A Medicinal Mushroom: *Phellinus Linteus*

Tongbo Zhu[1], Sung-Hoon Kim[2] and Chang-Yan Chen*,[1]

[1]*Department of Radiation Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School;* [2]*College of Oriental Medicine, Kyung Hee University, Seoul, South Korea*

Abstract: *Phellinus Linteus (Berkeley & M. A. Curtis) Teng* (PL) is a medicinal mushroom that has been practiced in oriental countries for centuries to prevent ailments as diverse as gastroenteric dysfunction, diarrhea, haemorrhage and cancers. In an effort to translate the Asian traditional medicines into western-accepted therapies, scientists have demonstrated that the extracts from fruit-bodies or mycelium of PL not only stimulate the hormonal and cell-mediated immune function and quench the inflammatory reactions caused by a variety of stimuli, but also suppress the tumor growth and metastasis. Mounting evidence from different research groups has shown that PL induces apoptosis in a host of murine and human carcinomas without causing any measurable toxic effects to their normal counterparts. Recently, research has been focused on the anti-tumor effect of PL, and in particular, on its ability to enhance some conventional chemotherapeutic drugs. These studies suggest PL to be a promising candidate as an alternative anticancer agent or a synergizer for existing antitumor drugs. Hereinafter, we summarize the present progress in elucidating the mechanisms underlying the potency of PL and its anti-tumor function. The fractionation and identification of the biologically active components from PL are also briefly introduced.

Keywords: *Phellinus linteus*, immunostimulation, cancer therapy, alternative anticancer agent.

INTRODUCTION

Phellinus Linteus (Berkeley & M. A. Curtis) Teng (PL) is a species of mushrooms belonging to the *Hymenochaetaceae Basidiomycetes* which is indigenous mainly to tropic America, Africa and East Asia. Being among a number of medicinal mushrooms that have been widely used in East Asia, especially Korea, China and Japan as health booster and ancient herbal medicine, PL has been described in Asian herbal medicine literature to be effective on a diversity of diseases, including improving blood circulation, enhancing detoxication and hepatoprotection of human body, combating allergy and diabetes, curing oral ulcer and alleviating gastroenteric disorder or lymphatic disease. In the last decade, PL has been investigated extensively for its extraordinary capacity of suppressing cancer or enhancing body immunity [1-11], which makes PL a prospective candidate for developing novel anti-cancer compounds from natural resources. The increasing interest in PL also comes from its ability to potentiate the efficacies of existing anti-cancer chemotherapeutical drugs [3,8].

Like other traditional Asian medicines, the traditional medication of PL is mostly based on empirical practice and deficient in providing any solid component-function data to support its clinical application in rigorously evidence-based western medical system without causing any safety concerns. As a result, researchers have been setting out to disclose the principles underneath the magic of PL to ward off multiple diseases with modern biomedical techniques. Their studies demonstrated that PL was not only an immuno-stimulator or immuno-regulator that was capable of stimulating the cell-mediated and innate immunity, activating T lymphocytes, B lymphocytes, native killer cells, dendritic cells (DCs) and macrophages, but also had direct cytotoxic activities against a wide spectrum of murine and human neoplasms including prostate cancer, lung cancer, colon cancer, epidermoid, hepacellular carcinoma, fibrosarcoma, neuroblastoma and melanoma [2-10,12-14]. From these investigations, the anti-metastatic, anti-angiogenic and anti-oxidant properties of PL have also been proven and indicate PL to be a powerful chemo-preventive agent or an adjunct chemotherapeutic agent.

THE EXTRACTION AND FRACTIONATION OF PL EXTRACT

PL is an herbal medicine composed of a mixture of various bioactive substances [15,19-22]. Although it is wildly believed in Asia that the interplay of different constituents of PL is responsible for its health-promoting effects, this notion, among many other theories of traditional Asian medicine, is mainly established on empirical clinical observations and lacks credibility proved by the contemporary medical science. The complicated chemical nature of botanical constituents in PL as well as the batch-to-batch inconsistency in PL culturing and processing further preclude standardization and hamper the development of PL by modern pharmaceutical programs. In order to reveal the bioactive constituents in PL, most researchers employed a routine procedure of Basidiomycete mushroom fractionation: from crude PL extraction by boiled-water or 80% ethanol, to bioactive fraction identification, then to further purification of bioactive compounds using a combination of such techniques as ethanol precipitation, fractional concentration, acidic precipitation, ion-exchange chromatography, gel filtration and affinity chromatography [3,10,15-18]. Various bioactive substances, including polysaccharides, proteoglycans (Figs. **1**-**4**), cyclophellitol (Fig. **5**), furan derivatives (Fig. **6**), hispidin (Fig. **7**) and hispolon (Fig. **8**) were thus identified and their bioactivities verified *in vivo* or *in vitro* [15,19-22]. For example, Kim *et al.* isolated a 150KD acidic proteoglycan from PL by hot water extraction, filtration, solvent precipitation, dialysis, and freeze-drying followed by chromatographing through anion-exchange column, which was demonstrated to possess both immunoregulatory and anticancer effects [15]. Although the exact chemical structure of this proteoglycan is yet to be resolved, compared to other polysaccharides or proteoglycans isolated from other Basidiomycete mushrooms with well-recognized antitumor properties, such as Lentinan from *Lentinus edodes* (Fig. **1**), D-fractions from *Grifola frondosa* (Fig. **2**), Schizophyllan from *Schizophyllum commune* (Fig. **3**), and Polysaccharide-K (PSK, or Krestin) or Polysaccharide-peptide (PSP) from *Trametes versicolor*, this acidic proteoglycan purified from PL was shown to be a (1,6) branched type (1,3) glycan (Fig. **4**) containing a mixture of monosacchride including mannose, glucose, arabinose and xylose with both α- and β- linkages, and a variety of amino acids including aspartic acid, glutamic acid, alanine, glycine and serine as well. Inagaki *et al.* fractionated mycelium of PL by sequential extraction with chloroform, ethy acetate, methanol, water and boiling water [23] and found the boiled-water fraction to be anti-allegically active. Min *et al.* determined two novel furan derivatives from the fruit-bodies of PL to be anti-complementary and named them Phellinusfurans A and B (Fig. **6**). Park *et al.* identified an antioxidant Hispidin (Fig. **7**) from mycelial cultures of PL. Recently, Chen *et al.* isolated an antitumor compound designated hispolon (Fig. **8**) from fresh PL fruit-bodies by ethanol extraction followed by silica gel column chromatography and reversed-phase HPLC and suggested it to be proapoptotic to human epidermoid KB cells [9]. Another compound designated cyclophellitol (Fig. **5**) iso-

