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Mycosphere Essays 7. *Ganoderma lucidum* - are the beneficial anti-cancer properties substantiated?

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Abstract

Ganoderma lucidum is a popular medicinal mushroom that has been used particularly in China, Japan and Korea for millennia to improve longevity and health. Research on various metabolic activities of *G. lucidum* have been performed in both *in vitro* and *in vivo* studies. There are a vast number of publications that show the abundance and variety of biological actions triggered by the primary metabolites of *G. lucidum* such as polysaccharides, proteins and triterpenes. However, it is debatable whether *G. lucidum* is a food supplement for health maintenance or is a therapeutic "drug" for medical purposes. There has been no report of human trials using *G. lucidum* as a direct control agent for cancers, however, some evidence showing the usage of *G. lucidum* as potential supplements for cancer patients and a small number of preclinical trials have suggested that it carries promising anti-cancer and immunomodulatory properties. In this review, the beneficial anti-cancer properties of *G. lucidum*, the evidence for medicinal uses and secondary metabolites, and the effects on human cancer are discussed. *G. lucidum* and related products can be used as a therapeutic drug, but more direct scientific evidence should be made available in the future. The efficiency of *G. lucidum* in clinical treatments can be proven by systematic translational research programs using, only standardized preclinically evaluated and biologically active *G. lucidum* extracts in alternative treatments. Hence, studies on *G. lucidum* should focus on improving methods and further clinical research on human subjects should be performed with more scientific reproducibility. Furthermore research should target pharmacologically active constituents of *G. lucidum* that contribute to positive immune responses, as well as the mode of action of *G. lucidum* at the molecular level at target organs.

Key words – Anti-cancer activity – clinical evidence – *Ganoderma lucidum* – Lingzhi

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Introduction

Lingzhi is the Chinese name, mainly applied to *Ganoderma lucidum* (Curtis) P. Karst. a woody polypore (Basidiomycota) that has been widely used in China for medicinal purposes for more than two thousand years (Sliva 2006). *G. lucidum* was introduced by Curtis (1871) based on material from Peckham, London, UK and the epithet was sanctioned by Fries (1821). *G. lucidum* has previously been treated as synonyms of *Boletus lucidus* (Fr.), *Polyporus lucidus* Curtis (Fr.), *Polyporus polychromus* Curtis (Fr.), *G. polychromum* Curtis (Fr.), *G. sessile* Curtis (Fr.) and *Fomes lucidus* (Curtis) Sacc. (Siwulski et al. 2015). Patouillard (1907) reported *G. lucidum* from China for the first time and Teng (1934) described collections of *G. lucidum* from different regions in China (Wang et al. 2012). Liu (1974) compiled a monograph of Traditional Chinese Medicinal Fungi, and he described *G. lucidum* as "Lingzhi" in his book. Since then, *G. lucidum* was accepted as the scientific binomial of "Lingzhi" in many reports on Chinese edible and medicinal mushrooms (Ying et al. 1987, Mao 1998, Dai et al. 2009). For over a century, "Lingzhi", the highly prized medicinal mushroom in East Asia, has been assigned to *G. lucidum*, a species originally described from Europe (Cao et al. 2012). Hawksworth (2005) suggested to conserve the name *G. lucidum* to an Asian type and introduce a new name for the European species (Yang & Feng 2013). Wang et al. (2012) proposed the name, '*G. lucidum'* as used for the Chinese species as incorrect and should be corrected to *G. sichuanense*. The validity of *G. lingzhi* and *G. sichuanense* has been recently debated. Wang & Yao (2009) proposed that *G. sichuanense* can represent '*G. lucidum'* in China. With the aid of molecular phylogeny, Wang et al. (2009) divided Asian specimens classified as *G. lucidum* into two clades; both clades were separated from the European *G. lucidum*. One clade, composed of tropical collections, represented *G. multipileum*, while the other clade is undescribed. As *G. sichuanense* had previously been described, Wang et al. (2009) proposed this name for *G. lucidum* in China. In parallel, Cao et al. (2012) found that the holotype of *G. sichuanense* was not conspecific with the unnamed clade, and proposed it as a new species called *Ganoderma lingzhi*, which was considered to be the most widely cultivated species in China. Yao et al. (2013) proposed *G. sichuanense* and *G. lingzhi* as synonymous based on morphological data from an epitype of *G. sichuanense*. However Zhou et al. (2014) again challenged this opinion, with *G. lingzhi* and *G. sichuanense* being an independent and taxonomically valid species by stressing that species types depends on geographical distributions. *G. lucidum* therefore is incorrectly recorded in China, and around the world. Hapuarachchi et al. (2015) reported *G. lucidum sensu lato* as a species complex whose taxonomy has been confused throughout history and is still debatable. Ryvarden & Johansen (1980) treated all names of the *G. lucidum* complex as the "*G. lucidum* group" because of the lack of specific morphological criteria to name species in this complex. Based on phylogeny by sequences of ITS, tef1a, rpb1 and rpb2 from worldwide samples, Zhou et al. (2015) made a systematical study on the *G. lucidum* species complex and demonstrated 13 species with color photos However the Chinese "Lingzhi" has continuously been referred to as *G. lucidum* in monographs of Ganodermataceae in China (Hapuarachchi et al. 2015). *G. lucidum* has been widely used for naming the commercialized "Lingzhi" products in the worldwide mushroom industry, since it has numerous health benefits (Lai et al. 2004). Hence, in this review we assigned the general name of *G. lucidum* "Lingzhi", as applied in the previous publications and discuss the beneficial anti-cancer properties, the evidence for medicinal uses, secondary metabolites, and the effects on human cancer.

Ganoderma lucidum is thought to be widely distributed in both tropical and temperate geographical regions, growing as a pathogen or saprotroph on a wide variety of hardwoods (Dai et al. 2007, Dong & Han 2015). It has large, perennial, and woody basidiocarps (Dong & Han 2015). *Ganoderma lucidum* is widely used in Traditional Chinese Medicine and food products as it is believed to increase human longevity and maintenance of vivacity (Sliva 2006). Lingzhi is viewed as the "herb of spiritual potency" or "mushroom of immortality", and symbolizes sanctity, success, goodness and longevity (De Silva et al. 2012a). Chinese herbal medicine has been practiced for thousands of years and is used increasingly in Western countries in conjunction with or in place of allopathic medicine (Yuen & Gohel 2005). *G. lucidum* is not used in cooking because of its bitter

