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Minireview

Antitumor polysaccharides from mushrooms: a review on the structural characteristics, antitumor mechanisms and immunomodulating activities

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ABSTRACT

Mushrooms are popular folk medicines that have attracted considerable attention because of their efficient antitumor activities. This review covers existing research achievements on the mechanisms of isolated mushroom polysaccharides, particularly (1→3)-β-D-glucans. Our review also describes the function in modulating the immune system and potential tumor-inhibitory effects of polysaccharides. The antitumor mechanisms of mushroom polysaccharides are mediated by stimulated T cells or other immune cells. These polysaccharides are able to trigger various cellular responses, such as the expression of cytokines and nitric oxide. Most polysaccharides could bind other conjugate molecules, such as polypeptides and proteins, whose conjugation always possess strong antitumor activities. The purpose of this review is to summarize available information, and to reflect the present situation of polysaccharide research filed with a view for future direction.

1. Introduction

In Asian countries such as China and Japan, polysaccharides extracted from mushrooms have played an important function as food and medicinal agent in the treatment of cancer. Numerous studies have reported that, through the ability of medicinal mushrooms to cure diseases, dietary intake of these mushrooms could be beneficial to humans.[1,2] Consumption of fresh mushrooms or dried mushroom powder could prevent breast cancer in pre- and postmenopausal women.[3] Mushroom with distinctive fruiting bodies, which exerts an effect in curing cancer, belongs to the class of Basidiomycetes, and sometimes in Ascomycetes. The primary taxa traditionally used are *Ganoderma lucidum* (*G. lucidum*), *Lentinus edodes* (Shiitake, *L. edodes*), *Tremella fuciformis* (*T. fuciformis*), *Griflola frondosa* (common name: Maitake, *G. frondosa*), *Hericium erinaceus* (*H. erinaceus*), *Agaricus blazei* Murrill (*A. blazei* Murrill), *Flammulina velutiper* (Fr.) Sing (*F. velutiper*), *Coriolus versicolor* (*Trametes versicolor*, *C. versicolor*, *T. versicolor*), *Inonotus obliquus* (*I. obliquus*), *Pleurotus ostreatus* (*P. ostreatus*), *Sparassis crispa* (*S. crispa*) and *Poria cocos* Wolf (*P. cocos* Wolf) among others. All of these species belong to the class Basidiomycetes, whereas *Cordyceps militaris* (*C. militaris*) is in the class of Ascomycetes. In 1957, the antitumor activity of the Basidiomycetes has been first demonstrated by Lucas. A substance isolated from *Boletus edulist* has been found to exert a significant inhibitory effect against Sarcoma 180 tumor cells in mice.[4] In recent years, Basidiomycetes mushrooms have been widely studied and used for their antitumor potential.[5] These Basidiomycetes mushrooms along with several species of the Ascomycota were used in traditional medicine for treatment of cancer, AIDS or universal immunosuppression.[1]

G. lucidum is a well-known medicinal fungus for the treatment of a variety of cancers.[6] As mentioned above, the benefits of *G. lucidum* are mainly reflected in antitumor activities, including cell-cycle arrest, induction of apoptosis, inhibition of motility, anti-angiogenesis, and anti-mutagenesis.[7] Polysaccharides from *G. lucidum* possessed preventive effects against the development of chemical carcinogen-induced aberrant crypt foci (ACF), colon adenoma, colon adenocarcinoma, and pulmonary adenocarcinoma in rats.[8] These polysaccharides are also used as a radio-protective agent that significantly prolongs animal survival,[9] and as a potential preventive agent against the side effects of chemotherapy.[10] Another important antitumor mushroom polysaccharide named lentinan was isolated from *L. edodes*, which is widely consumed as a nutritional health food worldwide. Lentinan activated the human immune system to perform antitumor function.[11] Lentinan was applied for clinical use in Japan since 1985, functioning as an immune adjuvant in conjunction with chemotherapy for stomach cancer treatment.[12] The equally important grifolan, which is extracted from *G. frondosa*, exhibits antitumor activity in gastrointestinal, lung, liver and breast cancers.[13,14] This polysaccharide is a macrophage activator that augments cytokine production without dependence on endotoxins. In addition, grifolan increased the expression of IL-6,

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IL-1 and tumor necrosis factor-alpha (TNF-α) of macrophages.[15] Maitake D fraction functions as an apoptosis inducer and immune enhancer against cancer.[16] A study showed that 69% of breast cancer patients consuming whole Maitake powder significantly suppressed the development of cancer.[17] In addition, Maitake could decrease metastatic progression, reduce the expression of tumor markers and enhance natural killer cell activity in breast cancer patients.[18,19] Schizophyllan (sizofiran or SCH/SPG), which is isolated from the inedible Chinese mushroom *S. commune*, is a highly potent antitumor polysaccharide that works against solid S-180 tumors.[20] In humans, schizophyllan is not only effective in suppressing gastric cancer, but also could prolong survival in patients who suffered head and neck cancer.[21] At the same time, schizophyllan is supposed to increase immune responses and acts as biological response modifiers *in vivo*.[22] One dose of schizophyllan is used to reduce the probability of mammary tumor, and decrease the progression of mammary carcinoma.[23] *S. crispa* is an edible medicinal mushroom. β-glucan from *S. crispa* is regarded as a good source of antitumor polysaccharide.[24,25] Meanwhile, in China, Japan, Korea, and North America, *P. cocos* Wolf is a well-known traditional medicine that grows around the roots of pine trees. Polysaccharide from *P. cocos* Wolf possesses antitumor potential and pharmacological properties, and could relieve the gastritis, edema, nephrosis, among others.[26] In addition, heteropolysaccharides from *P. cocos* Wolf which are cultured in a corn steep liquor medium exhibited higher antitumor activities against S-180 *in vivo* compared with that cultured in bran extract media.[27] Pleuran (β-1, 3-D-glucan), which is isolated from the oyster mushroom *P. ostreatus*, has been proven to retard the development of precancerous ACF lesions in the colon of the male Wistar rats.[28] *P. ostreatus* is a traditional Chinese medicinal and edible fungus that is distributed in Heilongjiang Province of China. Another study showed that polysaccharide isolated from *P. ostreatus* could increase the proliferation of lymphocyte caused by concanavalin A (Con A, a T-lymphocyte mitogen) or lipopolysaccharide (LPS, a B-lymphocyte mitogen).[29]

The majority of these antitumor polysaccharides are homoglycans or heteroglycans, which can be converted to glycopeptides, proteoglucans or glycoproteins when connected with other proteins.[30,31] In addition to the basic structure of polysaccharides, a higher structure such as chain conformation performs a key function in antitumor activities.[32] Polysaccharides that act as adjuvant medicines are more commonly used in combination with chemotherapy/radiotherapy to treat various cancers.