*Address correspondence to this author at the Department of Radiation Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, 21-27 Burlington Ave. Room 553C, Boston, MA 02215, USA; Tel: 617-632-8513; Fax: 617-632-0635; E-mail: cchen6@bidmc.harvard.edu

0929-8673/08 $55.00+.00

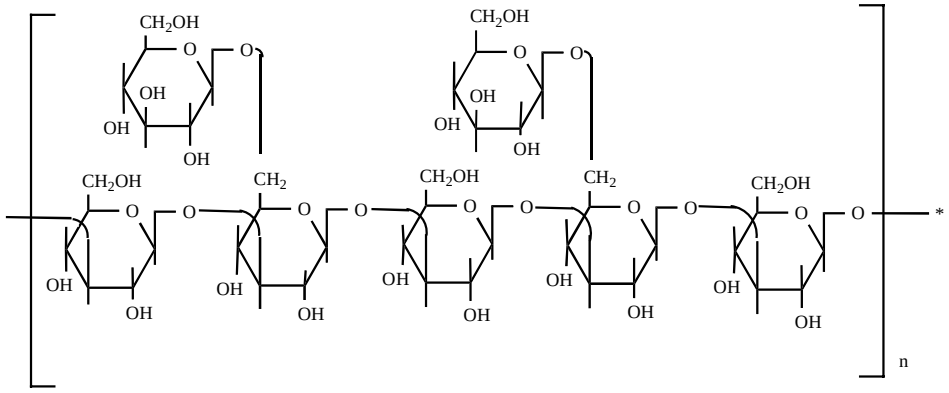

Fig. (1). Chemical structure of Lentinan isolated from *Lentinus edodes* with a fundamental structure of a unit of two β-(1,6) branches per five glucose residues of straight chain [59].

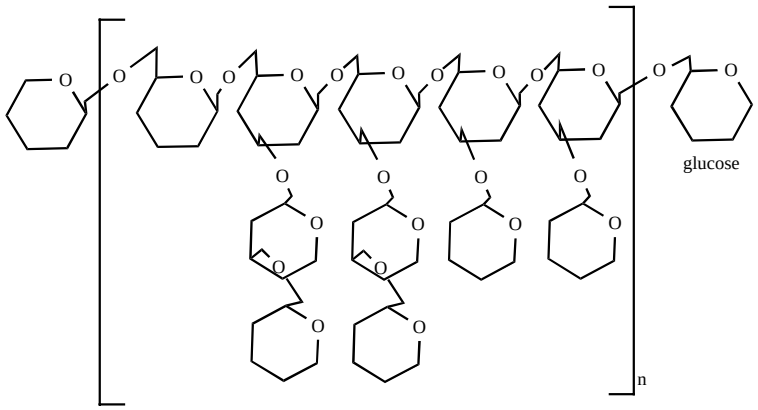

Fig. (2). Chemical structure of D-fraction isolated from *Grifola frondosa* [60].

lated from the culture filtrate of Phellinus sp. was also indicated to be able to restrict the metastatic potential of tumor cells or inhibit the infection of human immunodeficiency virus (HIV) by interfering with the synthesis of the cell surface carbohydrate [57,58]. Furthermore, Kim *et al.* decided that the ethyl acetate fraction, instead of polysaccharide-rich n-butanol or aqueous fraction of PL manifested high hepatoprotective activity [24].

THE IMMUNO-STIMULATING AND IMMUNO-REGULATORY PROPERTIES OF PL

The most extensive studies of the health beneficiary effects of PL are concentrated on its immuno-stimulating and immuno-modulatory efficacies. It was documented that PL differentially activated the immune system when challenged by a variety of extracellular stimuli [10,17-18,21,23,25-40]. For example, the *in vivo* study demonstrated that the administration of extracts from PL fruit-bodies affected the balance of T Helper type-1 (Th1) and type-2 (Th2) lymphocytes in mice [10,25,39]. In this process, PL in-

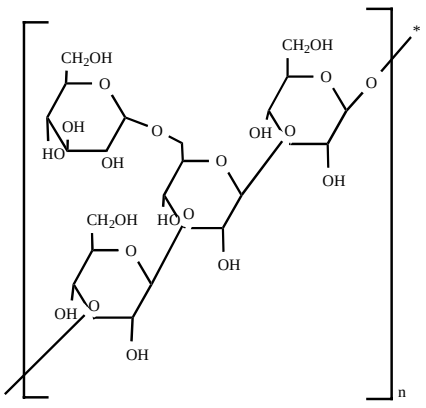

Fig. (3). Chemical structure of repeat unit of Schizophyllan isolated from *Schzophyllum commune* [61].