taste and a wooden texture (Chang 1996), however it is consumed in various forms. In recent decades, much attention has been placed on *G. lucidum* and many studies have reported that a vast number of pharmacological compounds are produced by *G. lucidum* (Paterson 2006). The pharmacological effect of *G. lucidum* is based on its strong immune-modulating action and immune potential capability, which supports and enhances the overall immune function (Singh et al. 2015). Studies have revealed that *G. lucidum* contains approximately 400 different bioactive compounds (Dong & Han 2015) with polysaccharides, steroids and triterpenes as the major groups (Boh et al. 2007), followed by alkaloids, fatty acids, glycoproteins, inorganic elements, lignin‟s, nucleosides, nucleotides, peptides, phenols, proteins, sterols and vitamins (Paterson 2006, Li et al. 2013). Bioactive compounds from *G. lucidum* hold an enormous structural and chemical diversity which is incomparable to any synthetic library (Li et al. 2013). These bioactive constituents are reported to be responsible for the anti-cancer, anti-inflammatory, anti-tumor, anti-oxidant, immunomodulatory, immunodeficiency, anti-diabetic, anti-viral, anti-bacterial, anti- fungal, anti-hypertensive, anti-atherosclerotic, anti-aging, anti-androgenic, anti-hepatotoxic, radical scavenging property, neuroprotection, sleep promotion, cholesterol synthesis inhibition, hypoglycemia, inhibition of lipid peroxidation/oxidative DNA damage, hepatoprotective properties, maintenance of gut health, prevention of obesity, and stimulation of probiotics (Paterson 2006, Cao et al. 2013, De Silva et al. 2012a, b, De Silva et al. 2013, Baby et al. 2015, Bishop et al. 2015, Liu et al. 2015). *G. lucidum* has been used as a functional food to prevent and treat many immunological diseases, such as anorexia, arthritis, asthma, bronchitis, cardiovascular problems, constipation, diabetes, dysmenorrhea, gastritis, hemorrhoids, hepatitis hypercholesterolemia, hypertension, insomnia, lupus erythematosis, migraine, nephritis, neurasthenia, neoplasia and tumorigenesis (Liu et al. 2002, Paterson 2006, Wang et al. 2012, Tan et al. 2015). The anti-cancer effect of *G. lucidum* is a widely studied topic (Liu et al. 2015). Yu & Shen (2003) reported Lingzhi (*G. lucidum*) was cultivated successfully for the first time in 1969 in China and currently, the annual sale of products derived from *G. lucidum* is estimated to be more than US$ 2.5 billion in Asian countries, including China, Japan, and South Korea (Li et al. 2013). *G. lucidum* products, from different parts of its fruiting bodies, mycelia or spores, are sold in the form of coffee, powder, tea, dietary supplements, spore products, drinks, syrups, tooth pastes, soaps and lotions and have been commercialized as food and drug supplements which enhance the body's immune system and improve metabolic functions (Chang & Buswell 1999, Lai et al. 2004, Singh et al. 2013). Although *G. lucidum* has many reported medicinal properties, the mechanisms of their actions have been poorly described. Improved research techniques that can demonstrate the health benefits of *G. lucidum* more effectively are now becoming increasingly possible (Bishop et al. 2015). Secondary metabolites have been isolated from various *Ganoderma* species with 240 isolated from *G. lucidum* alone (112 C30 Ganoderic acids, 55 other C30 lanostanes, 27 C27 Lucidenic acids, 18 other C27 lanostanes, three C24 lanostanes, one meroterpenoid, 23 steroids and one benzofuran). Since “Lingzhi” has been used as a traditional Chinese medicine for over two millennia, it has become an important research topic and is very popular in China. However, it is not clear if the claimed benefits of taking “Lingzhi” in various forms are substantiated. In the following part of this review, we discuss various bioactive compounds produced by the mushroom and their anti-cancer activities. We present and discuss experimental evidence in connection with “Lingzhi” and its beneficial medicinal properties.

Ganoderma lucidum as an anti-cancer agent

Cancer is a worldwide health problem and latest statistics of the World Cancer Research Fund International, reveals there are an estimated 14.1 million cancer cases around the world in 2012, and this number is expected to increase to 24 million by 2035 (http://www.wcrf.org/). There were 1, 665,540 new cancer cases and 585,720 cancer deaths estimated in the United States in 2014, and certain malignant tumors are a major cause of death (Cheng & Sliva 2015). Cancer is an abnormal growth of cells which proliferates in an uncontrolled way and, in some cases, metastasizes or spreads (Borchers et al. 2004, Zaidman et al. 2005, Ruddon 2007, NCI 2011, De

Silva et al. 2013). Uncontrolled cell proliferation can be induced by many factors, including chemical, physical, or biological agents (Anand et al. 2008, NCI 2011, WHO 2011). Chemotherapy and radiotherapy are two commonly used conventional treatments for cancer patients, however, a variety of adverse events are associated with these two treatments. Hence, oncological practices have looked for alternative cancer medications over the past few decades (Jin et al. 2012). Cycle-phase-specific anti-cancer drugs have either cytotoxic or cytostatic activities. Hence, cytotoxic drugs, including most of the chemotherapeutics, are toxic to cells and usually cause DNA damage, resulting in programmed cell death (apoptosis and autophagy) (Cheng & Sliva 2015). Cytostatic drugs however, can stop the rapid proliferation of cancer cells, instead of killing them directly (Cheng & Sliva 2015). Recent laboratory and preclinical studies *in vitro* and *in vivo*, concluded that the anti-cancer activity of *Ganoderma lucidum* can be attributed to a variety of different mechanisms. The direct cytotoxic and anti-angiogenesis mechanisms of *G. lucidum* have been established by *in vitro* and animal studies (Yuen & Gohel 2005). *G. lucidum* has been positively demonstrated as a treatment for most human and murine cancer cell lines tested specifically for lung, liver, breast, prostate, sarcoma, colon, bladder, cervix, and leukemia lines. Furthermore, studies on hepatoma and sarcoma cells with *G. lucidum*, have been extended to *in vivo* animal models which resulted in inhibition of tumor growth and metastasis (Yuen & Gohel 2005). Jin et al. (2016) demonstrated that there is insufficient evidence available to justify the use of *G. lucidum* as a first-line treatment for cancer. It is still debatable whether *G. lucidum* prolongs long-term cancer survival. *G. lucidum*, however could be administered as an alternative supplement to conventional treatment because it has potential to enhance tumor response and stimulate host immunity. Furthermore, *G. lucidum* was generally well tolerated by most participants with only a small number of minor adverse effects and no major toxicity. Although there have been small number of reports on the minor effects of *G. lucidum*, the use of its extract should be cautious, especially after thorough consideration of cost benefit and patient preference. Jin et al. (2012) found that patients who used *G. lucidum* extracts in their anti-cancer treatments were 1.27 times more likely to respond to chemotherapy or radiotherapy. However, the study failed to demonstrate significant effects on tumor shrinkage when *G. lucidum* extract was used alone. *G. lucidum* extracts could stimulate host immune functions by considerably increasing CD3, CD4 and CD8 lymphocyte percentages, however natural killer (NK) cell activity, which is suggested to be an indicator of self-defense against tumour cells, was marginally elevated. Patients were found to have a relatively better quality of life after the treatment with *G. lucidum* extract. Minor side effects were reported following *G. lucidum* treatment, including nausea and insomnia. Tables 1 and 2 show the cytotoxic effects of the concentration that inhibits 50% of cell proliferation (IC 50) and the lethal dose that causes 50% of cell death (LD 50) varied from 1 to 5,000 µg/ml with various components of *G. lucidum*. Furthermore, pure compounds of *G. lucidum* showed cytotoxic activity at very low concentrations. *G. lucidum* can exhibit anti-cancer activity on different types of cancers from different origins**.** Some *G. lucidum* products on the market are labeled as "immunomodulating" agents, which act potentially as a supplement to support cancer patients, but are substantiated by only two clinical trials (Yuen & Gohel 2005). Some evidence presented by some research groups and the mechanisms of anti-cancer agents are not explicitly documented (Yuen & Gohel 2005). There are some *in vitro* and *in vivo* animal studies that confirm *G. lucidum* has anti-cancer activity, but further research is essential, with human trials on direct anti-cancer activity. To date *G. lucidum* appears to be beneficial to cancer patients and can possibly become a new anti-cancer agent in the future assuming satisfactory clinical trials are performed.

Active anti-cancer compounds of *Ganoderma lucidum*

Triterpenoids

Triterpenes isolated from *Ganoderma lucidum* species have been identified as potential anti-cancer agents and have structural similarity to steroid hormones and exhibit a broad spectrum of anti-cancer properties (Paterson 2006, Cheng et al. 2010, De Silva et al. 2012a, Wu et al. 2012,