This article reviews recent work in this field with focus on the mechanisms of polysaccharide antitumor activities, structural features, physical properties, and adjuvant therapy.

2. Structure of polysaccharides

2.1. Relationship between the structure and antitumor activities of polysaccharides

Antitumor polysaccharides differ greatly in their chemical structure and physical properties. Polysaccharides are composed of certain amounts of monosaccharide residues. The primary structure of polysaccharides is defined by placement of the monosaccharide residues, position of glycosidic linkages, and the sequence of monosaccharide residues. These three factors result in the highest potential structural variability. The greatest structural variability could induce the highest polysaccharide capacity for carrying biological information. In general, numerous polysaccharides extracted from mushrooms possess immunomodulating activities. Multiple kinds of polysaccharides include β-glucans,[33] hetero-β-glucans, glycans and heteroglycans.[34] Various β-glucans generally possess different structural characteristics, mainly involving the degree of branching (DB), molecular weight (MW), and conformation, like triple helix, single helix, and random coil structures (Table 1).[35] β-glucans strengthen phagocytosis and trigger the expression of a series of cytokines, such as TNF-α and various types of interleukins.[36] In addition, several natural antitumor mushroom polysaccharides are bound to protein or peptide residues, such as glycopeptides, proteoglucans or glycoproteins. Based on different structures, different polysaccharides possess different MW, and the MW is closely related to the antitumor activities of polysaccharide.

A polysaccharide from *A. blazei* Murrill named ABP-W1, possess a triple helix in water solution. Most polysaccharides in triple strand helical chain conformation often possess stronger anticancer capacity than those in random coils or lines.[38] In addition, β-glucan (β-1, 3, β-1, 6 linked glucan) from *A. blazei* Murrill has been reported to act as an immunoaccelerator against cancer cells.[32] Polysaccharides of *Antrodia camphorate* (also called *Antrodia cinnamomea*, *Cinnamomum kanehirae* mushroom, camphor mushroom, camphor chamber mushroom and yin-yang mushroom) show antitumor activity, and their helical structure may be important in resisting tumors.[42] In addition, polysaccharide from *C. militaris* adopts a random coil conformation and exhibits considerable antitumor activity.[46,47] An alkaline-soluble antitumor polysaccharide from *Flammulina velutipes* exhibits strong antitumor activity against sarcoma S-180 *in vivo* but not *in vitro*. This polysaccharide is converted to random coils from single helices with increasing pH.[75] The majority of *G. lucidum* polysaccharides are glucans, and (1→3)- and (1→6)-β-D-glucans possess antitumor activity and superior absorption than others in *G. lucidum*.[52] However, several hetero-β-D-glycans, such as glucurono-β-D-glucan, arabinoxylo-β-D-glucan, xylo-β-D-glucan, manno-β-D-glucan and xylomanno-β-D-glucan, show strong antitumor properties as well.[51] These polysaccharides could enhance the antitumor, antibacterial, antiviral, anticoagulatory and wound healing activities.[52] HEB-AP Fr I was found to act as an immunostimulant through the activation of macrophages, which is isolated from *H. erinaceus* and show a β-mannan with a laminarin-like triple helix conformation.[55] FII-1 (from *H. erinaceus*) also shows good antitumor effect.[57] HE (from *H. erinaceus*) acted as an enhancer to increase the intracellular Dox accumulation.[58] The purified endo-polysaccharide isolated from *I. obliquus* mycelia is an α-fucoglucomannan, which is a specific activator of B cells and macrophages.[59] The water soluble polysaccharide from *I. obliquus* sclerotia is a heteropolysaccharide.[60] IOPS-F and IOPS-H are polysaccharides that are extracted from *I. obliquus*. The two polysaccharides possess a triple helix structure, and exhibit superior antioxidant capacity.[77] Lentinan is considered as bioactive immunomodulator agent and classified as a β-glucan, the conformation of which was important for immunostimulating activity.[52,60] The helical conformation is commonly believed to play an important function in enhancing immunopotentiating activity, but increasing data presently suggest that not only triple helical structures but also the distribution of the branch units along the backbone chain are the main mechanisms for activity as well.[52] In general, the most active polymers possess between 20% and 33% DB. The DB of lentinan is 40%.[78] More recently, a study on the correlation between branching and bioactivity showed that debranching of lentinan could enhance its biological proficiency. However, maximal immunomodulating and antitumor activities are achieved with a DB of 32%.[79] The main constituent of polysaccharide isolated from *P. cocos* Wolf is β-pachyman with DB of 1.5% to 2%.[64,65] Schizophyllan, an efficient polysaccharide isolated from *S. commune*, is another extensively studied mushroom-derived polysaccharide with immunopotentiating activity with a DB of 33%.[78] The highly water soluble SCG from *S. crispa* possesses a triple helix conformation and possibly exists in an irregular single helical conformation. This conformation promotes cytokine production and the synthesis of subsequently iNOS, NO and type I collagen.[73,80] Polysaccharopeptide krestin (PSK), an antitumor polysaccharide that is isolated from

Table 1
Some antitumor polysaccharides from mushrooms and their higher structure, degree of branching and molecular weight