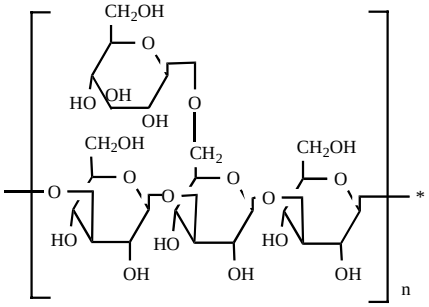

Fig. (4). Diagram of mushroom β-D-glucan [62].

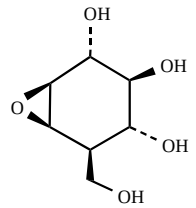

Fig. (5). Chemical structure of cyclophellitol [58].

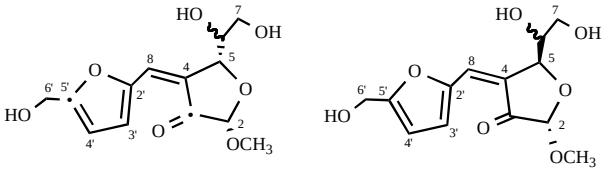

Fig. (6). Chemical structures of phellinusfurans **A** and **B** [63].

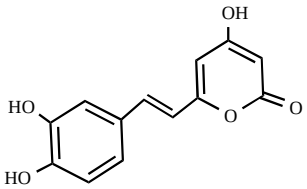

Fig. (7). Chemical structures of hispidin [64].

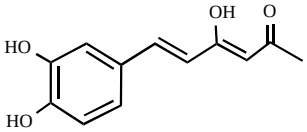

Fig. (8). Chemical structures of hispolon [9].

creased the expression/secretion of cytokines of Th1 cells [such as interferin γ (IFN-γ)], and constrained Th2 dependent-cytokines [for example, Interleukin 4 and 10 (IL4, 10)], resulting in inhibition of Th2 cell differentiation and subsequently hindering the IL4-mediated Immunoglobin E (IgE) production in B cells [25]. Since IgE is the primary mediator in Type I hypersensitivity reactions to food allergens, the PL extract appears to be a good, natural source for anti-allergy agents. Choi *et al*. reported recently that water extract of PL fruit-bodies prevented the release of histamine from peritoneal mast cells in response to allergenic stimuli in an IgE-dependent or independent fashion, in which the level of intracellular cAMP or calcium uptake was augmented [26]. This finding is convergent with an earlier discovery that the boiled-water extract from the mycelium of PL could tune down the skyrocketing vascular permeability incited by passive cutaneous anaphylaxis and histamine, as well as the ear swelling induced by tumor necrosis factor alpha (TNF-α) in the mouse allergic reaction model, and subside the hypersensitivity of asthma or allergic rhinitis [23]. Interestingly, the ability of PL to regulate the immune system seems to be versatile and largely dependent on physiological context of cells. In the face of different challenges, PL has been shown to mobilize diverse cellular responses to help restore the balance of immune system [10,17-18,21,23,25-40]. For example, the anti-inflammatory effect of proteoglycan from PL fruit-bodies was rendered on mice through altering the ratio between Th1 and Th2 cells when the mice were treated with collagen to induce autoimmune arthritis. The expression of pro-inflammatory cytokines (such as TNF-α and IFN-γ) was decreased and the level of anti-inflammatory cytokines [for instance, IL-10 and transforming growth factor-beta (TGF-β) was increased, resulting in subsidence of the autoimmune response in the joints of mice [27]. A similar reaction was observed to control the lipopolysaccharide (LPS)-induced septic shock in mice after the oral administration of polysaccharides isolated from PL fruit-bodies [28]. In this process, the expression of TNF-α was found to be down-regulated by up to 68%, which coincided with the decrease of major histocompatibility complex II (MHC II) expression in peritoneal macrophage and B cells. Since the overexpression of TNF-α and MHC II is responsible for the hyper-activated cellular immune response, these results suggested that PL also functioned against inflammation and was a potential remedy for treating autoimmune rheumatoid arthritis as well as bacterial sepsis.

Similar to the versatile immune responses induced by PL, the innate or cell-mediated immunity stimulated by PL manifests a non-specific pattern, with characteristics of activating diverse immunological members in response to various extracellular stimuli [10,17-18,21,23,25-40]. For instance, PL was found to induce the maturation of DCs from endocytotic antigen-capturing cells to antigen-presenting cells (APCs) which in turn migrated to lymphoid organs and provoked T cell-mediated immune responses [17,29]. In this process, the expression of the cell surface molecules, such as MHC I, MHC II, CD80, CD86, and intracellular cytokines IL-12 were dramatically elevated, paralleled by predominant DC migration into lymphoid tissues, a hallmark of the maturation of DCs [17]. Although the precise mechanisms underlying PL-induced DC maturation is still unclear, the loss-of-function experiments suggested potential involvement of two surface receptors: toll-like receptor 2 and 4 (TLR-2, 4) of DCs, and plasma membrane–associated kinases [protein tyrosine kinase (PTK) and protein kinase C (PKC)], as well as signal transducers (such as NF-κB, ERK and p38 MAPK) in this process.