Cheng & Sliva 2015). Wu et al. (2013) have been identified fifteen triterpenoids and furthermore two new triterpenoids reported by Zhao et al. (2015) from fruiting bodies of fungus *G. lucidum*. Triterpenoids such as Ganoderic acids, lucidimols, ganodermanondiol, ganoderiol F and ganodermanontriol, exert cytotoxic effects on various cancer cells (Chen & Chen 2003, Sliva 2003, Chang et al. 2006, Tang et al. 2006a, Weng & Yen 2010, Chin et al. 2011, De Silva et al. 2013). Cytotoxic triterpenoids inhibit human cervical cancer cells, and act as an alternative dietary approach for the prevention of colitis associated cancer (Cheng et al. 2010, Xu et al. 2010, De Silva et al. 2013). Ganoderic acids have been found to have direct cancer cell cytotoxicity on a wide variety of cancer cell lines, such as murine Lewis lung carcinoma (LLC) and Meth-A, and many of them have been suggested to counter angiogenesis and metastasis (Min 2000). Yue et al. (2008) reported that treatments with *G. lucidum* triterpenes regulated expression of 14 proteins in human cervical carcinoma cells. These proteins play important roles in cell proliferation, cell cycle, oxidative stress, and apoptosis. *G. lucidum* triterpenes induced sensitization of cells to the chemotherapeutic doxorubicin by increasing oxidative stress, DNA damage, and apoptosis (Cheng & Sliva 2015). *G. lucidum* triterpene extract suppressed proliferation of human colon cancer cells and also inhibited tumor growth in a xenograft model, which was associated with the cell cycle arrest at the G0/G1 phase and induction of the programmed cell death Type II autophagy (Cheng & Sliva 2015). Jiang et al. (2008) reported that Ganoderic acid A can suppress proliferation and colony formation of Cdk4 in breast cancer cells through inhibition of transcription factors AP-1 and NF-κB, which result in the down-regulation of expression of Cdk4. Furthermore, Ganoderic acid A inhibited phosphorylation or activation of transcription factor STAT3 which results an enhanced sensitivity of hepatoma cells to cisplatin. Apoptosis is induced by Ganoderic acid A in lymphoma cells through caspase-3 and caspase-9, and enhanced HLA class II-mediated antigen presentation and CD4+ T-cell recognition (Radwan et al. 2015). Ganoderic acid DM inhibited cell proliferation, induced the DNA damage, cell cycle arrest at the G1 phase, and apoptosis in human breast cancer cells and further improve the antigen presentation and CD4+ T-cell recognition in melanoma cells (Radwan et al. 2015). The growth of hepatoma cells is inhibited by triterpene enriched extracts from *G. lucidum* via suppressing protein kinase C and activating mitogen-activated protein kinases (Lin et al. 2003). Ganoderic acid T (12) induces apoptosis of metastatic lung tumor cells, through an intrinsic pathway related to mitochondrial dysfunction (Tang et al. 2006b). Semisynthetic modification of Ganoderic acid T produced more effective anti-cancer agents (Liu et al. 2012c). Cytotoxicity of a Ganoderic acid fraction called GA-Me has been tested on human colon cancer cells (Chen et al. 2008) and activation of the intrinsic mitochondria-dependent apoptotic pathway was identified and it was found that GA-Me is a potential natural apoptosis inducing agent for human colon tumors (Zhou et al. 2011). Ganoderic acid DM effectively inhibits cell proliferation and colony formation in MCF-7 human breast cancer cells. It exerted its anti-proliferative effect by inducing cell cycle (G1) arrest and apoptosis in MCF7 cells (Liu et al. 2012d, Wu et al. 2012, De Silva et al. 2013). Lucidenic acids A, B, C, and N have been isolated from fruiting bodies of *G. lucidum* and the extracts have anti-invasive effects on hepatoma cells, owing to extraordinary highs level of lucidenic acids (Weng et al. 2008). A new Ganoderic acid named 3α, 22β-diacetoxy-7α-hydroxy-5α- lanosta-8, 24 E-dien-26-oic acid was isolated from *G. lucidum* mycelia with substantial cytotoxic activity (Li et al. 2013). *G. lucidum* AF (antlered form of *G. lucidum*) contains a higher amount of triterpenes than normal *G. lucidum* giving potent immunomodulatory and anti-tumor effects (Nonaka et al. 2008, Watanabe et al. 2011, De Silva et al. 2013). Two new lanostane triterpenes, 3α, 12, 15 β -triacetoxy-5α -lanosta-7, 9(11), 24-trien-26-oic acid and 5α -lanosta-8, 24-diene-26, 27-dihydroxy-3, 7-dione were isolated from fruiting bodies of a Vietnamese strain of *G lucidum* having potential cytotoxic activities against human non-small cell lung adenocarcinoma (A549), breast adenocarcinoma (MCF-7), and prostatic small cell carcinoma (PC-3) (Nguyen et al. 2015). Treatment of cancer cells with *G. lucidum* extracts causes down-regulation of cell cycle associated proteins resulting in cell cycle arrest (Jedinak et al. 2011, Sliva et al. 2012, WHO 2014, Wu et al. 2013). Important triterpenoids, extracts, actions, IC 50 and LD50 values of *G. lucidum*, are listed in Tables 1 and 2.

Table 1 Anti-cancer activities of some important triterpenoids and *Ganoderma lucidum* extracts with IC 50 values

Triterpene	Cancer cells	Mechanism of action	Target	IC 50 (µg/ml)	References
Ganoderic acid A	Breast: MDA MB-231	Inhibition of cell proliferation and colony formation, suppression of cell adhesion, migration, invasion	NF-κ B, AP-1, Cdk4, u PA, uPAR	291	Jiang et al. 2008
	Liver: Hep G2	Increased sensitivity to cisplatin	STAT3	N/A	Yao et al. 2012
	Lymphoma: NALM-6	Induction of apoptosis	Caspase-3, -9, BIM, BAX	N/A	Radwan et al. 2015
Ganoderic acid DM	Breast: MCF-7, MDA-MB-231	Inhibition of cell proliferation and colony formation, cell cycle arrest, induction of apoptosis	Cdk2-4, cyclin D1, pRb, c-Myc, PARP	352	Wu et al. 2012
	Melanoma: J3, HT-144	Induction of apoptosis and autophagy	Caspase-3, -9, Bax	N/A	Hossain et al. 2012
Ganoderic acid H	Breast: MDA-MB-231	Inhibition of cell proliferation and colony formation, suppression of cell adhesion, migration, invasion	NF-B, AP-1, Cdk4, u PA, uPAR	N/A	Jiang et al. 2008
Ganoderic acid Jc	Leukemia: HL- 60 Breast: MCF-7	Cytotoxic	N/A	N/A	Liu et al. 2012
Ganoderic acid Mc	Cervix: HeLa Lung: 95D	Cytotoxic	N/A	N/A	Li et al. 2013
Ganoderic acid - Me	Breast: MDA-MB-231	Inhibition of cell proliferation, angiogenesis and invasion. Induction of apoptosis,	NF-κB, c-Myc, cyclin D1, Bcl-2, MMP-9, VEGF, IL-6, -8	N/A	Li et al. 2012
	Lung: 95D	inhibition of adhesion and migration of cells	MMP-2/9	N/A	Chen et al. 2008
	LLC implanted C57BL/6	Inhibition of lung metastasis	IL-2, and IFN-c, NK cells	N/A	Weng & Yen 2010
Ganoderic acid Mf/S	Cervix: He La	Cell cycle arrest, induction of apoptosis	Caspase-3, -9, Bax	N/A	Liu et al. 2011
	Lung: 95D	Cytotoxic	N/A	N/A	
Ganoderic acid T;	Cervix: He La	Inhibition of cell	NF-κB, MMP-9, i NOS	281	Liu et al. 2012