Systematic name	Antitumor agents	Higher structure	Degree of branching (DB)	Molecular weight (MW)	Biological effects	Reference
Agaricus blazei Murrill	ABP-W1 ((1→6)-α-D-galactopyranosyl and (1→2, 6)-α-D-glucopyranosyl main chains with one single terminal (1→)-α-D-glucopyranosyl branch at the O-2 position of (1→2, 6)-α-D-glucopyranosyl along the main chain in the ratio of 1:1:1)	Triple helix in water solution	~50%	390 kDa	Act as an immunoaccelerator against cancer cells	32,37,38
	(1→6)-β-glucan backbone with (1→3)-β-glucan side branches attached at O-3 for every three backbone residues					39,40
	(1→4)-α-glucan backbone with (1→6)-β-glucan side branches			20 kDa		32
Antrodia camphorate (*Antrodia cinnamomea*)	(1→3)-β-D-dextran main chain having (1→6)-β-dextran side branches	Helical structure	Unknown	10–10^3 kDa	Inhibited the proliferation of cancer cells; enhanced the NK/LAK activity.	41,42
Auricularia auricula-judae	AAG ((1→4)-linked D-glucopyranosyl main chain with (1→6)-linked D-glucopyranosyl branch at O-6)	Semi-stiff chains in 0.1M NaCl aqueous solution	65.5%	34–288 kDa	Induced apoptosis of cancer cell	43–45
	AF1 ((1→3)-β-D-glucan main chain with two (1→6)-β-D-glucosyl residues for every three glucose residues)	Comb-branched structure in the aqueous solution				
Cordyceps militaris	(1→6)-β-D-mannopyranosyl backbone with (1→4)-α-D-glucopyranosyl and (1→6)-β-D-galactopyranosyl side branches at O-3, and terminated with β-D-galactopyranosyl residues and α-D-glucopyranosyl residues	Random coil conformation	25%	26–50 kDa	Induced apoptosis of cancer cell	46,47
Flammulina velutipes	(1→3)-β-D-glucan	Single helix	Unknown	200 kDa	Increased the expression of cytokines	48
Ganoderma lucidum	(1→3)-, (1→4)- and (1→6)-β-D-glucan; hetero-β-D-glycans (glucurono-β-D-glucan, arabinoxylo-β-D-glucan, xylo-β-D-glucan, manno-β-D-glucan and xylomanno-β-D-glucan)	Triple helix	33%	80–200 kDa	Induced cell-cycle arrest, apoptosis; increased the cytokines expression, etc	49 50,51
	(l→3)-β-D-glucans with (1→6)-β-D-glucopyranosyl branches attached by (1→6) linkages as single unit					52
Griflola frondosa	Maitake D-fraction ((1→6)-β main chain with (1→4)–β branches, (1→3)–β main chain with β-(1→6) branches)	Triple helix	33%	800 kDa	Increased the expression of cytokines	53,54
Hericium erinaceus	HEB-AP Fr I (β-mannan)	Triple helix	20%	>100 kDa;	Enhanced the doxorubicin-mediated apoptotic signaling; induced the secretion of cytochrome c, etc	55
	HEP3 ((1→3)-β-D-glucopyranosyl main chain with one single unit β-D-glucopyranosyl branch at O-6 position for every three backbone units)					56
	FII-1 (glucoxylan with (1→3)-β and (1→6)-glucosidic linkages);					57
	FIII-2b (galactoxyloglucan-protein complex included (1→3)-β and (1→6)-glucosidic linkages);			13 kDa		57
	HE (1, 3-branched-β-1, 6-glucan)					58
Inonotus obliquus	α-linked fucoglucomannan Heteropolysaccharide (xylogalactoglucan consisted of glucose, mannose, galactose, xylose, arabinose, and fucose.)	Triple helix	Unknown	1000 kDa	Induced apoptosis	59,60
Lentinus edodes	Lentinan ((1→3)-β-glycan main chain with two (1→6)-β-D-glucopyranoside branches for every five (1→3)-β-D-glucopyranoside linear linkages)	Triple helix	40%	400–800 kDa	Recovered the activation of immune cells; increased the expression of cytokines, etc	52,60,61
Pleurotus ostreatus	(1→3)-β-D-glucan, heteroglucans	unknown	25%	2200–2900 kDa	Increased the proliferation of lymphocyte	62,63
Poria cocos Wolf	β-pachyman	Single helix	1.5~2%	26–268 kDa	Promoted the immune reaction; increased the expression of cytokines	64,65
	β-glucan					66
	(1→3)-α-D-glucan					67,68
	(l→3)-β-D-glucan (PC3)	Liner structure				69
Schizophyllum commune	Schizophyllan (sizofiran or SPG/SCH)	Reversible coiled-helix in water	33%	10^2–10^4 kDa	Increased the immune responses; increased the expression of cytokines; increased NK cells' activity, etc	70,71
	((1→3)-β-D-glucan main chain with (1→6)-β-D-glucopyrano branch at every three repeating)	Random coil conformation in dimethylsulfoxide				
Sclerotinia sclerotiorum	SSG	Triple helix	50%	Unknown	Improved the development of Th1 cells	72
Sparassis crispa	SCG ((1→3)-β-D-glucan main chain with single (1→3)-β-D-glucosyl side branching units at every three residues)	triple helix, irregular single helix	Unknown	2000 kDa	Enhanced the hematopoiesis; promoted the production of cytokine	24,73
Trametes versicolor (*Coriolus versicolor*)	PSK (Krestin, a protein-bound β-glucan. Sugar part was characterized by the main chain created by glucose units combined with (1→3)-β-glycosidic bonds, while in the side branches with (1→6)-β-glycosidic bonds)	Triple helix	20%	94 kDa; 28 kDa	Increased the expression of cytokines; stimulated the macrophage phagocytes, etc	60,70,74
Tremella fuciformis	Acidic glucuronoxylomannan ((1→3)-α-D-mannan backbone with β-linked D-glucuronic acid, and single or short chains of (1→2)-β-linked-xylose residues were attached at the C-2 position)	Triple helix	Unknown	53–1000 kDa	Induced human monocytes to express interleukines	32,75,76

Trametes versicolor, consists of β-glucan and peptide and stimulates the activity of cytokines.[70] The polysaccharide from *T. fuciformis* induces human monocytes to express interleukines, such as IL-1 and IL-6, and TNF *in vitro*.[81] Evidently, the main chain and additional β-(1→6) branch points of β-(1→3) linkages glucan had been indicated as important factors in antitumor action. However, polysaccharides that mainly possess (1→6) linkages β-glucans invariably possess lower activity. The structure shows a characteristic spectrum of β-(1→3) and (1→6)-glucosidic linkages and exhibits immunostimulating activity, including macrophage activation, expression of IL-1β and TNF-α, and nitric oxide (NO) production.[57] In addition, several polysaccharides are classified as α-mannan or α-glucan, which also possess anticancer activity. In addition to the primary structure, helical conformation is also an important structure that is found in antitumor mushroom polysaccharides. (1→3)-β-glucans exhibit a variety of biological and immunopharmacological activities related to their triple helix. As seen in Table 1, ten mushroom polysaccharides possess triple helix. Among these ten polysaccharides, schizophyllan[82] and lentinan[83] have been used in clinical applications. However, the exact mechanisms of the effect of the triple helix on antitumor action remain unclear. The single helix, random coil conformation, reversible coiled-helix, and comb-branched structure also exhibit antitumor capacities. A linear water-soluble (1→3)-β-D-glucan from *A. auricula* that exists as a single helical chain in solution shows extremely strong antitumor activity.[84]

