋆
PSG-1 (100 mg/kg)	230.57±7.10⋆	18.35 ± 0.77⋆	136.57 ± 4.48⋆⋆	25.12±1.09⋆⋆
PSG-1 (150 mg/kg)	248.82±6.07⋆⋆	15.32 ± 1.72⋆⋆	146.92 ± 3.20⋆⋆	30.33±1.91⋆⋆
Vitamin C	239.32±10.04⋆	16.20 ± 0.71⋆⋆	136.24 ± 1.74⋆⋆	26.96±4.08⋆

Note: mean ± *SD*; ▲*P* < 0.05, ▲▲*P* < 0.01 vs. control; ⋆*P* < 0.05, ⋆⋆*P* < 0.01 vs. aged control

and the reduction of lipid peroxidation [16]. Li WJ et al. had the same findings about PSG-1, the main constituent of *G. atrum*, that it had a protective effect on the brain against oxidative stress induced by D-gal in vivo [17].

Liu K et al. reported the anti-aging effect of recombinant *G. lucidum* immunomodulatory protein (rlz-8) on D-gal-induced aging mice. Studies showed that D-gal injected subcutaneously significantly decreased the levels of SOD, CAT, and GSH-px in serum and skin tissue of mice, increased the MDA level, and significantly decreased the serum melatonin (MT) level. It means that the aging changes caused by D-gal are related to oxidative stress. However, only high dose of rlz-8 (1320 μg/kg) can significantly increase the all above antioxidant substances and MT level and makedly reduce the MDA level. This finding suggests that rlz-8 can be adopted to enhance the antioxidant effect of aging mice and delay their aging [18].

The dysfunction of antioxidant enzymes accelerates the aging process as they are the first line of defense for protecting biological macromolecules against oxidative stress. GSH is the richest nonprotein thiol molecule in tissues and possesses the ability to prevent cerebral ROS accumulation through a direct reaction with ROS

and electrophilic metabolites. *G. lucidum* could increase the activity of antioxidant enzymes in the cardiac, hepatic, and cerebral mitochondria of aged mice [19].

Li X and Liang J (2013) studied the postponing effect of *G. lucidum* polysaccharides on the skin aging by antioxidant mechanism in D-gal-induced aging mice. The result of histopathological observation of skin structure showed that the epidermal and dermal thickness were reduced significantly in the aging model group compared with normal control group. The epidermal and dermal thickness in *G. lucidum* polysaccharide group and the vitamin E group (positive control) were increased markedly when compared with the aging model group. Compared with vitamin E group, only epidermal thickness was significantly enhanced in *G. lucidum* polysaccharide group. The SOD activity in the skin tissues of aging model group was decreased compared with normal control group. The SOD activity in the *G. lucidum* polysaccharide group was more than that in the aging model group and vitamin E group (Table 13.6). The decreased degree of CuZu-SOD mRNA cycle threshold value in the *G. lucidum* polysaccharide group was lower than that in aging model group and vitamin E group CuZu-SOD mRNA (Table 13.6). *G. lucidum* polysaccharide can repair the severe oxidative damage of skin tissue which cannot be repaired by vitamin E, indicating its potential clinical value in aging [20].

Cell senescence is a complex pathophysiological process involving multiple genes under the regulation of cell cycle. Wei et al. (2009) observed the effect of *G. lucidum* polysaccharide (GLP) on gene of CyclinD1, Rb, CDK4, and $p16^{NK4a}$ and the change of phosphorylated Rb (pRb) in aging human fibroblast (HDF) cell induced by H_2O_2. This study found that cultivated HDF cells grew well with long prismatic form and no keratinization in young group, while cells in the aging group arranged irregularly, with larger volume, more intracellular granules and vacuoles. GLP could reduce the intracellular vacuoles, but aging phenotype was not obvious. Compared with the young group and the control group, the cell viability of the aging model group significantly decreased, the activity of beta-galactosidase increased significantly (Table 13.7), the expression of CyclinD1 gene significantly increased, the expression of CDK4 gene decreased significantly, the expression of p16INK4a increased (Table 13.8), the expression of Rb increased, and Rb phosphorylation