Triterpene	Cancer cells	Mechanism of action	Target	IC 50 (µg/ml)	References
ganoderic acid derivatives		proliferation, induction of apoptosis			
	HCT-116 human colon carcinoma cells	Inhibition of cell aggregation, adhesion and migration	N/A	N/A	Weng & Yen 2010
Ganoderenic acid D	Liver: Hep G2 Cervix: He La Colon: Ca Co-2	Cytotoxic	N/A	N/A	Ruan et al. 2014
Ganodermanontriol	Breast: MDA-MB-231	Inhibition of cell proliferation and colony formation, suppression of cell adhesion, migration, invasion	CDC20, u PA, u PAR	348	Jiang et al. 2011 Yuen & Gohel 2009
	Colon: HCT-116, HT-29	Inhibition of cell proliferation	β-catenin, cyclin-D1, Cdk4, PCNA, E-cadherin	N/A	Jedinak et al. 2011
Ganoderiol F	Leukemia: HL-60 Breast: MCF-7	Cytotoxic	N/A	N/A	Grienke et al. 2014
Ethyl lucidenates A	Leukemia: HL-60	Cytotoxic	N/A	N/A	Li et al. 2013
Lucidenic acid A, B, C & N	Liver: Hep G2	Inhibition of cell invasion	NF-κ B, AP-1, ERK1/2, MMP-9, c-Jun, c-Fos52	280A), 354(B), 332(N)	Weng et al. 2008,
		Induced apoptosis	caspase-9 and caspase-3		Weng & Yen 2010
3α, 22β-diacetoxy-7α-hydroxy-5α-lanosta-8, 24 E-dien-26-oic acid	Cervix: He La; lung: 95D	Cytotoxic	N/A	N/A	Li et al. 2013, Weng & Yen 2010
3α, 12, 15 β -triacetoxy-5α -lanosta-7, 9(11), 24-trien-26-oic acid and 5α -lanosta-8, 24-diene-26, 27-dihydroxy-3, 7-dione	A549 MCF-7 PC-3	Cytotoxic	N/A	N/A	Nguyen et al. 2015
Ergosta-7,22-diene-2β,3α,9α -triol	PLC/PRF/5 KB	Cytotoxic	MTT	1.2 0.9	Lin et al. 1991
5α,8α-Epodioxyergosta-6,22-dien-3β-ol	PLC/PRF/5 KB	Cytotoxic	MTT	11.0 9.8	Lin et al.1991
Crude methanolic extract	L1210 LLC	Cytotoxic	MTT	15 10	Tomasi et al. 2004
Commercial extract X	MDA-MB 123 PC-3	Cytotoxic	MTT	NA 250	Jiang et al. 2004
Sporoderm-broken spores alcoholic extract	He La	Cytotoxic	MTT	4460	Zhu et al. 2000
Fruiting bodies ethanolic extract	MCF-7	Cytotoxic	MTT	NA	Hu et al. 2002

Triterpene	Cancer cells	Mechanism of action	Target	IC 50 (μg/ml)	References
	MTC-11		MTT	129.3	Lu et al. 2004
			HTIA	113.0	
Fruiting bodies water extract	HUC-PC	Cytotoxic	MTT	509.0	Lu et al. 2004
			HTIA	990.0	
spores ethanolic extract	HUC-PC	Cytotoxic	MTT	274.7	Lu et al. 2004
			HTIA	234.0	
Spores water extract	HUC-PC	Cytotoxic	MTT	365.0	Lu et al. 2004
			HTIA	465.0	

A549: human non-small cell lung adenocarcinoma; NA: not available; HTIA: 3H thymidine incorporation assay; HUC-PC: Human uroepithelial cell line; MTC-11: low-grade bladder cancer cell line; MTT: tetrazolium method; MCF-7: breast adenocarcinoma; PC-3: prostatic small cell carcinoma; IC50-concentration that inhibits 50% of cell proliferation (Yuen & Gohel 2005).

Table 2 Anti-cancer activities of *Ganoderma lucidum* triterpenes and extracts with LD 50 values

Ganoderma lucidum product/extract/pure compound	Cell Line	LD50(μg/ml)	Method	Reference
Triterpene-enriched mycelial ethanolic extract	Huh-7	450	ACP 32	Lin et al. 2003
Sporoderm-broken spores alcoholic extract	He La	4,700	TB 30	Zhu et al. 2000
Culture broth of mycelia	Hep3B	NA	TB	Chung et al. 2003
Ganoderic acid G 18	Meth-A	6.8	SRB	Min et al. 2000
Ganoderic acid γ	NA	15.6	NA	Yuen & Gohel 2009
Ganoderic acid ε	NA	12.2	NA	Yuen & Gohel 2009
Ganoderic acid θ	NA	5.7	NA	Yuen & Gohel 2009
	LLC	15.2		
Ganoderic acid C1		17	NA	Yuen & Gohel 2009
Ganoderiol F 6	Meth-A	4.4	NA	Yuen & Gohel 2009
Ganodermanontriol	LLC	9.6	NA	Yuen & Gohel 2009
Ganolucidic acid A	NA	15.5	NA	Yuen & Gohel 2009
Lucidenic acid α	NA	17.8	NA	Yuen & Gohel 2009
Lucidumol A	Meth-A	2.3	NA	Yuen & Gohel 2009
Lucidumol B	LLC	8.5	NA	Yuen & Gohel 2009
		16.6		
Ganodermanondiol	Meth-A	12.5	NA	Gao et al. 2002
	NA	3.4	NA	
	NA	9.2	SRB	
	LLC	14		
	T-47D	4.7		
	S-180	11		
Ganodermnonol	T-47D	10	NA	Gao et al. 2002

	NA	4.8		
	Meth-A	2.8		
Ganodermadiol	NA	10.3	NA	Yuen & Gohel 2009
Lucialdehyde A	NA	10.4	NA	Yuen & Gohel 2009
Lucialdehyde C	LLC	10.7	NA	Yuen & Gohel 2009
	T-47D	4.7		
	S-180	7.1		
Lucialdehyde B	NA	4	NA	Yuen & Gohel 2009
	NA	NA		
	T-47D LLC	15		
		14.3		

Table 3 Main polysaccharides from *Ganoderma lucidum* with anti-cancer activities

Polysaccharides	**Major monosaccharides**	**Cancer cell type**	**Mechanism of action**	**References**
Se GLP-2B-1(Se-enriched *G. lucidum* polysaccharide)	Glucose mannose	Different cancer cells	Proliferation of different cancer cell lines in vitro and induces mitochondria-mediated apoptosis	Zhang et al. 2011
***G. lucidum* polysaccharides fraction 3 (F3)**	Glucose, mannose	Human leukemia THP-1 cells	Cytotoxic. Induces macrophage-like differentiation and apoptosis	Cheng et al. 2007
LZP-F3	Glucose, galactose, mannose	Bladder urothelial carcinoma cell N/P (14) and N/As (0.5)	Modulating the expression and activation of apoptosis correlated proteins	Huang et al. 2010
GLIS	Glucose,	Tumor growth cancer cells	Inhibitory effect on tumor growth and dramatically enhanced both humoral and cellular immune functions in a mouse model	Zhang et al. 2010
***Gl*PS**	Fructose, glucose	Tumor growth cancer cells	Inhibited tumor growth in a murine sarcoma 180 model and the adhesion ability of tumor cells to HUVECs via the up-regulation of serum amyloid Immunomodulation effect (on lymphocytes) and cyclophosphamide induced immunosuppression	Zhu et al. 2007

Polysaccharides	Major monosaccharides	Cancer cell type	Mechanism of action	References
			in mice	
		Lung cancer	Promoted cancer cells to induce lymphocyte proliferation and activation	Sun et al. 2011
			fully or partially reversed lymphocyte suppression	Sun et al. 2013
***Gl*PP**	Xylose, Fructose, Glucose	Human lung cancer cells	Inhibit the proliferation of HUVECs by inducing cell apoptosis and decrease the expression of secreted VEGF	Cao & Lin 2006
GLPP	Glucose	Tumor growth cancer cells	Inhibited the growth of inoculated S180, Heps, and EAC tumor cells in mice with an enhanced host immunofunction, and prevented the immunosuppression induced by cyclophosphamide treatment and 60 Co radiation in mice	Pang et al. 2007
		Gastric cancer	Reduction of IL-6 and TNF-α levels and increased concentration of IL-2, IL-4, and IL-10 in serum of rats with 65	Pan et al. 2013
***Ganoderma lucidum* polysaccharide derivatives (S-GL and CM-GL)**	Glucose	NA	Induced cell cycle arrest at the G2/M phase and suppressed the growth of sarcoma tumor cells in vitro and in vivo with low toxicity to animals	Wang et al. 2009
Ethanol extracts of *G. lucidum*	Glucose	Human gastric carcinoma cells	Induction of apoptosis	Jang et al. 2010
(LZ- D-7)	Glucose	Tumor	Activates the B-cells and acts as immunostimulatory drug	Ye et al. 2011
GSG	Glucose	Murine resident peritoneal macrophages	induce IL-6 and TNF-α secretion Dectin-1, MAPKs- and Syk- dependent pathway	Guo et al. 2009