2.2. Effect of molecular weights

For a long time, MW has been recognized as a critical parameter that dictates the antigenicity of a molecule.[85] Moreover, high MW glucans are commonly believed to exhibit higher bioactivity (Table 1).[32,60,70,86] To trigger antitumor events, the polysaccharides must initially bond to receptors or proteins. Presumably, high MW polysaccharides may exhibit more connections to receptors or proteins. In addition, larger polysaccharides possess more repeating units and thus higher variability, and increased variability infers higher MW. However, several antitumor mushroom polysaccharide exceptions, such as (1→3)-α-glucuronoxylomannans, are not strongly dependent on MW. Their hydrolyzed fractions, which contain glucuronoxylomannans with MW from 53–1000 kDa also possess antitumor activity as the aforementioned fractions.[87]

Analysis on the four fractions of PSK shows that the highest MW fraction displays the greatest potential immunomodulatory activity. A (1→3)-β-glucan that was extracted from *G. frondos* showed changes in biological activities related with various MWs. The fraction with the highest MWs (800 kDa) showed the strongest antitumor and immunomodulatory activities.[88] The water-soluble glucan fraction, isolated from *H. erinaceus* possessed MW exceeding 100 kDa. This glucan exhibits anti-artificial pulmonary metastatic tumor and immunoenhancing effects.[89] Schizophyllan is similar to lentinan in terms of the triple helix structure and biological activity, but not physicochemically. Schizophyllan possesses an MW of 450 kDa[61] and thus exerts a marked antitumor effect.[85] The MWs of certain schizophyllans ranging from 100 kDa to 10^4 kDa exhibit a markedly antitumor effect.[90] Lentinan owns MWs of about 400–800 kDa.[61] The MW of *F. velutipes* polysaccharide was estimated to be about 200 kDa and thus exhibit potent antitumor activity against sarcoma S-180 *in vivo*.[75] Another immune-mediated antitumor polysaccharide SCG (polysaccharide from *S. crispa*) possesses an MW exceeding 2000 kDa.[73] This polysaccharide directly targets macrophages and dendritic cells (DCs) to produce TNF-α and IL-12 and indirectly targeted on $CD4^+$ T cells via the production of IFN-γ and granulocyte macrophage-colony stimulating factor (GM-CSF).[91]

Like higher molecular weights, some low MW polysaccharides, such as schizophyllan, present the same antitumor activity against S-180.[61] As found based on seven antitumor polysaccharide–protein complexes isolated from *Ganoderma tsugae*, polysaccharides exhibiting antitumor ability that originate from fruiting bodies mainly include heteropolysaccharides, with MWs of about 10 kDa.[92] Münzberg, Rau, and Wagner showed that schizophyllan with an MW range of 1–5 kDa had the largest and most effective biological fraction.[93] A low MW fraction with an MW of 20 kDa that is extracted from the fruiting body of *A. blazei* Murill possesses tumor-specific cytocidal and immunopotentiating effects.[94] Another polysaccharide with MWs ranging from 53 to 1000 kDa was isolated from *T. fuciformis*. This polysaccharide induced human monocytes to express interleukin-6 as efficiently as the non-hydrolyzed fraction.[75] The polysaccharides from *P. cocos* at low dosage with relatively high MWs exhibit strong antitumor activities *in vivo*. MWs ranging from 26 to 268 kDa and the extended chain conformation could enhance the antitumor activity by increasing the interaction between polysaccharides and the immune system.[95] A branched β-glucan from *Sclerotium rolfsii* contains a fraction with a low MW. This β-glucan exhibits strong immunostimulatory activity, increases the secretion of TNF-α, and stimulates the proliferation of lymphocytes.[96]

From what has been described above, schizophyllan exhibits considerable antitumor ability regardless of high or low MW. Differences in MW exert no evident influence on medicinal properties. In addition, other mushroom polysaccharides rarely exhibit this phenomenon. For most mushroom polysaccharides, large molecular weight polysaccharides have better anti-cancer mechanism. However, according to the data listed above, numerous low MW polysaccharides possess considerable anti-cancer mechanism. More research that focus on the correlation between MW and the anticancer mechanism of mushroom polysaccharides are required.

3. Anticancer mechanism

Previous studies showed that polysaccharides exert their antitumor activity indirectly via host's immune system instead of a direct cytotoxic effect.[52] Polysaccharides assist the host to endure adverse biological stresses and to increase immunity against the development of cancer cells, thereby increasing immunity through the stimulation of some or all of major biological systems. We denote these polysaccharides as biological response modifiers.[4] Polysaccharides from mushrooms could activate the innate immune system and exert antitumor activity by accelerating the host's defense mechanisms. Polysaccharides activate effector cells, such as macrophages, T-lymphocytes, B-lymphocytes, cytotoxic T-lymphocytes (CTL), and natural killer cells[97] to express cytokines, such as TNF-α, IFN-c, and IL-1β. Cytokines invariably possess antiproliferative activity, cause apoptosis and differentiation in tumor cells, and also secrete products like reactive nitrogen, oxygen intermediates, and interleukins (Fig. 1).[98] In 1957, Byerrum et al first reported that mushroom polysaccharides possess antitumor property.[99] Since then, numerous polysaccharides have been isolated from mushrooms, and these polysaccharides have been proven to be associated with antitumor property. At the same time, the antitumor activities of polysaccharides have been extensively studied. For example, lentinan has also been proven to improve the human immune system.[100] In addition, the activation of mechanisms for fungal polysaccharides against cancer cells is composed of a complex series of reactions, which induces innate and adaptive immune systems.[101]

3.1. Immunomodulating activities

3.1.1. Adaptive immunity

The adaptive immune system is also called the acquired immune system/specific immune system. Fungus polysaccharides could enhance the cytotoxic activity of NK cells and improve the expression