Table 13.6 Superoxide dismutase activity and relative expression of CuZu-superoxide dismutase mRNA in mouse skin tissues [Ref. 20]

Group	n	Superoxide dismutase activity (U/mg prot)	Relative expression of CuZu-SOD mRNA (Ct)
Normal control	10	58.43±3.83	21.78±1.12
Aging model	11	$41.68±5.44^{a}$	$25.63±0.78^{a}$
Vitamin E	11	$48.07±3.71^{a,b}$	$24.39±0.99^{a,b}$
Ganoderma lucidum polysaccharide	11	$54.17±5.61^{c,d}$	$22.79±1.21^{c,d}$

[a]$P < 0.01$ vs. normal control group; [b]$P < 0.05$; [c]$P < 0.01$ vs. aging model group; [d]$P < 0.05$ vs. vitamin E group

Table 13.7 Effects of hydrogen peroxide (H_2O_2) on cell viability and beta-galactosidase activity and function of *G. lucidum* polysaccharide. [Ref. 21]

Group	Cell viability	Beta-galactosidase activity (%)
Young	1.485 ±0.055	1.48 ±0.75
Control	1.319 ±0.050	38.89 ±4.50
Old	0.375 ±0.068[a]	93.75 ±5.80[a]
GLP 50 mg/L	0.895 ±0.061[a, b]	38.95 ±5.61[c]
GLP 100 mg/L	1.301 ±0.053[c, d]	30.11 ±5.31[c, d]
GLP 150 mg/L	1.270 ±0.069[c, d]	35.70 ±4.69[c, d]

Compared with young group and control group, [a]$P < 0.01$; compared with old group, [b]$P < 0.05$, [c]$P < 0.01$; compared with GLP 50 mg/L group, [d]$P < 0.01$

Table 13.8 Effects of hydrogen peroxide (H_2O_2) on CyclinD1, CDK4, and p16 ^{INK4a} mRNA and function of *G. lucidum* polysaccharide. [Ref. 21]

Group	CyclinD1/β-actin	CDK4/β-actin	p16 ^{INK4a} /β-actin
Young	0.502 ±0.051	0.610 ±0.080	0.279 ±0.030
Control	0.729 ±0.080	0.581 ±0.060	0.330 ±0.030
Old	0.890 ±0.102[a]	0.270 ±0.030[a]	0.934 ±0.102[a]
GLP 50 mg/L	0.690 ±0.061[c]	0.372 ±0.061	0.593 ±0.061[c]
GLP 100 mg/L	0.592 ±0.050[c, e]	0.450 ±0.050[c, d]	0.536 ±0.050[c]
GLP 150 mg/L	0.581 ±0.050[c, e]	0.461 ±0.050[c, e]	0.542 ±0.060[c]

Compared with young group and control group, [a]$P < 0.01$; compared with old group, [b]$P < 0.05$, [c]$P < 0.01$; compared with GLP 50 mg/L group, [d]$P < 0.05$, [e]$P < 0.01$

decreased. The difference with the aging model group is that GLP at medium and high doses could restore above senescence changes of HDF cells induced by H_2O_2 (Tables 17.7 and 17.8). In conclusion, protective effect of GLP on senescence of HDF cells induced by H_2O_2 may associate with regulation of cell proliferation cycle [21].

Lin and Pan (2009) also found that *G. lucidum* polysaccharide (0.5~2.0 g/kg) could significantly increase reducing the hydroxyproline and the SOD content of skin in 16-month-old rats [22].

13.4 Prolonged Effect of *Ganoderma* on Life Span of *Caenorhabditis elegans* and Yeast

Anti-aging studies are commonly performed in a variety of organisms, such as yeasts, nematodes, fruit flies, mice, and rats. In *Caenorhabditis elegans*, *G. lucidum* effectively promoted the nematodes to resist the oxidative stress and significantly prolonged the life span of the nematodes.