PTK and PKC were also suggested to participate in PL-induced activations of murine peritoneal macrophage [18,21] and B lymphocytes [30]. Following treatment with acidic proteoglycan isolated from PL fruit-bodies, an upregulation in the Nitric Oxide (NO) production and TNF-α expression, along with increased expression of cell surface molecule CD80, CD86 and MHC II were detected in murine peritoneal macrophages, implicating an improved phagocytostic potential and predisposition to initiate the T-cell mediated immunity against malignant cells. Likewise, PL-sourced proteoglycan treatment preferably activated CD19+ B cells over CD3+ T cells in murine splenic lymphocytes, concurrent with upregulation of co-stimulatory molecules CD86 and CD80 in B cells, indicating this PL proteoglycan exerted immunostimulatory effect via selective B-cell activation. In both cases, the PL-mediated activations of macrophage and B lymphocyte were shown to be PTK and PKC dependent.

THE ANTI-CANCER PROPERTY OF PL

The extraordinary preventative or inhibitory effect of PL against cancer is one of the most appreciated properties of PL. It is also the main reason why PL attracts so much attention. In Japan, it has been reported that a patient suffered from hepatocellular carcinoma with multiple lung metastases underwent a complete tumor regression 6 months after taking the extract of PL mycelium, independent of any other therapies [13]. In another case, the ingestion of PL coupled with radiation therapy led to spontaneous remission of hepatocellular carcinoma in a Korean patient which had metastasized to the skull [14]. Furthermore, after taking the extract of PL, the hormone refractory prostate tumor in a Japanese patient with the bone metastasis shrank tremendously [12]. All these cases suggested a linear relationship between the intake of PL and tumor regression. Additionally, results from *in vivo* studies using mouse models and *in vitro* experiments employing cultured cell lines have reached to the consensus that PL possesses the anti-cancer potency against a variety of murine and human carcinomas.

PL has been demonstrated to act both directly and indirectly against tumors. For example, in mice transplanted with MCA-102 fibrosarcoma, administration of proteoglycan from PL fruit-bodies was determined to enhance the transition of bone marrow-derived DCs into their maturation status. The matured DCs were loaded with tumor antigens and subsequently migrated to lymphoid organs where they primed naive T cells to promote T cell-mediated tumoricidal responses [10]. This PL-originated proteoglycan also induced a Th1 dominant immune state in mice by elevating the expression of Th1-dependent cytokines (such as IFN-γ) and diminishing Th2-regulated cytokine IL-4, which cooperatively contributed to the suppression of MCA-102 tumor growth in mice in the

absence of causing any detectable adverse effects like weight loss [10]. Another example of PL's indirect anti-cancer property comes from the observation that PL could function as antagonist against H_2O_2-induced gap junctional intracellular communication (GJIC) inhibition in WB-F344 rat liver epithelial cells [1]. The intracellular communication is indispensable in maintaining tissue homeostasis, controlling cell growth or differentiation. Disruptions of GJIC have been indicated to be involved in numerous tumorigenesis processes [41-47]. Therefore, the PL-mediated cellular response to neutralize the H_2O_2-induced GJIC inhibition was suggested as auxiliary in countering carcinoma proliferation.

Aside from indirect anti-cancer effect, PL has also been demonstrated to hold direct cytotoxic activities towards different carcinomas [1-4,7-14]. The aqueous extract of PL fruit-bodies was capable of eliciting both caspase8-mediated and ER-stress-related apoptotic signaling pathways in hormone responsive prostate cancer LNCaP cells [2]. In the advanced, hormone refractory prostate cancer PC3 cells, only caspase8-mediated caspase cascade was activated, resulting in less intensive, yet noticeable apoptotic responses. Studies also showed that low concentrations of extracts from PL fruit-bodies were able to cause cell growth arrest in murine or human lung cancer cells, which was concurrent with decreased activities of cyclin-dependent kinases CDK2, 4 and 6. In contrast, high concentrations of PL induced apoptosis, which was characterized by activation of caspase cascade, loss of mitochondria membrane potential and cytochrome c release, leading to loss of clonogenecity [3]. PL was also able to trigger apoptosis in human colon cancer SW380 cells [4], in which the cells transiently arrested in G2/M phases of the cell cycle and subsequently underwent apoptosis in a does-dependent manner. In this process, cyclin B1 and Bcl-2 were down-regulated and cytochrome c was released from the mitochondria to the cytosol. Furthermore, a study showed that the mycelium of PL upregulated the expression of pro-apoptotic Bax and activated caspase 3, thereby causing programmed cell death in neuroblastoma SK-N-MC cells [5]. A recent report of the cytotoxic effect of PL was ascribed to a bioactive component isolated from ethanol extract of PL fruit-bodies designated hispolon (Fig. **8**), which was shown to does-dependently hamper the proliferation of human epidermoid KB cells and disrupt the mitochondria potential and consequently induce apoptosis [9]. However, it was unclear whether the cytotoxicity of hispolon extracted from PL equally affected normal and malignant cells.