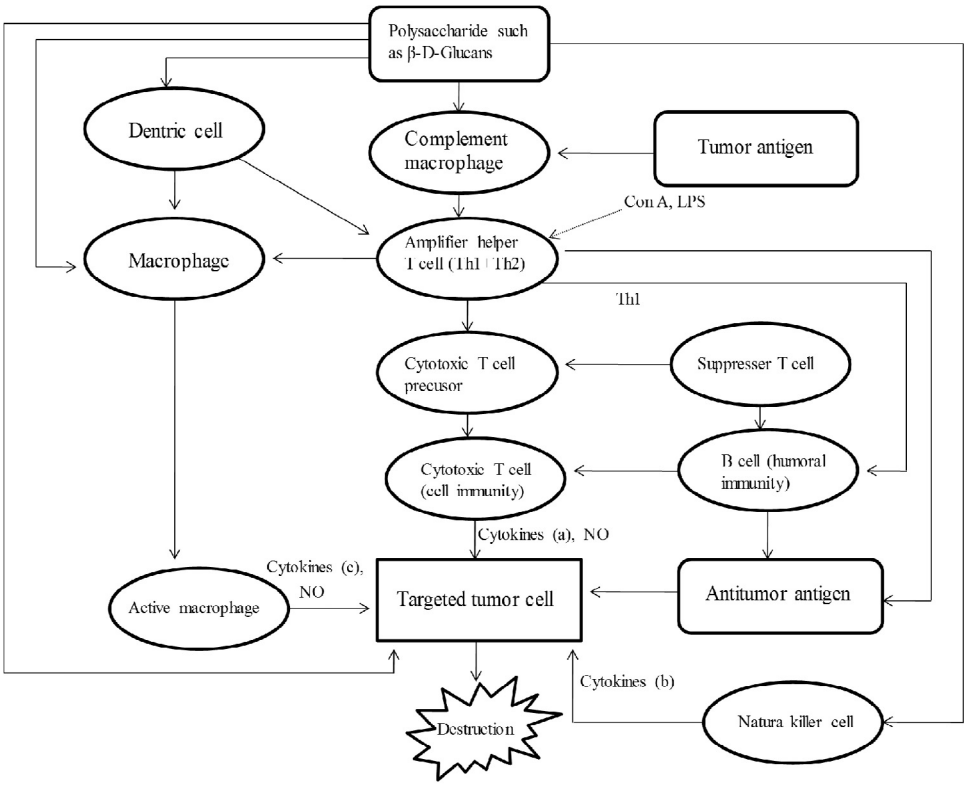


Fig. 1. Possible immune mechanism cytokines (a): Fungus polysaccharides increased the production of IL-2, 3, 4, 6, 8, and 12, TNF-α, IFN-γ, IL-1α, and IL-1β generated from T-cells. Cytokines (b): Mushroom polysaccharides increased the production of IL-1, 2, 3, 12, TNF-α, and IFN-γ of NK cells. Cytokines (c): Fungus polysaccharides could improve the release of IL-6 and 8, IL-1β, TNF-α, and IFN-γ from macrophages. ConA was used as a specific T-cell activator and LPS was used as a general activator of B cells, macrophages, and DCs.

of TNF-α and IFN-γ from macrophages and lymphocytes, respectively.[102] Meanwhile, the immunopotentiation of polysaccharide could be evaluated by thymus, macrophage phagocytosis, humoral antibody production, and delayed-type sensitivity response.[103] Further study also reported that polysaccharides from mushroom fruiting bodies could induce cytokine expression via a toll-like receptor-4-modulated protein kinase signaling pathway.[104]

Splenocytes from a polysaccharide of *Antrodia camphorata* (AC-PS)-treated mice group exhibited a higher spontaneous proliferation, whether oral or intraperitoneal administration. This proliferation was further enhanced by phytohemagglutinin treatment. IL-12 production in AC-PS-treated mice was increased significantly, and the other cytokines like IL-6, TNF-α and IFN-γ were moderately increased by AC-PS treatment.[41]

Meanwhile, treatment of mice with polysaccharide obtained from *P. cocos* named CS-PCS3-II caused the promotion of the immune reaction to antigen. Administration of CS-PCS3-II exhibited an enhanced phagocytic index and a significantly dose-dependent increase in humoral antibody production. CS-PCS3-II could stimulate T cell-mediated immunity, leading to the secretion of various cytokines. CS-PCS3-II administration may be less toxic than 5-Fluorouracil (5-Fu), which kills tumor cells as well as normal cells.[105] Pretreatment of endothelial cells with PC-II, another *P. cocos* polysaccharide, suppressed IFN-c-induced IP-10 protein release in a dose-dependent manner, indicating that PC-II may function in regulating inflammation.[106] CS-PCS3-II and PC-II as fungus polysaccharides exert a positive effect on cancer treatment.

In addition, polysaccharides isolated from fresh fruiting bodies of *G. lucidum* are used to potentiate the production of cytokines which are generated from human monocytes, namely, macrophages and T-lymphocytes. In the treatment of *G. lucidum* polysaccharide mice groups, stronger immunomodulatory activities and generation of cytokines (IFN-γ, IL-4 and IL-6) from spleen lymphocytes are observed.[107,108] A fucose-containing glycoprotein from *G. lucidum* accelerates the proliferation of spleen cells and increases the expression of various cytokines, such as IL-1, IL-2, and IFN-γ.[109] Most recently, numerous researchers found that a proteoglycan fraction obtained from *G. lucidum* activates B-cells and improves immune response of tumor patients as an immunostimulatory drug.[110] Most of *G. lucidum* polysaccharides increased the production of cytokines that could improve cellular and humoral immune activity.

The equally important antitumor Maitake D fraction performs a key function in regulating the balance between Th1 and Th2 and improving the activation of helper T-cells; this polysaccharide could enhance cellular immunity and act as an efficient immunotherapeutic agent for cancer patients.[17]

Intramuscular administration of schizophyllan to mice enhances the generation of cytotoxic T lymphocytes *in vivo*.[111] The antitumor effect of schizophyllan was caused by the enhanced production of cytokines such as IL-1, -2, and -3 and IFN-γ, which activate NK cells, spleen cells, and lymphoid cells, as well as bone marrow cells.[112] Therefore, we conclude that the main anticancer activity of schizophyllan occurs via stimulated cytokines.

Lentinan and schizophyllan are significantly similar in composition, biological activity and the mechanisms of antitumor action.[113] Lentinan could recover the activation of immune cells to the normal level. In addition, lentinan could stimulate immune cells to express cytokines and other biologically active substances that enhance the body's resistance to malignant transformation.[114] Lentinan has been described as an oriented adjuvant that is focused on T cells,[115] and regulates the balance between Th1/2 and Th1 by a significant increase in IL-12 production.[116] Furthermore, in the treatment of

patients suffering from stomach cancer, lentinan could inhibit the synthesis of prostaglandin, which could lead to a slowdown of T-lymphocyte differentiation as well as inhibition of Treg cells activity.[117] Under the action of lentinan, the increased production of activated cytotoxic T-lymphocytes was observed in the spleen.[100] Furthermore, peripheral blood mononuclear cells significantly produce IL-1α, IL-1β, and TNF-α.[114] Other cytokines like colony stimulating factor (CSF), TNF-α, interferon (IFN), IL-1, and IL-3, which are generated from T cells and macrophages, could be increased by the administration of lentinan, and result in maturation, differentiation and proliferation of immunocompetent cells for host defense mechanism.[114,118] In addition, lentinan regulates infiltration of activated immune effector cells, such as NK cells and cytotoxic T-lymphocytes, to destroy the tumors.[119] Lentinan regulates immune cells, such as cytotoxic T-lymphocytes, NK cells and macrophage, to release cytokines, leading to the elimination of tumors.