Cuong VT et al. (2019) used the *Caenorhabditis elegans* as an animal model to study the antioxidative stress and anti-aging effects of *G. lucidum* water extract. Results showed that *G. lucidum* effectively promoted the nematodes to resist the oxidative stress of paraquat and heavy metal Cr^{6+} and significantly prolonged the life span of the nematodes. Further investigation found that *G. lucidum* protected the nematode against the insults of paraquat and heavy metals through the diet restriction pathway and the mTOR/S6K signaling pathway, respectively, whereas the effect of *G. lucidum* on the longevity of the nematode mainly depended on the germline signaling pathway. Microarray assays were conducted to reveal the gene expression profiles. The expression levels of 2746 genes were significantly changed during the aging process, 2082 genes downregulated and 664 upregulated, of which 34 genes were reversed in their expression by the treatment of *G. lucidum* in aged nematodes.

Among them, 25 genes that were downregulated during the aging were significantly increased after the treatment with *G. lucidum* extract, and 9 genes upregulated during the aging were inhibited after the extract administration. More than half of the genes are not well studied or without any known functions. Only the smg-1, a serine-threonine kinase which plays a conserved role in nonsense-mediated mRNA decay (NMD) in worms and mammals, has been reported involved in longevity of *C. elegans* [23].

Weng Y et al. (2010, 2011) purified four novel anti-aging ergosterol derivatives, ganodermasides A, B, C, and D, from *G lucidum* spores and studied the anti-aging activity of these compounds on yeast. Results showed that these compounds significantly extended the replicative life span of the K6001 yeast strain, and the anti-aging activity on yeast is comparable to a well-known substance, resveratrol. Ganodermasides A, B, C, and D regulated the expression of the gene for *UTH1* to prolong the replicative life span of yeast [24, 25].

13.5 Conclusion

Take in all, *Ganoderma* and its variety of extracts have some anti-aging properties and exert their anti-aging effects mainly through immunomodulation, anti-oxidation, and free radical scavenging activities, increasing mitochondrial dehydrogenases and complex I and II of electron transport chain, and anti-neurodegeneration by antioxidative stress [26, 27], and so on (Fig. 13.1). Based on its longevity effects, *Ganoderma* has some other healthy promoting properties such as anti-diabetes and anticancer. Therefore, it is important to find its effective anti-aging ingredients and extracts in *Ganoderma*. Plenty of evidence is required to provide a comprehensive explanation on its effects and mechanisms underlying the anti-aging property.

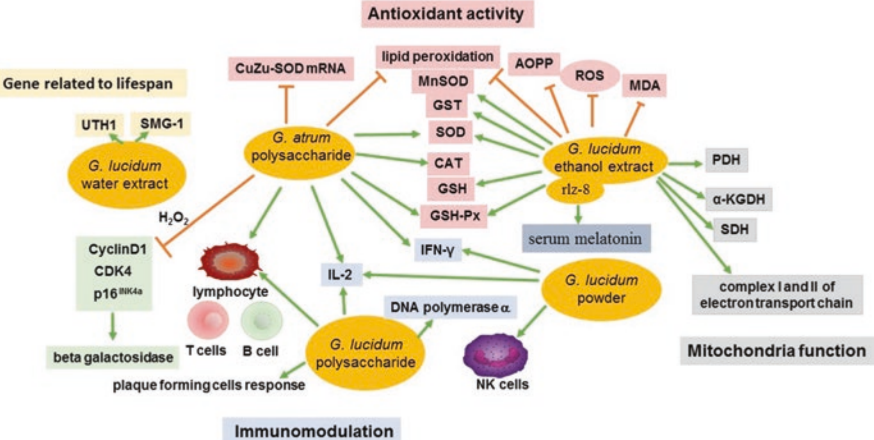

Fig. 13.1 The schematic diagram on anti-aging effects and mechanism of *Ganoderma*. *Ganoderma* exert anti-aging effects through radical scavenging activities and ferric-reducing antioxidant power to produce antioxidant effects, enhancing mitochondria function, immunomodulatory effect and enhance longevity gene expression. *G. lucidum*: *Ganoderma lucidum*; *G. atrum*: *Ganoderma atrum*; rlz-8: recombinant *G. lucidum* immunomodulatory protein; blue arrows mean increase the activity or expression of molecules; red T shape arrow means decrease the activities or expression of molecules

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Chapter 14
Preventive and Therapeutic Effect of *Ganoderma* (Lingzhi) on Skin Diseases and Care