From studies of PL in cultured cells, it has been shown that metastatic melanoma B16F10 cells, following treated with acidic polysaccharide from PL fruit-bodies, displayed impaired ability to adhere to and invade through the extracellular matrix, which is an index of deteriorated metastatic potential of tumor [6]. This observation was in concert with the *in vivo* pulmonary metastasis assay in the mice transplanted with B16F10 melanoma cells, in which a significant reduction in lung metastasis was witnessed after administration of polysaccharide prepared from mycelial culture of PL. This anti-metastatic effect of PL against melanoma was also present in another study using a similar animal model, where urokinase type plasminogen activator (uPA), matrix metalloproteinase 2 (MMP-2) and tissue inibitor of metalloproteinase 2 (TIMP-2) were examined [7]. The treatment of aqueous extract of Cambodian PL induced downregulation of uPA while the levels of MMP-2 and TIMP-2 remained unchanged. Moreover, the chick embryo chorioallantoic membrane (CAM) assay suggested prominent anti-angiogenic activities rendered by PL extract [48].

Carcinogenesis is a complicated pathological process involving profound genetic and epigenetic changes within intracellular signaling molecules and their communication with cues embedded in extracellular microenvironment, with the result of autonomous cell proliferation, angiogenesis promotion, and tumorigenesis. Inhibition or reverse of different phases in this process is the target of most therapeutic modalities. PL, with its many bioactive constituents, possesses multifunctional anti-tumor properties and blocks tumor growth at various stages of malignancy. These establish PL as a promising candidate for developing novel anti-cancer therapies. Moreover, PL has been shown to be able to potentiate the effects of other conventional anti-tumor pharmaceuticals, as exemplified by the finding that low concentrations of PL could synergize with doxorubicin at its non-cytotoxic dose range to induce apoptosis in prostate or lung cancer cells [3,8], implicating that PL can also function as an adjunct in cancer treatment to reduce doses of anti-cancer drugs routinely used clinically to avoid the cytotoxicity.

OTHER HEALTH-BENEFICIARY PROPERTIES OF PL

Like other medicinal mushrooms, the bioactive substances in PL are manifoldly health-beneficiary. Other than being immunoregulatory and anticancerous, PL is an excellent herbal source of antioxidants and liver-protectants [24,49-50]. In a screening of natural product-derived agents against dementia, a compound designated hispidin (Fig. **7**) was identified to be a non-competitive inhibitor of β-secretase (β-site amyloid precursor protein cleaving enzyme, BACE1) with much less inhibitory effects on α-secretase (tumor necrosis factor-alpha converting enzyme, TACE) and other serine proteases such as chymotrypsin, trypsin and elastase [22]. In addition, hispidin showed a potential for scavenging reactive oxygen species including super oxide anion radical, hydroxyl radical and stable free radical 1,1-diphenyl-2-picrylhyrazyl (DPPH), but not hydrogen peroxide radical. In concord, an evaluation of the antioxidant effect of PL showed that the ethanolic extract of fresh PL fruit-bodies had comparable efficacy as vitamin C in removing DPPH, and was able to block lipid peroxidation (LPO) and oxanthine oxidase activities as well [48].

Although the exact mechanism responsible for PL antioxidant activity is still elusive, in primary rat hepatocytes, the anti-free radical capacity of PL has been suggested to partially contribute to its protective effect of ameliorating iron-overloading-mediated oxidative stress [51]. Such hepatoprotective effect of PL has also been evident in cultured rat hepatocytes in which extract of dried PL fruit-bodies was shown to help maintain or restore the hepatic glutathione (GSH) levels and uridine incorporation into RNA [52]. Another investigation demonstrated that the carbon tetrachloride (CCl_4)-induced liver damage could be suppressed by treatment with boiled-water extract of PL cultured on germinated brown rice [49]. Furthermore, PL is a potential diabetes controller, hinted by improvement of insulin resistance and insulin secretion in 90% of pancreatectomized rats that were treated with water extract of PL fruit-bodies [53].

SAFETY ISSUE

For centuries, medication of herbal medicines including medicinal mushrooms has been practiced to combat a wide range of diseases and is still a significant means of medical treatment in parallel with western medicines in Eastern Asian countries [54,55]. Although their efficacy and non-toxicity have been indicated by numerous *in vivo* or *in vitro* studies over many years, the lack of western standard clinical evidence as well as clear-cut structure-function relationship demonstrations impedes their acceptance by western treatment regimes. In particular, unlike highly purified pharmaceutical compounds, herbal medicines often consist of multiple naturally derived ingredients, arousing the safety concern about the possible side-effects caused by interactions among different components. Nevertheless, traditional Asian medicines represent a golden mine of naturally resourced agents confronting various diseases including cancers. With the advances in chemical- and bio- technologies, development of novel, highly effective pharmaceuticals from these resources is gaining increasing attention. Along with these efforts, some compounds isolated from oriental herbal medicines that possess the antitumor activities have proceeded