PSK could also improve both cellular and humoral immunity,[120] which increases the expression of cytokines, such as TNF-α, IL-1, IL-6, and IL-8.[121] These cytokines could result in the intensification of T-cell cytotoxicity against tumor cells, stimulation of antibody production from B-cells, or the promoted expression of receptors for IL-2 on T-cells.[122] SSG, a homoglucan from *Sclerotinia sclerotiorum*, improved the development of Th1 cells via the IL-12 pathway.[123] SCG from *Sparassis crispa* could enhance the response of hematopoiesis.[24]

All these data suggest that mushroom polysaccharides could influence adaptive immunity. By activating the B-cells and T-cell function and interfering with tumor angiogenesis, mushroom polysaccharides can inhibit promotion and progression of tumors.

3.1.2. Innate immunity

Innate immunity, which is also known as non-specific immunity and first line of defense, includes macrophages, neutrophils, NK cells and DCs as gatekeepers. Innate immunity is always regulated by chemical-messengers and cytokines. In addition, innate immunity is stimulated by activating inflammatory and acute phase responses.[114,124] β-glucans, isolated from fungus, act as pathogen-associated molecular patterns on cell membrane receptors, as well as stimulate immune function.[125] Dectin-1, a type II transmembrane protein receptor, recognizes a variety of β-(1→3) and β-(1→6)-glucans, which are the most important activators of innate immune responses in macrophages.[126] The process of dectin-1 involves the combination with β-glucans, which stimulate several signaling pathways to enhance innate immune responses. A series of biochemical reactions include the activation of phagocytosis, the production of reactive oxygen species, and the induction of inflammatory cytokines.[127] CR3 receptor (also called Mac-1 or CD11b/CD18) is commonly found on the surface of immune effector cells, such as macrophages, neutrophils and NK cells. CR3 receptor is the most important β-glucan receptor.[128]

3.1.2.1. Anticancer mechanism of macrophages. Antitumor immune response has been well documented to be regulated by macrophages. Antitumor response can induce a series of events, starting with inflammation mediated by macrophages, which stimulate NKs and DCs and finally activating the cytotoxic lymphoid system. Macrophages may be the first line of defense in tumors. Oral administration of β-glucans would diffuse the polysaccharide to the proximal small intestine to be recognized by macrophages *in vivo* or *in vitro*.[101] β-glucans would decomposed into micromolecular fragments and transported to the bone marrow and endothelial cells. Then macrophages would release micromolecular fragments, which adopt by circulating granulocytes, monocytes and DCs.[129] Finally, innate immune response is triggered by β-glucans.[101] The activated macrophages preferentially act on dead cells and intracellular pathogens,[130] as well as produce cytokines that activate NK cells and T-lymphocytes, both of which are cytotoxic to tumor cells, by using different mechanisms. NK cells released chemical substances that destroyed tumor cells via disrupt cell membranes.[129] Polysaccharides from *G. lucidum*, which regulate the host's immune system, activate bone marrow-derived macrophages in a dose-dependent manner. These activated macrophages significantly enhance phagocytotic ability and elevate the release of IL-1β and NO.[11] In addition, polysaccharides from *G. lucidum* treated mononuclear cells, which are obtained from human umbilical cord blood, would significantly differentiate themselves into macrophages and NK cells.[131] Treatment of human acute monocytic leukemia cell line with these polysaccharides resulted in enhanced macrophage differentiation by activating caspases and p53 (also known as tumor protein p53, cellular tumor antigen p53, phosphoprotein p53, or tumor suppressor p53). Researchers utilized the changes in cell adherence, cell cycle arrest, increased expression of differentiation markers, and down-regulation of myeloperoxidase (MPO) to prove the differentiation.[132] MPO is an enzyme that is solely synthesized in myeloid and monocytic cells and the down-regulation of MPO activity is a sign of macrophage differentiation.[133] For the stimulation of *G. lucidum* polysaccharides, the IL-6 released by macrophages is more sensitive in comparison with TNF-α and IL-1β.[107] Macrophage phagocytosis is intensified by lentinan. Lentinan would increase the expression of cytokines like TNF-α.[134] In addition, macrophage phagocytosis was stimulated by PSK.[21] Schizophyllan improved the production of macrophages and lymphocytes,[111] as well as the activation of phagocytes, strongly increased release in reactive oxygen species and proinflammatory cytokines IL-6, IL-8 and TNF-α, and also increased the expression of CD11b and CD69L markers on the leukocyte surface.[90] Grifolan, a novel macrophage activator isolated from *G. frondosa*, increases the gene expression of IL-6, IL-1, and TNF-α *in vitro*.[113] A polysaccharide from *L. lepideus* could efficiently restore a bone marrow system that is damaged by radiation, and increased the expression of IL-1, IL-6, and GM-CSF. Galactomannan, which is isolated from *Morchella esculenta*, increases macrophage activity and enhances NF-κB-directed luciferase expression in THP-1 human monocytic cells.[30] The activation of transcription factor NF-κB regulates cell survival and suppresses the apoptotic potential of chemotherapeutic drugs.[135] Fucogalactan, which is extracted from *Sarcodon aspratus*, augments the gene expression of TNF-α and NO in macrophages of mice *in vitro*.[30] The induction of NO and TNF-α production and gene expression by activated macrophages could exert a cytotoxic impact on malignant cells.[136] HEB-AP Fr I, which is isolated from *H. erinaceus*, upregulates cytokine expression (TNF-α and IL-1β) and NO release effectively, thereby suggesting that the polysaccharide could activate macrophages.[55] WEHE, which is also obtained from *H. erinaceum*, could increase the steady-state mRNA expression of iNOS. This increase could produce micromolar concentration of NO, stimulate macrophages, and induce the activation of NF-κB.[137] NF-κB activation led to the production of enzymes like cyclooxygenase-2, which enhances the production of reactive oxygen species and lead to further DNA damage.[138] In addition, ATOM extracted from *A. blazei* causes an increase in the number of peritoneal macrophages in tumor-bearing mice.[30]

3.1.2.2. The anticancer mechanism of natural killer cells. As an antitumor cellular component, NK cells effectively inhibit tumor development, growth, and metastasis.[139] A polysaccharide from *H. erinaceum* activated NK cells of splenocytes is responsible for the lysis of Yac-1 cells,[140] which are sensitive to NK cells.[141] The polysaccharide activates NK cells indirectly by promoting other immunomediators or cellular components.[140] Powder from *P. linteus* was orally administered on mice that suffer from the Hep3B hepatoma cell line. The result shows that immune-modulatory and antitumor effects are intensified by the increased release of IL-12, IFN-γ and TNF-α,

which subsequently enhances the activity of NK ells.[142] Finally, intramuscular administration of schizophyllan to the mice group significantly increased NK cell activity in spleen cells.[111]