Table 4 Pharmacologically active proteins isolated from *Ganoderma lucidum*

Protein product	Effects/activity	Cell line	Applications	Patent, year	References
Antiangiogenic *G. lucidum* fraction from fruit body, mycelia, or spores	Inhibition of angiogenesis and inhibition of endothelial cell growth, inhibition of pathological neovascularisation in a tissue	Angiogenesis inhibition by endothelial cell culture assay Anti-angiogenic activity determination on HEP-2 and calf pulmonary-arterial endothelial (CPAE) cell lines Cytolytic/cytotoxic assay on CPAE cells Matrix metalloproteinase assay Endothelial cell tubule/cord formation assay Endothelial cell migration assay Testing on fertilized chicken eggs: inhibition of blood vessel development on chorioallantoic membrane	Pathological neovascularisation: cancer, rheumatoid arthritis and osteoarthritis, neovascular based dermatological conditions, diabetic retinopathy, Karposi's Sarcoma, agerelated macular degeneration, restenosis, telangectasia, glaucoma, keloids, comeal graft rejection, wound granularization, angiofibroma, Osler Webber Syndrome, myocardial angiogenesis, scleroderm	Wong 2003	Boh 2012
LZ-8 protein, LZ-8 protein-fused antigen	Enhancement of innate and adaptive immunity by activating dendritic cells and macrophages, to produce cytokines (TNF-, IL-1, IL-6, IL-10 and IL-12) or chemokines, maturation of dendritic cells achieved through activation of mitogen-activated protein kinase pathway or NF- B, T cells activation and proliferation to produce cytokines (IL-2, IL-4 and IFN-γ)	Stimulation of bone marrow derived dendritic cells (BMDCs) to produce cytokines and chemokines Promotion of BMDCs maturation Activation of T cells Activation of mitogen-activated protein kinase pathway and NF- B Activation of macrophages Activation of human monocyte-derived dendritic cells	Enhancing innate and adaptive immunity by activating dendritic cells and macrophages by administering LZ-8 protein Enhancing the immuno-genicity of an antigen, by administering a LZ-8 protein-fused antigen	Chu & Chen 2009	Boh 2012
Recombinant *G. lucidum* immunoregulat	Increasing the number of leukocytes	Human leukemia cells: growth inhibition, lethal effect, apoptosis induction	Anti-cancer medication for lung, pancreatic, liver, intestinal, prostatic, uterus, bone and mammary cancers, lymphoma, leukemia	Sun & Shang, 2011	Boh 2012

y protein (rLZ-8)	Prevention of systemic allergic reactions and immune rejection after organ transplantation	*In vivo* animal tests: tumour growth inhibition in mice. Influence on normal tissue cells in rats and rabbits	Treatment of leucopoenia, neutropoenia and granulocytopoenia	
		Leukocyte-related treatments in mice Prevention and treatment related to radiation in mice	Treatment of graft rejective reaction and reversion of immunosuppression resistance	
Vaccines consisting of an antigen e.g. protein of a cancer cell or virus, or DNA; and LZ-8 protein as an adjuvant	Immunomodulation and anti-cancer activity	TNF- production in dendritic cells; T cell activation; cytotoxicity on LN inguinal cells	Pharmaceutical vaccine and a method for augmenting the immunogenicity of a mammal / human	Chu and Boh 2012 Chen, 2009
	Elimination of pathogens and neoplastic cells	*In vivo* testing on mice: tumor suppression efficacy; immunization / antitumor effect of DNA-LZ-8 vaccine		

Published medical investigations with *Ganoderma lucidum* preparations, case studies and clinical trials

Table 5 Clinical trials carried out with *Ganoderma lucidum* preparations

Clinical trial	Cancer type	Dose	Effect	Reference
Non randomized clinical studies of 140 cancer patients, all with metastasis	Breast cancer	6 g of *G. lucidum* essence per day for 6 months	Six patients with breast cancer showed promising results, a woman over 50 with breast cancer metastasized to the lung and after the treatment her lung tumor disappeared,	Morishige 1988 (Information taken from Chen & Seleen 2007)
		9 g per day of *G. lucidum* essence with a high dosage of vitamin C for 2 months, the dosage increased later to 20 g per day	cancer patient with metastasis to the bone, has improved considerably	
A patient diagnosed with hepatoma treated with *Ganoderma* spores	Hepatoma (at the portal vein region of the liver)	High dose of *Ganoderma* spores treatment from May to August1999 (*Ganoderma* spore treatment only)	Tumor size 5.1 × 6.6 × 7.7 cm in May 1999, reduced to 3.5 × 3.4 × 3 cm in August, 1999	Liu & Chung 2001
Chinese *G. lucidum* essence for 547 cancer patients	Medium and late phase cancer	A continuous 2-3-month active long treatment with a daily dosage of 4-6g of *G. lucidum* essence, further dosage of 2g per day continuously after the third	Significantly lower death rate of patients in the long-term treatment	Shi & Quing 2001

Clinical trial	Cancer type	Dose	Effect	Reference
		month of therapy		
33 patients with androgen-dependent prostate cancer (ADPCa) and 37 patients with androgen-independent prostate cancer (AIPCa) were treated with PC-SPES (Prostate Cancer-SPES contained eight herbs including *G. lucidum*)	Prostate cancer	nine capsules daily (quantity undetermined)	87% of patients had decreased PSA levels after 2 months; 78% still had decreased PSA levels after 6 months. Side effects included nipple tenderness (6%)	Small et al. 2000
Phase I/II open-label clinical trial, Ganopoly (*G. lucidum* polysaccharide) treatment for 143 previously treated advance stage cancer patients	Advance stage all cancer types	1800 mg daily three times a day, orally, 12 weeks	27 patients were lost to follow up or refused to further theraphy before 12 weeks, 38 patients had stable disease for 12 weeks, 46 patients had progressive disease, 16 had developed progressive disease at 6^{th} week. There were 32 patients in stable condition for at least 12 weeks, a statistically significant increase in lymphocyte myogenic reactivity and natural killer cell activity, Ganopoly may have an adjunct role in the treatment of patients with advanced cancer	Gao et al. 2002
Double-blind and placebo-controlled randomized trial, Ganopoly treatment for 68 advanced stage lung cancer patients	Lung cancer	600 mg, three times daily orally, before meals for 12 weeks	13 patients had stable disease for lymphocyte mitogenic reactivity to concanavalin A, number of treated patients with stabilized cancer at 12 weeks was 35.1% vs. 22.6% of control patients, Treatment resulted in a statistically significant increase in Karnofsky performance scores (a method of measuring patients' performance of daily living activities) in 50% vs. 14.3% in the control group, CD 3 and natural killer cell activity significantly increased in Ganopoly treated patients, immune responses were significantly increased in treated patients but not control patients	Gao et al. 2003a

Clinical trial	Cancer type	Dose	Effect	Reference
Ganopoly treatment for 34 advanced stage cancer patients	Lung, breast, liver, colon, prostate, bladder or brain advance stage cancers	Treated orally with 1800 mg Ganopoly, three times daily, before meals, for 12 weeks	Enhanced the immune responses Significantly increased mean plasma concentrations of IL-2, IL-6, and IFN- γ Levels of IL-1 and TNF- γ significantly decreased Variability among patients observed in the numbers of each lymphocyte subset at baseline PHA responses enhanced in most patients A significant increase in the mean NK activity observed	Gao et al. 2003b
26 patients treated with hot water extract of *G. lucidum*, *Coriolus versicolor* and *Panax ginseng*	Severe cancers (stages 3 or 4)	150 ml each, three times a day for 35 days	Alleviation of complications and body pain, increased appetite, reduced stress, improvement in sleep and body functions, With 5 patients 50 % regression of carcinoma (cervical, lung, ovaries, breast and cutaneous carcinoma), and regression of metastasis of cutaneous and breast cancer, others no special response	Goino 2004
4 patients treated with hot water extract of *G. lucidum*, *Coriolus versicolor* and *Panax ginseng* **Patient 1: no other medications.** **Patient 2: with chemotherapy.** **Patient 3: with radiotherapy** **Patient 4 (control): not administered any treatment**	Cancer stage 4 Patient 1: breast Cancer Patient 2: lung cancer Patient 3: sarcoma, Patient 4 (control): intestine cancer	150 ml each, 3 times a day for 35 days	Improvement of blood and immunological conditions of Patients 1, 2 and 3 Alleviation of body pain, increased appetite, improved sleep and body functions, with alleviation of complications and better recovery Improved immunological conditions of patients 2 and 3	Goino 2004
Open-label clinical study of Ganopoly 30 patients, treated orally	Advanced-stage lung cancer	Ganopoly 5.4 g per day, for 12 weeks	Not statistically significantly alter the mean mitogenic reactivity to phytohemagglutinin, mean counts of CD3, CD4, CD8, and CD56, mean plasma concentrations of IL-2, IL-6, and IFN- γ, or NK activity,	Gao et al. 2005 a