through clinical trial and licensed as anti-cancer drugs in Asia, such as Kanglaite from coix of Traditional Chinese Medicine, and various mushroom-derived polysaccharides or proteoglycans like Lentinan from *Lentinus edodes*, Grifron-D from *Grifola frondosa*, Schizophyllan from *Schizophyllum commune*, PSK and PSP from *Trametes versicolor*. Some of them are also under clinical trial in the United States or European countries [54,55]. Not surprisingly, PL, with its abundantly documented anticancer efficacies, becomes a prospective candidate in the program of novel chemotherapeutic agent development. Of especially importance is PL's potential to act as a synergizer to potentiate the antitumor activities of other conventional chemotherapeutic drugs while tuning down the side-effects on patients by decreasing the necessary drug doses. Nonetheless, while the effects of PL in immuno-promotion and cancer suppression have been repeatedly confirmed by different research studies, the caution about the safety of broad use of this herb medicine has also been brought forward in light of the potential complications PL may bring forth through interacting with such drugs as immunoregulatory antibodies or cytokines. Another potential risk of PL intervention was implicated by the finding that administration of PL extract worsened benign prostatic hyperplasia in rats [56]. Therefore, the worldwide acceptance and license of PL not only entail a more accurate definition of the bioactive components of PL, a more reproducible culturing process to produce PL enriched with bioactive compounds, as well as a more standardized purification process of bioactive compounds from PL, but also call for extensive efforts in testing and evaluating the overall safety of incorporating PL into treatment regimes, including determinations of acute, subacute or subchronic toxicity, mode and dosage quantities of administration, *et cetera*.

In summary, more sufficient anti-cancer therapies are in demanding. Current investigations demonstrate that PL possesses multiple functions against various diseases, especially against various types of cancer. The mechanistic studies at molecular level provided evidence that PL differentially affected normal versus malignant cells, which might render different sensitivity to its toxicity. By identifying and modulating intracellular targets of PL, this herbal medicine can be developed for clinical usage.

ACKNOWLEDGEMENTS

We thank Drs. G-F. Hu (Harvard Medical School, Boston, MA) and S. Calderwood (Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA) for their critical comments.

ABBREVIATIONS

PL	=	Phellinus linteus
PSK	=	Polysaccharide-K
PSP	=	Polysaccharide-peptide
Th1	=	T Helper type-1
Th2	=	T Helper type-2
IFN	=	Interferin
IL	=	Interleukin
IgE	=	Immunoglobin E
TNF	=	Tumor necrosis factor
TGF	=	Transforming growth factor
LPS	=	Lipopolysaccharide
MHC	=	Major histocompatibility complex
APC	=	Antigen-presenting cell
DC	=	Dendritic cell
TLR	=	Toll-like receptor
PTK	=	Protein tyrosin kinase
PKC	=	Protein kinase C
NO	=	Nitric oxide
GJIC	=	Gap junctional intracellular communication
CDK	=	Cyclin-dependent kinase
uPA	=	urokinase type plasminogen activator
MMP	=	Matrix metalloproteinase 2
TIMP	=	Tissue inhibitor of metalloproteinase
BACE	=	β-site amyloid precursor protein cleaving enzyme
TACE	=	Tumor necrosis factor-alpha converting enzyme
DPPH	=	1,1-diphenyl-2-picrylhyrazyl
LPO	=	Lipid peroxidation
GSH	=	Glutathione
CCl	=	Carbon tetrachloride