Zhuming Yin, Baoxue Yang, and Huiwen Ren

Abstract *Ganoderma lucidum* (*G. lucidum*, Lingzhi), a kind of mushroom with various bioactivities, is recently revealed to improve skin quality and treat skin diseases. Traditionally, polysaccharides and ganoderic acids have been reported as the major functional metabolites of *Ganoderma* possessing antioxidant and anti-inflammatory functions. Based on our research and other studies, *Ganoderma* extracts, such as *Ganoderma* polysaccharides, have been used in promoting skin wound healing, mitigating postburn infection, and preventing skin flap ischemia-reperfusion injury. *Ganoderma* extracts have also been used in skin care, because of their roles in skin photoaging and skin whitening. Meanwhile, the anti-inflammatory effect of *Ganoderma* in atopic dermatitis and cutaneous sarcoidosis is also elaborated in this chapter. Finally, the potential use of *Ganoderma* in skin carcinoma is introduced. In brief, the dermoprotective effect of *Ganoderma* will be summarized in this chapter.

Keywords *Ganoderma* · Lingzhi · Polysaccharides · Triterpenes · Skin care · Skin diseases

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14.1 Effect of *Ganoderma* on Skin Injury

As the largest organ of the human body, the integrity of the skin plays an important role in immune surveillance, sensory detection, and self-healing. A variety of injuries, such as frostbite, burn, infections, and surgical interventions, can impair skin integrity and metabolic balance [1, 2]. *Ganoderma* extracts have been used in promoting skin wound healing and mitigating skin injuries.

14.1.1 Therapeutic Effect of Ganoderma on Skin Wound Healing

Wound healing is a complex process with the major characteristics as regulated collagen deposition, which is initiated and promoted by the mechanism of inflammation, fibroplasia, and scar maturation in response to skin injury [3, 4]. The diabetic foot ulcer is one of the most common and severe complications of diabetes mellitus because of impaired wound healing [5, 6]. *Ganoderma* polysaccharide (GL-PS) treatment at the dose of 10, 50, and 250 mg/kg intragastrically administered to STZ-induced type 1 diabetic mice can effectively improve the restoration of full-thickness wounds. 250 mg/kg GL-PS administration can promote the rate of wound closure to about 61.34% compared to the control (39.51%). Meanwhile, GL-PS does not affect blood glucose and body weight on STZ-induced type 1 diabetic mice. 50 mg/kg and 250 mg/kg GL-PS treatment significantly inhibits Ser36 phosphorylation of p66Shc and Pin1 levels to reduce the accumulation of p66Shc in mitochondria. This treatment markedly increases iNOS activity and thus activates the pro-inflammatory process. Furthermore, GL-PS also accelerates diabetic wound healing by enhancing MnSOD activity and promoting angiogenesis [7].

Fibroblasts can differentiate into myofibroblasts, which are responsible for collagen synthesis and wound contraction. 80 μg/ml GL-PS increases the viability and migration ability of primary human skin fibroblasts in vitro. Because of the fibroblast proliferation and activation, the rate of wound healing in the Kunming mice model with full-layer skin resection trauma increases to about 70 and 80%, respectively, with the treatment of 20 mg/kg and 40 mg/kg of GL-PS. In the process of wound healing, GL-PS can activate Wnt/β-catenin signaling pathway and increase the expression of TGF-β1, which may serve to intensify the migration ability of fibroblasts and upregulate the collagen deposition [8]. In the cohort study on the treatment of diabetic foot, the injection of GL-PS can significantly ameliorate intermittent claudication and reduce the incidence of hypesthesia compared with control [9].

The polyporate structure, permeability, and solubility characteristics of nanostructured lipid carriers (NLCs) make it capable for accommodation of the novel drug. Besides using *Ganoderma* directly, NLCs seem to be a good carrier for the application of *Ganoderma* on wound healing. The accumulative amount in epidermis was increased by 7.76 times in the group of *Ganoderma* with NLCs compared with *Ganoderma* only.

Not only the improvement of permeation, *Ganoderma* with NLCs is stable at room temperature for as long as 3 months without color changes or phase separation. In the sulfadiazine zinc ointment (SZO)-induced frostbitten rabbit model, the edema rate of rabbit's ears is decreased to about 7.32%, and the percentage of survival area is increased to about 90.13% compared with SZO control group, which display 41.23% and 54.56%, respectively [10–12]. Therefore, *Ganoderma* with NLCs may be developed to a novel application of *Ganoderma* for the treatment of skin would healing.