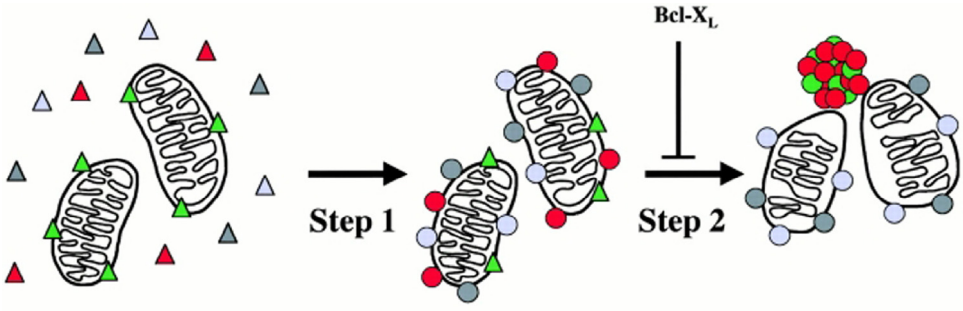
3.1.2.3. Anticancer mechanism of DCs. Lentinan activates DCs to improve immunomodulation and antitumor activity. Moreover, DCs combined with K cells perform a key function in the elimination of tumor cells.[114] The effect of *A. camphorata* polysaccharide (GF2) on the maturation of DC cells exhibit up-regulated expression of MHC class II and CD86 molecules, as well as increased production of IL-10 and IL-12.[143] Meanwhile, sparan (SCG) promotes MHC-II expression and NO production in DCs and macrophages. In addition, SCG increases the gene expression of pro-inflammatory cytokines, such as IL-12, IL-1β, TNF-α, and IFN-α/β by DCs. Among these cytokines, IL-12 is a major factor in the induction of Th1 immune response.[144] Administration of PSK could result in a direct interaction with tumor cells and induce an inflammatory response, which would trigger the elimination of transformed cells.[145] To whom PSK was injected would observe increased numbers of immunologically competent cells and a rise in DCs and Tc cell capacity. Moreover, PSK influenced the phenotypic and functional maturation of dendritic cells from human $CD14^+$ cells.[146]

3.2. Direct tumor inhibition activity

3.2.1. Induced apoptosis of cancer cell

The gene group of Bcl-2 encodes numerous proteins that regulate apoptosis (Fig. 2). According to the number of Bcl-2-homology domains, these proteins are divided into three sub-families: (A) a subfamily containing Bcl-2, Bcl-xL and Bcl-w exerting anti-apoptotic activities and sharing sequence homology; (B) a subfamily consisting of Bax, Bad and Bak and performing proapoptotic activity; (C) a subfamily containing Bik and Bid, and exhibiting pro-apoptotic activity.[148] In the apoptotic process, Bax performs a key function in forming oligodimers.[149] The polysaccharide from *C. militaris* (WECM) causes apoptosis in human lung carcinoma A549 cells, resulting in an increase in Bax in a dose-dependent manner, and a decrease in Bcl-2.[150]

Tumorigenesis is an imbalance between proliferation and apoptosis. A study indicated that the direct addition of *G. lucidum* polysaccharides B (GL-B) into tumor cell culture medium neither restricted the proliferation of S-180 and HL-60 nor induced apoptosis of both tumor cells *in vitro*. However, the serum from GL-B treatment of mice could inhibit S-180 and HL-60 cell proliferation and cause apoptosis *in vitro*. In addition, splenocyte and peritoneal macrophage conditioned medium along with GL-B all restricts HL-60 proliferation and induces apoptosis *in vitro*.[151] *G. lucidum* polysaccharide (GLP) inhibits the development of ACF in a dose-dependent manner, decreasing the total number of aberrant crypts (AC) and restricting cyst formation.[152] GLP reduces the expression of integrin to inhibit cancer cell adhesion and tumor cell proliferation. In addition, another study revealed that *Amauroderma rude* exhibits an ability of inducing cell apoptosis in comparison with the control, and treatment with *A. rude* significantly suppresses the development of colony formation.[153] Moreover, lentinan could increase the production of cytokine in a dose-dependent manner in immune cells and suppresses the expression of caspase-3 in mice with liver cancer, thereby reducing tumor growth.[116] Meanwhile, the mechanism of grifolin induction of apoptosis involves the up-regulation of death-associated protein kinase 1 (DAPK1) via the p53–DAPK1 pathway.[154] Most studies indicated that Maitake could induce apoptosis in MCF-7 breast cancer cells.[6] Previous studies showed that the polysaccharides from *I. obliquus* (Chaga mushroom) induce apoptosis by up-regulating caspase-3 in HT-29 colon cancer cells,[155] and in melanoma cells *in vitro* and *in vivo*.[156] Furthermore, schizophyllan could induce apoptosis along with an increase in the levels of caspase-3 protein, reducing the incidence of hepatocellular carcinomas in 7, 12 Dimethylbenz(a)anthracene (DMBA)-treated mice and in DMBA+ tamoxifen (TAM)-treated mice.[23] Mice treated with AAG (a water-soluble β-glucan isolated from *A. auricula-judae*) showed significantly decreased mRNA level of Bcl-2 and increased mRNA level of Bax. These results suggest that the AAG polysaccharide



	Cytosolic	Mitochondria bound	Apoptotic clusters
Bax	▲	●	●
Bak		▲	●
Bid	▲	●	
Bad	△	○	

Fig. 2. Bcl-2 family in apoptosis (taken from Amotz Nechushtan et al[147]). The image shows that Bcl-X_L is always circumscribed by the mitochondria, whereas Bax, Bak, Bid, and Bad exist in the cytosol of healthy cells. Following death signals, the over-expressed Bid and Bad do not translocate completely to the mitochondria in apoptotic cells. Instead, a significant portion of the cellular Bid and Bad remain in the cytosol, and Bid triggers Bax redistribution from cytosol to membrane. Then, Bax and Bak translocate to mitochondria during apoptosis. Finally, Bak and Bax leave the mitochondria and coalesce into the same clusters adjacent to mitochondria during apoptosis. However, Bid and Bad do not coalesce during apoptosis in contrast to Bax and Bak (the figure already got the authorization from the author and the press).

causes S-180 tumor cell apoptosis probably by increasing the expression of Bax to antagonize the effect of Bcl-2.[43] In addition, polysaccharides from *A. camphorate* named AC-PS remarkably inhibit the proliferation of human leukemic U937 cells, resulting in an inhibition rate of 55.3%. The antitumor activity of AC-PS against S-180 tumor in the ICR mice model is increased in a dose-dependent manner. NK/LAK activity is enhanced by AC-PS *in vivo*, resulting in cytolytic activity of splenocytes. These cytolytic activities were further enhanced by PHA-stimulation.[41] The direct tumor inhibition activity of mushroom polysaccharide mainly involves tumor apoptosis, and most polysaccharides inhibit the development of tumor in a dose-dependent manner. The Bcl-2 family, DAPK1, and caspase-3 occupy important positions in the process of direct tumor inhibition.