REFERENCES

[1] Cho, J.H.; Cho, S.D.; Hu, H.; Kim, S.H.; Lee, S.K.; Lee, Y.S; Kang, K.S. *Carcinogenesis*, **2002**, *23*, 1163.
[2] Zhu, T.; Guo, J.; Collins, L.; Kelly, J.; Xiao, Z.J.; Kim, S.H.; Chen, C.Y. *Br. J. Cancer*, **2007**, *96*, 583.
[3] Guo, J.; Zhu, T.; Collins, L.; Xiao, Z.X.; Kim, S.H.; Chen, C.Y. *Mol. Carcinog*., **2007**,*46*, 144.
[4] Li, G.; Kim, D.H.; Kim, T.D.; Park, B.J.; Park, H.D.; Park, J.I.; Na, M.K.; Kim, H.C; Hong, N.D. *Cancer Lett*., **2004**, *216*, 175.
[5] Choi, Y.H.; Huh, M.K.; Ryu, C.H.; Choi, B.T.; Jeong, Y.K. *Int. J. Mol. Med*., **2004**, *14*, 227.
[6] Han, S.B.; Lee, C.W.; Kang, J.S.; Yoon, Y.D.; Lee, K.H.; Lee, K.; Park, S.K; Kim, H.M. *Int. Immunopharmacol*., **2006**, *6*, 697.
[7] Lee, H.J.; Lim, E.S.; Ahn, K.S.; Shim, B.S.; Kim, H.M.; Gong, S.J.; Kim, D.K.; Kim, S.H. *Biol. Pharm. Bull*., **2005**, *28*, 27.
[8] Collins, L.; Zhu, T.; Guo, J.; Xiao, Z.J.; Chen, C.Y. *Br. J. Cancer*, **2006**, *95*, 282.
[9] Chen, W.; He, F.Y.; Li, Y.Q. *J. Ethnopharmacol*., **2006**, *105*, 280.
[10] Kim, G.Y.; Oh, W.K.; Shin, B.C.; Shin, Y.I.; Park, Y.C.; Ahn, S.C.; Lee, J.D.; Bae, Y.S.; Kwak, J.Y.; Park, Y.M. *FEBS Lett*., **2004**, *576*, 391.
[11] Han, S.B.; Lee, C.W.; Jeon, Y.J.; Hong, N.D.; Yoo, I.D.; Yang, K.H.; Kim, H.M. *Immunopharmacology*, **1999**, *41*, 157.
[12] Shibata, Y.; Kurita, S.; Okugi, H.; Yamanaka, H. *Urol. Int*., **2004**, *73*, 188.
[13] Kojima, H.; Tanigawa, N.; Kariya, S.; Komemushi, A.; Shomura, Y.; Sawada, S.; Arai, E.; Yokota, Y. *Radiat. Med*., **2006**, *24*,139.
[14] Nam, S.W.; Han, J.Y.; Kim, J.I.; Park, S.H.; Cho, S.H.; Han, N.I.; Yang, J.M.; Kim, J.K.; Choi, S.W.; Lee, Y.S.; Chung, K.W.; Sun, H.S. *J. Gastroenterol. Hepatol*., **2005**, *20*, 488.
[15] Kim, G.Y.; Park, H.S.; Nam, B.H.; Lee, S.J.; Lee, J.D. *Bioresour. Technol*., **2003**, *89*, 81.
[16] Nakamura, T.; Matsugo, S.; Uzuka, Y.; Matsuo, S.; Kawagishi, H. *Biosci. Biotechnol. Biochem*., **2004**, *68*, 868.
[17] Park, S.K.; Kim, G.Y.; Lim, J.Y.; Kwak, J.Y.; Bae, Y.S.; Lee, J.D.; Oh, Y.H.; Ahn, S.C.; Park, Y.M. *Biochem. Biophys. Res. Commun*., **2003**, *312*, 449.
[18] Kim, G.Y.; Oh, Y.H.; Park, Y.M. *Biochem. Biophys. Res. Commun*., **2003**, *309*, 399.
[19] Kim, G.Y.; Lee, J.Y.; Lee, J.O.; Ryu, C.H.; Choi, B.T.; Jeong, Y.K.; Lee, K.W.; Jeong, S.C.; Choi, Y.H. *Biosci. Biotechnol. Biochem*., **2006**, *70*, 1218.
[20] Min, B.S.; Yun, B.S.; Lee, H.K.; Jung, H.J.; Jung, H.A.; Choi, J.S. *Bioorg. Med. Chem. Lett*., **2006,** *16*, 3255.
[21] Kim, G.Y.; Choi, G.S.; Lee, S.H.; Park, Y.M. *J. Ethnopharmacol*., **2004**, *95*, 69.
[22] Park, I.H.; Chung, S.K.; Lee, K.B.; Yoo, Y.C.; Kim, S.K.; Kim, G.S.; Song, K.S. *Arch. Pharm. Res*., **2004**, *27*, 615.
[23] Inagaki, N.; Shibata, T.; Itoh, T.; Suzuki, T.; Tanaka, H.; Nakamura, T.; Akiyama, Y.; Kawagishi, H.; Nagai, H. *Evid. Based Complement. Alternat. Med*., **2005**, *2*, 369.
[24] Kim, S.H.; Lee, H.S.; Lee, S.; Cho, J.; Ze, K.; Sung, J.; Kim, Y.C. *J. Ethnopharmacol*., **2004**, *95*, 367.
[25] Lim, B.O.; Jeon, T.I.; Hwang, S.G.; Moon, J.H.; Park, D.K. *Biotechnol. Lett*., **2005**, *27*, 613.
[26] Choi, Y.H.; Yan, G.H.; Chai, O.H.; Lim, J.M.; Sung, S.Y.; Zhang, X.; Kim, J.H.; Choi, S.H.; Lee, M.S.; Han, E.H.; Kim, H.T.; Song, C.H. *Biol. Pharm. Bull*., **2006**, *29*, 1360.
[27] Kim, G.Y.; Kim, S.H.; Hwang, S.Y.; Kim, H.Y.; Park, Y.M.; Park, S.K.; Lee, M.K.; Lee, S.H.; Lee, T.H.; Lee, J.D. *Biol. Pharm. Bull*., **2003**, *26*, 823.