14.1.2 Effect of Ganoderma on Postburn Infection

Infection is one of the most deleterious factors that affect the wound healing after skin burn. Multidrug-resistant bacteria have obtained more prevalence and have been a major obstacle in the treatment of postburn infection [13]. Additionally, *Pseudomonas aeruginosa* can promote adherence and invasion ability of other bacteria by damaging the host's immune responses and forming a barrier to antibiotics [14]. The methanol extract of *G. lucidum* fruiting bodies has been proved to decrease a protease activity from 27 to 3 units/ml at the minimum inhibitory concentration of 2 mg/ml against extended-spectrum β-lactamase-producing and multidrug-resistant *Pseudomonas aeruginosa* clinical isolates. Although there needs a deep dive into the antibiotic mechanism of *Ganoderma*, the current study provides a promising therapeutic strategy against the *Pseudomonas* infection by administrating the potential drug without any antibiotic abuse [15].

14.1.3 Effect of Ganoderma on Skin Substitute

The dermal substitutes are basically made of a sheet of autologous keratinocytes, which are isolated from a donor and expanded in vitro. Traditionally, the production of the epidermal sheets is time-consuming and costly, and the resulting products usually have a short shelf life [16]. The Sacchachitin membrane, produced from the waste residue of the fruiting body of *Ganoderma tsugae*, has been reported to be an ideal skin substitute with great biocompatibility and clinical efficacy [17, 18]. Compared with gauze and Beschitin, it has been successfully applied in the management of excised wounds in guinea pigs with more efficiency compared with other materials such as gauze and Beschitin [19].

In clinical practice, the Sacchachitin membrane has been used to promote the wound closure of patients who are suffering from chronic skin ulcer for more than 7 months [20]. Further investigation revealed that the Sacchachitin membrane possesses the ability to induce type I collagen synthesis in keratinocytes. Thus, Sacchachitin membrane can promote the proliferation and migration of keratinocytes much faster (up to 2 days) than that of the cotton gauze group. Meanwhile, the Sacchachitin membrane of *Ganoderma tsugae* can reduce the activity of matrix

metalloproteinases (MMPs) in extracellular matrix degradation and facilitate the reestablishment of an extracellular matrix around wounds, which results in more rapid wound healing [20, 21].

14.1.4 *Therapeutic Effect of Ganoderma on Skin Flap Ischemia-Reperfusion Injury*

The ischemia-reperfusion injury during microvascular skin flap transfer is one of the major complications in plastic surgery, which may lead to flap compromise [22, 23]. *Ganoderma lucidum* polysaccharide peptide at the dose of 100 mg/kg/day could ameliorate the reperfusion damage and reduce the necrosis area by 40% in 7 days compared with sham-operation groups (Fig. 14.1). The burst of reactive oxygen species (ROS) is widely considered as the main culprit in the pathogenesis of ischemia-reperfusion, accompanied by an impairment of cellular redox homeostasis and extensive cell apoptosis. GLPP administration can largely attenuate oxidative stress and reduce the level of the early-stage apoptosis by 20% compared with sham-operated group. Thioredoxin-1, an endogenous redox signaling regulator, has been proved to protect free skin flaps by mitigating the oxidative stress and inhibiting the activation of the apoptosis signal-regulating kinase 1 (ASK-1) and mitogen-activated protein kinase (MAPK) pathway [24]. Later on, the hypothesis has been substantiated that GLPP may attenuate the skin flap ischemia-reperfusion injury by promoting thioredoxin-1-dependent antioxidant and antiapoptotic signaling transduction (Fig. 14.2). These preliminary results indicate that GLPP may represent a novel and potential preventive and therapeutic strategy in the practice of microsurgery [24].

14.2 Effect of *Ganoderma* on Skin Care

Maintaining and improving skin health and integrity are fundamentally essential for normal skin function. However, UVR stands on the opposite side of skin health, causing skin photoaging and dulling. A huge number of previous studies have reported various reasons of the pathological phenomenon, including the DNA damage, gene mutation, immunosuppression, oxidative stress, and inflammatory response which are all derived from UVR [25, 26].