Clinical trial	Cancer type	Dose	Effect	Reference
			Some cancer patients showed markedly modulated immune functions	
Clinical trial, 47 patients with advanced colorectal cancer, Ganopoly, 1 capsule contains 600 mg extract (provided by Encore International Co. Auckland, NZ)	Colorectal cancer,	3 capsules, 3 times per day before meals for 12 weeks	No statistical significance on mitogenic reactivity to phytohemagglutinin when a compared to baseline values and after 12-weeks of treatment	Gao, et al. 2005 c
Open- label study, 47 patients treated with *G. lucidum* polysaccharides	Advanced colorectal cancer	Ingestion 5.4 g per day, for 12 weeks	Increase mitogenic reactivity to phytohemagglutinin Responsible for of CD3, CD4, CD8 and CD56 lymphocytes, plasma concentrations of IL-2, IL-6 and IFN-γ, and NK activity Potential immunomodulating effect in patients with advanced colorectal cancer	Chen et al. 2006
Series of non-randomized clinical studies of *G. lucidum* polysaccharide extracts on breast cancer and other cancer patients, used along with other conventional, standard therapies	Breast cancer and other cancer types	500mg of *G. lucidum* polysaccharide extracts per capsule, 6 capsules, 3times a day	Obtained positive results, effective in helping tumor regression, highly effective in eliminating or reducing the expected side effects of radiotherapy and chemotherapy, including nausea, vomiting, sore throat, loss of appetite, and insomnia, all patients experienced improvement in their quality of life	Teow 1997, 2004, 2006 (Information taken from Chen & Sleen 2007)
Open-label clinical trial, 15 prostate cancer patients, Rokkaku Reishi (Suntory Holdings, Osaka, Japan)	Prostate cancer	3 packets per day for 6 months (Quantity undetermined)	No response in terms of prostate-specific antigen, no statistically significant anticancer activity and no serious adverse effects	Yoshimura et al. 2010
Pilot, randomized, placebo-controlled clinical trial, 48 patients with cancer-	Breast cancer	Data collected at baseline and after treatment of 4 weeks, 1000 mg, 3 times per day	Statistically significant improvements in physical well-being and fatigue, reported less anxiety and depression with better quality of life, No serious adverse effects	Zhao et al. 2012

Clinical trial	Cancer type	Dose	Effect	Reference
related fatigue undergoing endocrine therapy, treated with spore powder 1000 mg (Beijing Great Wall Pharmaceutical Factory, Batch number B20050008)			occurred	
Ganopoly TM (crude polysaccharide fractions extracted from *G. lucidum*,) 143 patients (83 men and 60 women) having advanced previously treated cancer, median age of 61 years	Advance tumor (stage III and IV)	600 mg, three times daily orally, before meals for 12 weeks	Two patients withdrew due to gastrointestinal toxicity, and 3 patients died of progressive disease , 27 patients were not assessable for responses and toxicity, 11 patients with stable disease at 6 weeks and after lost to follow up, Of the 100 fully assessable patients, 46 patients had progressive disease before or at the 6 week, 16 patients developed progressive disease between 6 and 12 weeks of therapy, No complete or partial response observed, but 38 of 143 patients had stable disease for 12 weeks or more, prostate-specific antigen levels in the 5 prostate cancer patients reduced significantly during stable disease	Zhou et al. 2014
Randomized double blind placebo control, 42 patients treated with water extract and spores' preparation with a placebo of high dose vitamin C	Gynecological cancer	Water extract 1,000 mg per pack, *G. lucidum* spores preparation 1,000 mg per pack and vitamin C 200 mg per pack, ingest one package with 200 ml of distilled water one hour before a meal twice a day for day 1 and 2, increase the dosage to two packages of drug ingested with 200 ml of distilled water before meals two times a day on day 3 and 4, after dosage increased to 3 packages for 12 weeks	2 patients in the water extract Lingzhi group and 3 patients in the spore lingzhi group achieved stability in the disease, all patients in placebo group showed progression in the disease	Suprasert et al. 2014
Randomized double blind placebo control, 60 patients who failed chemotherapy were treated with *G. lucidum* spore preparation			11,8 and 5 patients evaluated in water extract, spore and placebo arms respectively, stable disease that achieved 38.1% in the water extract arm, 50% in the spore arm and none in the placebo arm, after one-year overall survival was 63.6% in the water extract arm, 60% in the spore arm and 44% in the placebo arm, slightly improve the immune system with minimal side effects	Suprasert et al. 2015