[28] Kim, G.Y.; Roh, S.I.; Park, S.K.; Ahn, S.C.; Oh, Y.H.; Lee, J.D.; Park, Y.M. *Biol. Pharm. Bull*., **2003**, *26*, 1418.
[29] Kim, G.Y.; Han, M.G.; Song, Y.S.; Shin, B.C.; Shin, Y.I.; Lee, H.J.; Moon, D.O.; Lee, C.M.; Kwak, J.Y.; Bae, Y.S.; Lee, J.D; Park, Y.M. *Biol. Pharm. Bull*., **2004**, *27*, 1656.
[30] Kim, G.Y.; Park, S.K.; Lee, M.K.; Lee, S.H.; Oh, Y.H.; Kwak, J.Y.; Yoon, S.; Lee, J.D.; Park, Y.M. *Int. Immunopharmacol*., **2003**, *3*, 1281.
[31] Kim, B.C.; Choi, J.W.; Hong, H.Y.; Lee, S.A.; Hong, S.; Park, E.H.; Kim, S.J.; Lim, C.J. *J. Ethnopharmacol.*, **2006**, *106*, 364.
[32] Bae, J.S.; Ahn, S.J.; Yim, H.; Jang, K.H.; Jin, H.K. *Ann. Surg*., **2005**, *241*, 534.
[33] Bae, J.S.; Jang; K.H.; Jin, H.K. *World J. Gastroenterol*., **2005**, *11*, 810.
[34] Bae, J.S.; Jin, H.K.; Jang, K.H. *J. Vet. Med. Sci*., **2004**, *10*, 1205.
[35] Lim, B.O.; Yamada, K.; Cho, B.G.; Jeon, T.; Hwang, S.G.; Park, T.; Kang, S.A.; Park, D.K. *Biosci. Biotechnol. Biochem*., **2004**, *68*, 2391.
[36] Kang, H.S.; Choi, J.H.; Cho, W.K.; Park, J.C.; Choi, J.S. *Arch. Pharm. Res.*, **2004**, *27*, 742.
[37] Kim, H.M.; Han, S.B.; Oh, G.T.; Kim, Y.H.; Hong, D.H.; Hong, N.D.; Yoo, I.D. *Int. J. Immunopharmacol*., **1996**, *18*, 295.
[38] Song, K.S.; Cho, S.M.; Lee, J.H.; Kim, H.M.; Han, S.B.; Ko, K.S.; Yoo, I.D. *Chem. Pharm. Bull. (Tokyo)*, **1995**, *43*, 2105.
[39] Oh, G.S.; Lee, M.S.; Pae, H.O.; Kwon, J.; Lee, S.S.; Jeong, J.G.; Shin, M.K.; Kwon, T.O.; Chung, H.T. *Immunopharmacol. Immunotoxicol*., **2006**, *28*, 281.
[40] Kim, S.H.; Song, Y.S.; Kim, S.K.; Kim, B.C.; Lim, C.J.; Park, E.H. *J. Ethnopharmacol.*, **2004**, *93*, 141.
[41] Kang, K-S.; Sai, K.; Hirose, A.; Hasegawa, R.; Trosko, J.E.; Tohru, I. *Cancer Lett.*, **2001**, *173*, 163.
[42] Trosko, J.E.; Ruth, J.R.J. *Front. Biosci*., **1998**, *3*, 208.
[43] Trosko, J.E. *Eur. J. Cancer Clin. Oncol.*, **1987**, *23*, 19.http://carcin.oxfordjournals.org.ezp1.harvard.edu/cgi/external_ref?access_num=A1987F776000005&link_type=ISI
[44] Klaunig, J.E.; Ruch, R.J. *Lab. Invest*., **1990**, *62*, 135.
[45] Kang, K.-S.; Lee, Y.-S.; Kim; H.S.; Kim, S.H. *J. Toxicol. Environ. Health*, **2001**, *65*(Part A), 523.
[46] Mehta, P.P.; Hotz-Wagenblatt, A.; Rose, B.; Shalloway, D.; Loewenstein, W.R. *J. Memb. Biol.*, **1991**, *124*, 207.http://carcin.oxfordjournals.org.ezp1.harvard.edu/cgi/external_ref?access_num=A1991GV37400003&link_type=ISI
[47] Omori, Y.; Dagli, M.L.Z.; Yamakage, K.; Yamasaki, H. *Mut. Res.*, **2001**, *477*, 191.
[48] Song, Y.S.; Kim, S.H.; Sa, J.H.; Jin, C.; Lim, C.J.; Park, E.H. *J. Ethnopharmacol*., **2003**, *88*, 113.
[49] Jeon, T.I.; Hwang, S.G.; Lim, B.O.; Park, D.K. *Biotechnol. Lett*., **2003**, *25*, 2093.
[50] Shon, Y.H.; Nam, K.S. *Biotechnol. Lett*., **2003**, *25*, 167.
[51] Kim, S.H.; Lee, H.S.; Lee, S.; Cho, J.; Ze, K.; Sung, J.; Kim, Y.C. *J. Ethnopharmacol*., **2004**, *95*, 367.
[52] Ye, S.F.; Hou, Z.Q.; Zhang, Q.Q. *Phytother. Res,*. **2007**, *21*, 948.;
[53] Choi, S.B.; Park, C.H.; Choi, M.K.; Jun, D.W.; Park, S. *Biosci. Biotechnol. Biochem*., **2004**, *68*, 2257.
[54] Sullivan, R.; Smith, J.; Rowan N. J. *Perspect. Biol. Med.*, **2006**, *49*, 159.
[55] Normile, D. *Science*, **2003**, *299*, 188.
[56] Shibata, Y.; Kashiwagi, B.; Arai, S.; Fukabori, Y.; Suzuki, K. *Urology*, **2005**, *66*, 455.
[57] Atsumi, S.; Iinuma, H.; Nosaka, C.; Umezawa, K. *J. Antibiot.*, **1990**, *42*, 1579.
[58] Atsumi, S.; Umezawa, K.; Iinuma, H.; Naganawa, H.; Nakamura, H.; Iitaka, Y.; Takeuchi, T. *J. Antibiot.*, **1990**, *43*, 49.
[59] Mizuno, T. *Int, J. Med. Mushrooms*, **1999**, *1*, 9.
[60] Kodama, N.; Komuta, K.; Sakai, N.; Nanba, H. *Biol. Pharm. Bull.*, **2002**, *25*, 1647.
[61] Leathers, T. D.; Nunnally, M. S.; Price, N. P. *Biotechnol. Lett.*, **2006**, *28*, 623.
[62] Kidd, P. M. *Altern. Med. Rev.*, **2000**. *5*, 4.
[63] Min, B. S.; Yun, B. S.; Lee, H. K.; Jung, H. J.; Jung, H. A.; Choi, J. S. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 3255.
[64] Park, I. H.; Chung, S.K.; Lee, K. B.; Yoo, Y. C.; Kim, S. K.; Kim, G. S.; Song, K. S. *Arch. Pharm. Res*., **2004**, *6*, 615.

Received: January 25, 2008 **Revised: April 01, 2008** **Accepted: April 03, 2008**