14.2.1 *Effect of Ganoderma on Skin Photoaging*

When the skin is exposed to the UV energy, ROS is exponentially generated through the interaction between intracellular chromophores and oxygen molecules. ROS may activate the extracellular signal-regulated kinases (ERKs), c-Jun N-terminal

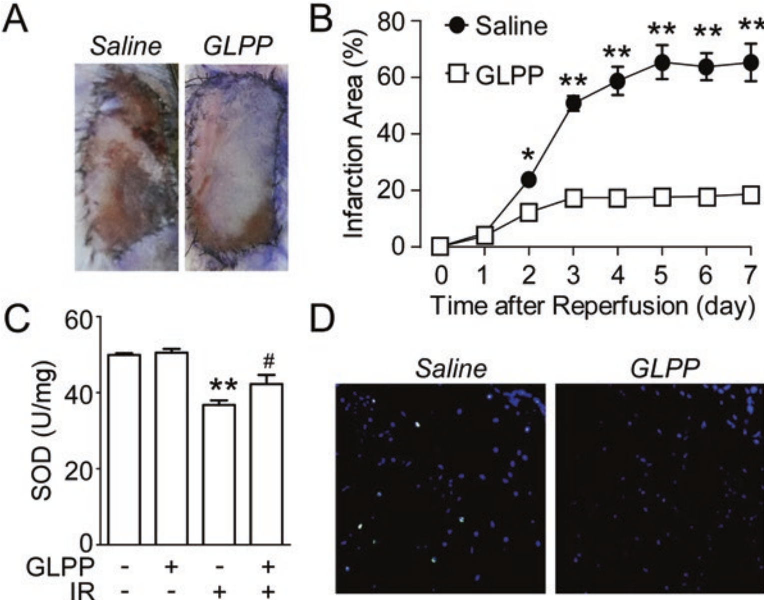

Fig. 14.1 GLPP protects the skin flap against ischemia-reperfusion injury in mice. (**a**) The representative skin flap infarction area on 7th day after skin ischemia-reperfusion. (**b**) The flap infarction area recorded daily for 7 days after ischemia-reperfusion in mice with or without GLPP treatment. The infarction area of the ischemia-reperfusion flap was significantly diminished in the GLPP-treated group. (**c**) Superoxide dismutase levels in mouse skin flaps after reperfusion for 24 h. (**d**) The representative photographs showing cell apoptosis degree evaluated by TUNEL (green fluorescence)

kinase (JNK), and p38 in the mitogen-activated protein kinase (MAPK) pathway, and then MMPs could be overexpressed [27, 28]. MMPs subsequently trigger the skin photoaging process by degrading the type I collagen in the extracellular matrix [29, 30].

G. lucidum has been confirmed to display an antioxidant effect in protecting murine skeletal muscles after exhaustive exercise. It has also been demonstrated to own potential antiaging ability to regulate the relative gene expression and induce cytokine secretion in aging skin cells [31]. Huang et al. demonstrate that 40 μg/kg *G. lucidum* can promote fibroblast viability and inhibit fibroblast aging following 60 mJ/cm^2 UVB exposure. During the UVR-induced photoaging process, *G. lucidum* can inhibit the MAPK signaling pathway and decrease the MMP-1 expression level, resulting in the alleviation of extracellar matrix degradation [32, 33].

Tsugarioside compound 3 is one of the extracts from the fruit bodies of *Ganoderma tsugae*. Tsugarioside compound 3 (IC_{50} value was 116.1 μM) has also been proved to increase the viability of HaCaT cells exposed to 15 mJ/cm^2 UVB and protect human keratinocytes against damage [34, 35]. Overall, *Ganoderma* may be a good candidate herbal medicine for protective extracts against UVR-induced photodamage. In rat aging model, 250 mg/kg GL-PS can increase the epidermal and