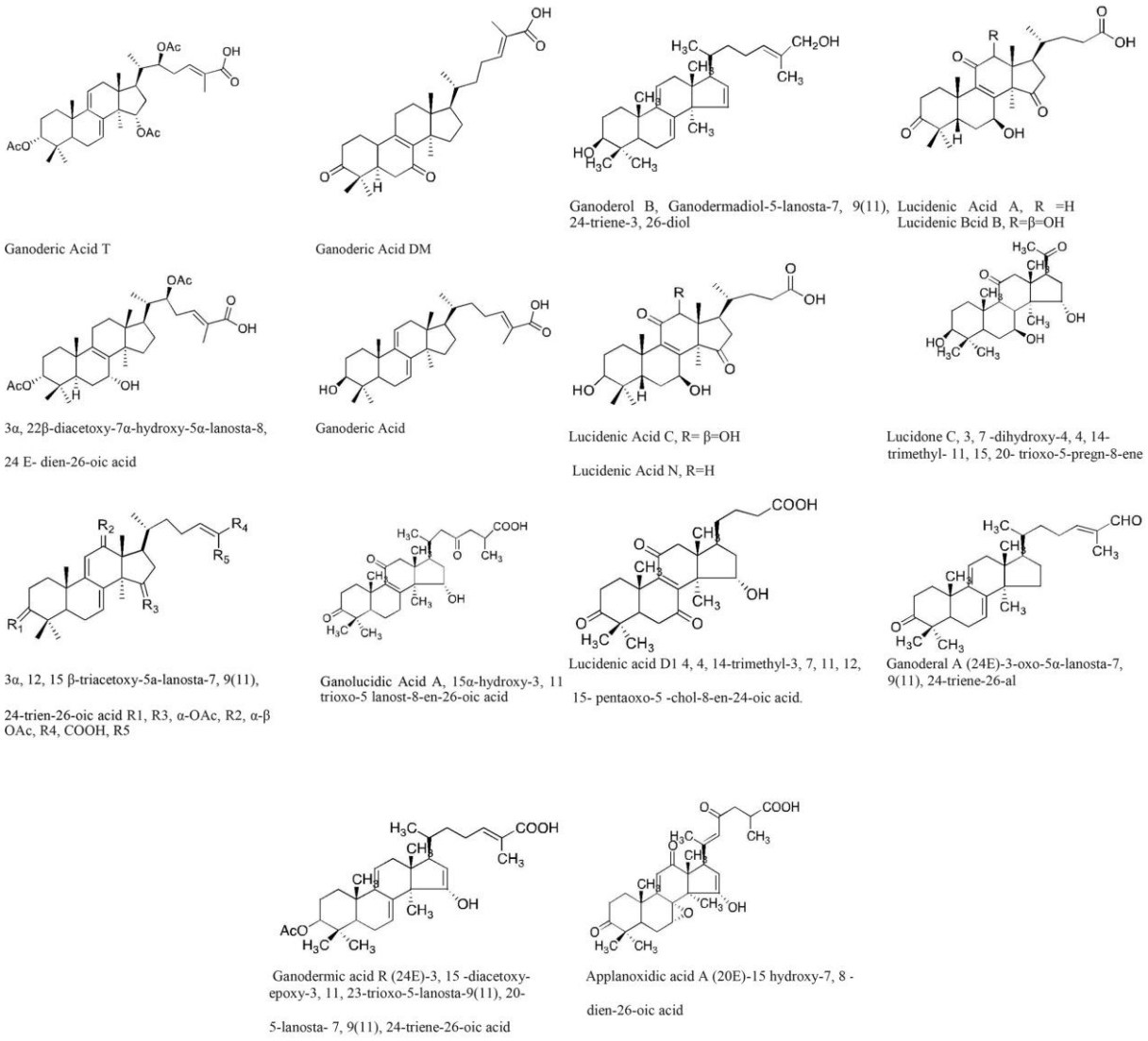

Fig. 1 – Chemical structures of selected triterpenoids with anti-cancer activities from *Ganoderma lucidum* (ChemOffice Ultra 2000, Strack 2001)

Polysaccharides

Several studies have revealed the medicinal potentials of crude polysaccharides isolated from *Ganoderma lucidum* (Gao et al. 2003, Shao et al. 2004, Stanley et al. 2005, Kuo et al. 2006). Hung et al. (2008) reported that *G. lucidum* polysaccharides are believed to initiate an indirect anti-tumor mechanism in the host immune system. Polysaccharides isolated from fruiting bodies contain (1→3) and/or β-(1→6)-D-glucans as main active ingredients and the latter are responsible for immune-modulating and anti-cancer properties (Jones 1992). The anti-cancer effect of polysaccharides arises from enhancement of the host‟s immune system, rather through direct cytocidal effects (Gao et al. 2000a, 2000b, Lu et al. 2004, Zhu et al. 2007). These intricate sugars stimulate or modulate the immune system by activating immune cells, such as macrophages (Gao et al. 2003, 2005, Shao et al. 2004, Akramiene et al. 2007, Ahmadi & Riazipour 2007), B cells (Shao et al. 2004, Manassila et al. 2005), natural killer cells (Gao et al. 2005, Ahmadi & Riazipour, 2007, Akramiene et al. 2007, Huang & Ning 2010), T cells (Gao et al. 2003, 2005, Manassila et al. 2005, Ahmadi & Riazipour 2007). Tumorigenesis was also suppressed by *G. lucidum* polysaccharides. (Xu et al. 2011). A new polysaccharide isolated from Selenium enriched *G. lucidum* suppressed proliferation of different cancer cell lines *in vitro* and induced mitochondria mediated apoptosis (Shang et al.

2009, 2011). Significant antitumor efficacy was observed through cytotoxic effects on tumor cells by *G. lucidum* polysaccharide nanoparticles in combination with *G. lucidum* polysaccharides (LZP-F3) (Li et al. 2010b) and arsenic trioxide showed synergistic growth inhibition of human urothelial carcinoma cells (Huang et al. 2010). Two water-soluble sulfated and carboxymethylated *G. lucidum* polysaccharide derivatives (S-GL and CM-GL) induced cell cycle arrest at the G2/M phase and suppressed the growth of sarcoma tumor cells *in vitro* and *in vivo* with low toxicity to animals (Wang et al. 2009). Polysaccharides were also reported to increase the immunoglobin levels to produce an elevated response to antigenic substances such as bacteria, viruses and tumor cells and stimulate TNF-α and IL-6 production, activate NF-κB and increase hepatotoxicity activity (Gao et al. 2003, Manassila et al. 2005, Kuo et al. 2006). Furthermore, the branched chains of beta-glucans act on complement receptor type 3 (CR-3) triggering a series of molecular pathways such as NF-κ B, mitogen-activated protein kinase (MAPK) and protein kinase C (PKC), which in turn, activate the host immune response for immune cell proliferations (Hong 2004).

Beta-glucans also act on dectin-1 receptor and toll-like receptor 2 (TLR-2) (Brown 2006, Gantner 2003). These actions are enhanced maturation of dendritic cells and increases the opsonic and non-opsonic phagocytosis in turn increase the cytokine production and splenic NK-cell cytotoxicity (Chan 2007). A crude extract of the polysaccharide from fruiting bodies has been used to induce cytokines expression via a toll-like receptors-4 modulated protein kinase signaling pathway (Guo et al. 2009, De Silva et al. 2013). Chemical components of *Ganoderma lucidum* spores have potential immunomodulatory effects and anti-tumor activities (Guo et al. 2009, De Silva et al. 2013). When compared to unmodified glucans, chemically modified α-D-glucan from spores of *G. lucidum* have been reported to increasing stimulation effects of lymphocyte proliferation and antibody production (Bao et al. 2001a, b, De Silva et al. 2013). Ganoderan is a biopolymer which has also been used as additional therapy in combination with anti-cancer drugs. It was able to increase the effectiveness of cytotoxic drugs and immunomodulators in patient with prostate cancer (Giavasis 2015). Glucose, galactose, mannose, rhamnose and fucose were detected in *G. lucidum* polysaccharide hydrolysate (Miyazaki & Nishijima 1981, Aryantha et al. 2002,). Furthermore, Chang & Lu (2004) separated polysaccharides of *G. lucidum* and found that polysaccharide groups composed glucose, D-mannose and D-galactose. Monosaccharaides and oligosaccharides such as glucose with small amounts of other sugar residues such as mannose, fucose, xylose and galactose were observed in highly anti-cancerous preparation (Ooi & Liu 2000). Fucose is reported to play a role in prevention and treating cancers in mammals (Listinsky et al. 2001, Moriwaki & Miyoshi 2010, Ale et al. 2011, Miyoshi et al. 2012, Liao et al. 2013). Hence, polysaccharides from *G. lucidum* can be used to produce carbohydrate-based vaccines with therapeutic efficacy for infectious disease and cancer treatment. *G. lucidum* has been used for its analgesic and muscle relaxing properties since ancient times (Zhang et al. 2011). Matsuzaki et al. (2013) have demonstrated that *G. lucidum* polysaccharides exhibit an anti-depressant-like effect and reduces anxiety-type behaviour in rats. Table 3 shows the main polysaccharides having anti-cancer activity.

Ergosterol

The pro-vitamin D2, ergosterol (ergosta-5, 7, 22-trien-3β-ol) is abundant in *Ganoderma lucidum* (Paterson 2006). Ergosterol in *G. lucidum* can be converted to vitamin D2 by ultraviolet (UV) irradiation and hence, produces a variety of photo irradiation products. The principal products are provitamin D2, tachysterol, and lumisterol Ergosterol peroxide (5α, 8α-epidioxy-22E-ergosta-6, 2 dien-3β-ol). The latter is a steroidal derivative of ergosterol and contributes to various biological activities with strong immunomodulatory and anti-tumor activities (Krzyczkowski et al. 2009). Pro-apoptotic activity of ergosterol peroxide and (22E)-ergosta-7, 22-dien-5α-hydroxy-3, 6-dione on prostate cancer cells indicate that these compounds can reduce the growth of prostate cancer cells (Russo et al. 2010).

Proteins

The fungal immunomodulatory protein, Ling Zhi-8 (LZ-8), has been regarded as one of the most important bioactive substances produced by *Ganoderma lucidum*. The recombined Ling Zhi-8 (rLZ-8) is known to improve immune modulatory effects on human monocyte-derived dendritic cells and induces significant activation and maturation of human dendritic cells (Lin et al. 2009). LZ-8 acts as a promising adjuvant that enhances the efficacy of a DNA cancer vaccine and hence, could be used in preventing and treating various cancers. Further, LZ-8 could activate murine macrophages and T lymphocytes, but other *Ganoderma* polysaccharides only activate the macrophages (Yeh et al. 2010, De Silva et al. 2013).

Discussion

Are the beneficial anti-cancer properties truly substantiated?