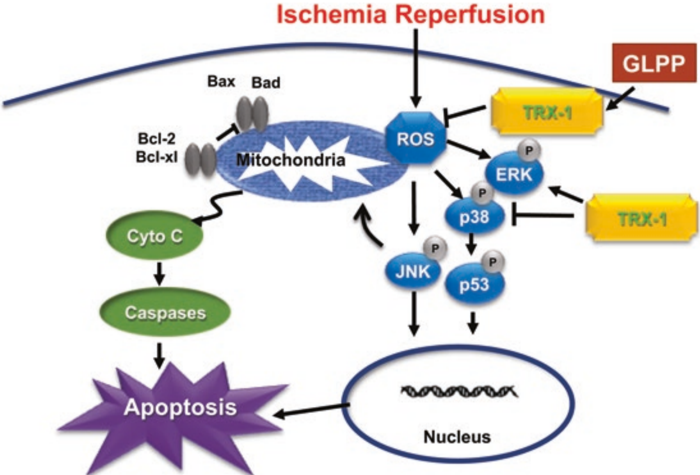

Fig. 14.2 The therapeutic mechanisms of *Ganoderma* on skin flap ischemia-reperfusion injury. Both classic mitochondrial apoptotic pathway and ASK-1-MAPK signaling pathway are activated by ROS in skin tissue after ischemia-reperfusion injury. Trx-1 can eliminate ROS level directly and inhibit ASK-1-MAPK signaling pathway. The treatment of GLPP significantly relieves the Trx-1 depletion. Then, GLPP rescues the cells from apoptosis by downregulating the expression levels of ASK-1, p-p38, and cleaved caspase-3. Therefore, Trx-1 mediates the antioxidant and antiapoptotic mechanisms of GLPP in the process of skin flap ischemia-reperfusion injury. GLPP, *Ganoderma lucidum* polysaccharide peptide; Trx-1, thioredoxin-1; ROS, reactive oxygen species; p-ERK1/2; phosphorylation of extracellular signal-regulated kinase, p-p38, p38 mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; p53, cellular tumor antigen p53

dermal thicknesses, leading to improving the skin tissue structure. Compared with 250 mg/kg 5% vitamin E oil, 250 mg/kg GL-PS can also increase the epidermal thickness by about 20.67%. This result proves that GL-PS is helpful to repairing the severe oxidative damage which cannot be done by vitamin E [36].

14.2.2 *Effect of Ganoderma on Skin Whitening*

The main determinant of skin color is melanin, a black pigment synthesized from tyrosine by epidermal melanocytes [37]. Tyrosinase, a multifunctional copper-containing oxidase, is considered the key enzyme that orchestrates melanogenesis in melanocytes [38].

The ganodermanondiol, an extract from *G. lucidum*, is reported to affect the melanin production by decreasing the expression of tyrosinase-related protein-1 (TRP-1), TRP-2, and microphthalmia-associated transcription factor in B16F10 melanoma cells. Moreover, the treatment of ganodermanondiol at 10 μM can increase the phosphorylation of ERK and JNK and suppress the phosphorylation of p38, suggesting that ganodermanondiol may inhibit melanogenesis by modulating

MAPK signaling pathways [39–41]. Another species called *Ganoderma formosanum* at the dose of 2 μm/mL exhibits high tyrosinase inhibition activity (IC_{50} value was 118.26 ± 13.34 ppm) in B16-F10 melanoma cells. *Ganoderma formosanum* (400 ppm) also shows a better depigmenting activity to reduce approximately 50% of melanin formation, compared with the classic depigmenting drug kojic acid (20 mM) in the model of zebrafish embryos [42, 43]. The studies about the major active compounds of ganodermanondiol provided new insights into the development of potential skin whitening ingredients from *G. lucidum*.

Currently, the extracts from *G. lucidum* have been commercially used in a variety of facial mask cosmetics as a kind of tyrosinase inhibitors [44]. The safety is an important reason for its prevalence, for *G. lucidum* shows no toxicity to human fibroblast cells in vitro and no side effect in volunteers' skin test [45–47]. But the clinical use of *G. lucidum* remains a long way to go. Although *G. lucidum* has been proved as a safe and effective medical and healthcare agent, there is still an accidental report that the local ingestion of *G. lucidum* can induce vesiculobullous skin lesions over the entire palms [48].

14.3 Effect of *Ganoderma* on Inflammatory Skin Diseases

As universally acknowledged, the skin is the first defensive barrier against infection. Human keratinocytes possess all inflammasome components, which would be produced as a series of protective and regenerative responses of the body [49]. Therefore, the inflammatory skin diseases are basically immune responses to the infections and dangers, as well as the hereditary disorders.

14.3.1 Therapeutic Effect of Ganoderma on Atopic Dermatitis

Atopic dermatitis is a skin disease with inflammatory, pruritic, chronic, and relapsing symptoms [50]. *Artemisia capillaris* (*A. capillaris*), derived from the solid-state fermentation with *G. lucidum*, can reduce the rate of ear swelling by about 40% in the mouse model of 2, 4-dinitrofluorobenzene-induced atopic dermatitis. *A. capillaris* can remarkedly decrease the expression of iNOS and eNOS and inhibit the MMP-2, MMP-7, MMP-9, MMP-12, MMP-14, and MMP-19 mRNA expression, leading to a less active inflammatory response in mouse ear. The study confirmed that *A. capillaris* may alleviate inflammation-related skin reaction associated with antioxidant capacity, displaying promising potential in the clinical application [51]. In the meantime, *Ganoderma* tea (30 g daily for 8 weeks) is also applied to relive patients from eczema syndrome (ClinicalTrials.gov identifier: NCT02533635), which is an autoimmune inflammatory skin disease [40].

14.3.2 *Effect of Ganoderma on Cutaneous Sarcoidosis*

Sarcoidosis is a systemic disease characterized by the pathological development of noncaseating epithelioid granulomas [52]. The symptoms involve infiltrated plaques, maculopapular eruptions, infiltration of old scars, and lupus pernio, and subcutaneous sarcoidosis is the most common category of cutaneous sarcoidosis. In a case report, a 44-year-old male patient had suffered from annular cutaneous sarcoidosis on his scalp for 4 years. Given the immunomodulatory, anti-angiogenic, and cytotoxic characteristics, the multiple plaque lesions almost disappeared by the treatment of *G. lucidum* with the goat milk-containing soap for 3 days. This report has been preliminarily confirmed as an effective maneuver against such skin lesions [53], providing a novel application of GL-PS for the management of skin sarcoidosis.

14.4 Effect of *Ganoderma* on Skin Carcinoma

Skin carcinoma is a group of malignancies commonly diagnosed in Caucasians. Skin cancers can be divided into nonmelanocytic skin cancer and malignant melanoma [54]. *Ganoderma tsugae* methanol extract (GTME) (3 mg/ml) can inhibit the proliferation of human epidermoid carcinoma A-431 cells up to 90% for 72 h. GTME inhibits PI3K/Akt/mTOR signaling pathway and downregulates vascular endothelial growth factor expression, resulting in suppressing the angiogenesis and growth in A-431 cells [55]. *Ganoderma* polysaccharide (4 μl/ml) is also reported to suppress cell proliferation and induce cell apoptosis in time- and dose-dependent manners in murine skin carcinoma cells (CH72) [56]. In a two-stage mouse skin carcinoma model, one compound of *Ganoderma* triterpene extract (85 nmol), 20-hydroxylucidenic acid N, delays the formation of papillomas by 55.56% in mouse skin and inhibits mouse skin carcinogenesis after the exposure to this compound 20 weeks [57]. This kind of MeOH extracts of *G. lucidum* may also be applied in combination with cancer chemotherapy and targeted therapy.

14.5 Conclusion

As an herbal medicine, *Ganoderma* has been widely used in Asian countries to ameliorate plenty of health problems and to prolong life span in the past 2000 years. However, clinical trials of *Ganoderma* did not go smoothly in the past decades. Although *Ganoderma* has been confirmed to have anti-fatigue, antioxidant, antiaging, and antitumor activities in skin diseases and skin care, there is a great gap between nutraceutical and pharmaceutical application. Meanwhile, it is still a hard scientific work to investigate the convincing proof for the novel applications in other

skin diseases and skin care. The identification of clear active pharmaceutical ingredients and biological mechanism would play an important role in the development of *Ganoderma*-based drugs. It should not be underestimated that the bioactive metabolites from *Ganoderma* are the suitable strategies for new skin-related drug development.

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