To date, the majority of research studies performed on the benefits of using *Ganoderma lucidum* in traditional Chinese medicine, have been restricted to *in vitro* and animal studies. Despite the fact that many scientists have postulated a positive association between *G. lucidum* and treatment of many diseases especially cancer, there are still skepticism and bias in connection with the medicinal potential of this fungus. Observational and anecdotal reports, have not as yet been substantiated by well controlled clinical trials or reliable scientific data as very few studies have been conducted with *G. lucidum* in human cancer patients. These *in vitro* and animal studies need to be applied to human studies and further clinical trials now as the majority of findings does not translate to the human condition. When going from most sophisticated animal studies into humans, candidates drop out at each level of study. However, some persons are unable or do not wish to tolerate the side effects of radiotherapy or chemotherapy. Hence, they need to turn to herbal remedies or dietary supplements. Their effectiveness, potential side effects, and possible interactions with other drugs are often unclear since these products are not studied and regulated in the same way as drugs. For many of these products, scientific studies are now underway. Public database of clinical trials in the US (Home - ClinicalTrials.gov, access in December 08, 2015) lists five studies of *G. lucidum* and only two were with cancer patients. The first published clinical trial for anti-cancer activity using *G. lucidum* was that of Morishige (1988) and 140 breast cancer patients with metastasis were treated with *G. lucidum* essence however only six patients showed promising results (Table 5). Liu & Chung (2001) reported only one patient diagnosed with Hepatoma, was treated with *Ganoderma* spores, but there was no standard dosage recorded and the treatment was short term (3-4 months) but there was a significant reduction of the size of the tumor (Table 5). There is a lack of information regarding long term treatment, standard dosage, number of patients treated, placebo control, age, gender and the side effects. Small et al. (2000) recorded a clinical trial (Table 5) treated with PC-SPES), a herbal combination remedy, and a Chinese herbal supplement which contains eight herbs including *G. lucidum.* It was manufactured by Botanic Lab of Brea, California, USA and introduced to men with prostate cancer in 1996 and decreased PSA levels of cancer. In 2002, this herbal medicine was recalled due to various side effects, such as loss of sex drive, difficulty with erectile function, breast enlargement and tenderness and reduction in body hair since PC-SPES produced an estrogen hormonal effect in the body as PC-SPES samples were found to be contaminated with US Food and Drug Administration (FDA) controlled prescription drugs (White 2002). Gao et al. (2002) has used Ganopoly (*G. lucidum* polysaccharide extract) treatment for 12 weeks on 143 previously treated advanced stage cancer patients and the results (Table 5) revealed that Ganopoly was effective for patients, however 46 patients showed progressive disease after 6^{th} week and another 16 patients had developed progressive disease between 6^{th} and 12^{th} week. This research could have been improved by increasing the sample size of the patients, selecting effective dosage, long term treatment and using a standard placebo control. Furthermore, toxicity should be tested since there were side effects such as nausea, insomnia, sweating and diarrhea. Gao et al. (2003a) assessed the effectiveness of Ganopoly treatment on 68 advanced

stage lung cancer patients in a double-blind and placebo-controlled randomized trial (Table 5). Thirteen patients showed stable disease and lymphocyte mitogenic reactivity to concanavalin A, furthermore, CD 3 percentage and natural killer cell activity significantly increased in Ganopoly treated patients (Gao et al. 2003a). These results demonstrated that Ganopoly can be considered as a supplement for treatment of lung cancer patients. Further Ganopoly treatments with 34 advanced stage cancer patients enhanced immune responses and significantly increased the population of T cells and natural killer cell activity compared to baseline (Table 5, Gao et al. 2003b). Gao et al. (2005) also investigated Ganopoly treatment again as open-label clinical study for 30 advanced stage lung cancer patients with the same dose as Gao et al. (2003a). Results showed that subgroups of cancer patients are responsive to Ganopoly along with chemotherapy or radiotherapy. Further studies should be continued to determine suitable dosage of the drug, and more trials are needed with a large group of patients, along with radiotherapy and chemotherapy for long term treatments with gender and age base for better results in immune responses. The toxicity of drug should be studied thoroughly since there were side effects such as nausea and insomnia. If Ganopoly treatment is being given along with desired doses of chemotherapy or radiotherapy, then the question arises as to which factor really contributes to positive association with immuno-stimulating effects. Clearly defined protocols, medical standards on exact bioactive compounds and carefully designed experimental controls such as double-blinded, placebo-controlled studies with large trial populations should be performed, so that only Ganopoly variable accounts for better immune responses and not chemotherapy or radiotherapy. Studies on the identification of active ingredients, isolation and purification of individual compounds should be continued and this will enable the active ingredients within Ganopoly to be measured and to understand whether the beneficial compounds in *G. lucidum* act synergistically or independently.

Hot water extract of three herbs including *Ganoderma lucidum* was used to treat a few severe stage cancer types (Table 5) along with radiotherapy and chemotherapy under control conditions. The results revealed that it enhanced the blood and immunological conditions of cancer patients (Goino 2004). *G. lucidum* polysaccharides were taken orally by advanced colorectal cancer patients (Table 5) and results suggested that they have a potential immunomodulating effect in patients (Chen et al. 2006). Teow (1997, 2004, 2006) performed a series of clinical trials on breast cancer patients using *G. lucidum* polysaccharide capsules and obtained positive results (Chen & Sleen 2007). Yoshimura et al. (2010) used Reishi powder packets and performed open label clinical trial for 15 breast cancer patients and obtained no statistically significant anti-cancer activity. Zhao et al. (2012) treated 48 breast cancer patients with a spore powder of *G. lucidum* (Table 5). Ganopoly TM was applied to advanced tumor (stage III and IV) patients by Zhou et al. (2014) (Table 5). This medical investigation was particularly standard since there was information on age, gender, dosage and adequate patient sample size and most of the patients were not assessable for the treatments. However most of the clinical trials lack information regarding the progress of long term treatments (Goino 2004, Chen et al. 2006, Teow 1997, 2004, 2006, Yoshimura et al. 2010, Zhao et al. 2012, Zhou et al. 2014), have small sample sizes (Goino 2004, Yoshimura et al. 2010, Zhao et al. 2012, Zhou et al. 2014), age (Goino 2004, Teow 1997, 2004, 2006, Yoshimura et al. 2010, Zhao et al. 2012) and placebo control (Teow 1997, 2004, 2006). Suprasert et al. (2014, 2015) carried out successful clinical trials using *G. lucidum* water extracts and *G. lucidum* spores for gynecologic cancer patients who failed chemotherapy (Table 5). After long term treatment with Lingzhi, 60% of the patients showed progressive disease and 40% showed stable disease (Suprasert et al. 2014). The survival rate was nearly 60% for both water extract and spores after 1 year of continuous treatment (Suprasert et al. 2015). The results revealed that the drug controlled the disease and slightly improved the immune system with minimal side effects. But it is unclear whether the patient‟s survival depends on less advance stage of the disease or due to result of treatments. *Ganoderma lucidum* has a long history as a medicinal mushroom but there is a lack of information corroborating the traditional uses. Hence, to reach its economic and medicinal potential a number of challenges need to be addressed.

Conclusion

Accurate scientific evidence is needed for establish the safe and efficient use of *Ganoderma lucidum* as an integrative therapy for cancers. Experimental, epidemiological, and clinical studies should identify the molecular targets of the cancers and resolve the association between *G. lucidum* intake and cancer risk. Furthermore, the efficacy dosage, efficacy of the drug, and safety, alone or in combination with chemotherapy or radiotherapy should be researched. Investigations should be carried out to determine which pharmacologically active constituents from *G. lucidum* contribute to positive immune responses and exploit the mechanisms of action of *G. lucidum* at the genetic level or molecular level and with target organs, to fully comprehend their mode of action in cancer treatments. Furthermore, a wider array of toxicity tests should be performed to weigh the pros and cons of cancer treatment among patients and investigate whether the quality of life is improved. More rigorous, reproducible and well-designed scientific studies with adequate sample size and reliable statistical data are also needed to support the associations.

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