3.2.2. Anti-angiogenesis

Angiogenesis is a multistep process that occurs early in tumor growth and is a rate-limiting step for tumor progression.[157] Polysaccharides of *G. lucidum* possess anti-angiogensis ability and could inhibit the production of NO, an inducing agent of angiogenesis over expressed in tumors.[158] Meanwhile, a study showed that a polysaccharide peptide (Gl-PP) of *G. lucidum* suppressed the development of human umbilical cord vascular endothelial cells (HUVEC) in a dose-dependent manner. In HUVECs, Gl-PP would decrease the expression of Bcl-2 and increase the expression of Bax, inducing apoptosis in vascular endothelial cells. Gl-PP may suppress angiogenesis by inhibiting the release of pro-angiogenic factors and decreasing the development of vascular endothelial cells.[151] Polysaccharides of *G. lucidum* could modulate mitogen-activated protein kinase (MAPK) and Protein Kinase B signaling to inhibit prostate cancer angiogenesis. Polysaccharides could alter the phosphorylation of extracellular signal-regulated kinases 1/2 and Akt kinases. Moreover, another study showed that *G. lucidum* inhibits the morphogenesis of capillary by preventing the release of angiogenic factors, namely, vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β1, constituting a key step in angiogenesis in relation to cancer development.[159] In addition, a water-soluble polysaccharide of *G. lucidum* spore possesses characteristics of potential immunomodulatory and antitumor activities along with proliferative response of splenocytes and inhibits the development of Lewis lung cancer cells in mice. The polysaccharide is an effective modifier of the MAPK pathway and spleen tyrosine kinase Syk-dependent TNF-α and IL-6 release in murine resident peritoneal macrophages.[104] In addition, polysaccharides from *A. cinnamomea* significantly suppresses VEGF interaction with VEGF receptor 2, which induces the inhibition of VEGFR2 phosphorylation and cyclin D1 expression, and leading to anti-angiogenic effects in endothelial cells.[160] Meanwhile, a study revealed that a polysaccharide from *Phellinus linteus* (*P. linteuscan*) could induce anti-angiogenic activity, revealing a novel inhibitor of angiogenesis, especially for tumor treatment and prevention. This finding indicates that *P. linteuscan* acts as an immune potentiator and as a direct inhibitor of cancer cell adhesion.[58]

3.2.3. Cell cycle arrest

Cell cycle progression is regulated at several irreversible transition points. Cyclins and cyclin-dependent kinases (CDKs) are key regulators in the eukaryotic cell cycle by controlling the activity of CDKs.[161] Studies on molecular targets and the signaling mechanism underlying the antitumor effect revealed that grifolin significantly induces cell cycle arrest in the G1-phase because of the restriction of the ERK1/2 or the ERK5 pathway.[162] Meanwhile, schizophyllan induces a gradual dose-dependent accumulation of cells at the G2/M phase along with a decrease in the population of cells in G1 phase. Schizophyllan significantly improves the tyrosine15 (Y15) phosphorylation of CDK1 without regulating the levels of CDK1 protein, and induces the accumulation of p53.[163] In mitosis, CDK1 is an important regulator that is bound to cyclin B.[164] Therefore, schizophyllan improves the increase in Y15 phosphorylation via deactivating CDK1, which may be the major mechanism inducing an accumulation of cells in the G2/M cell cycle phase. In addition, schizophyllan improves the accumulation of p53, which protects mammals from neoplasia by inducing apoptosis, DNA repair, and cell cycle arrest in response to a variety of stresses.[165] Moreover, a polysaccharide of *A. blazei* induces an arrest at the G2/M cell cycle phase in human gastric epithelial AGS cells.[166] Furthermore, a polysaccharide isolated from *C. versicolor* could cause the arrest of the G0/G1 phase in tumor cells.[167] Akihisa et al reported that compounds 18 and 23, which are isolated from *P. cocos* Wolf, shows an inhibitory effect on calf DNA polymerase α and rat DNA polymerase β.[168]

4. Antitumor activities of polysaccharides together with other substances

4.1. Chemical modification

The biological activity of polysaccharides that show antitumor activity and immunopotentiation could be improved by chemical modification.[61] Various chemical modifications of polysaccharides produced sulfated, methylated, carboxymethylated, hydroxylated, formylmethylated, or aminoethylated derivatives.[70] For instance, chemical modifications of the α-D-glucan from *G. lucidum* spores demonstrate increasing stimulating effects of antibody production and B lymphocyte proliferation in comparison with unmodified glucans.[169] As mentioned above, the main chemical component of *P. cocos* Wolf is a water-insoluble β-(1→3)-D-glucan. This polysaccharide hardly showed bioactivities, but could exhibit antitumor property after chemical modification.[78]

Sulfation of polysaccharides results in more effective antitumor activities.[170] Typically, a series of sulfated or carboxymethylated derivatives of the polysaccharides could enhance antitumor activities.[171] For instance, sulfated lentinan could enhance antitumor efficacy to improve the human immune response to vaccines by enhancing the population of anti-body and white blood cells.[172] All sulfated derivatives present significantly higher antitumor activities than the native non-sulfated polysaccharide *in vivo* and *in vitro*. These sulfated derivatives act against human hepatoma cell HepG2 and S-180 tumor cell and exhibit substantially lower toxicity than 5-Fu. Through up-regulation of Bax and down-regulation of Bcl-2, sulfated derivatives accelerate apoptosis in S-180 tumor cell.[173] Endothelial cell tube formation was attenuated by treatment with sulfated polysaccharides from *A. cinnamomea*.[170] In addition, a carboxymethylated-sulfated β-(1–3)-D-glucan from *P. cocos* exhibits five times stronger antitumor activity in comparison with native polysaccharides.[105] The chemically modified derivatives of methylated polysaccharide possess the potential to suppress the development of tumor cells.[174] Formyl methylated and aminoethylated derivatives of schizophyllan cause increased production of tumor regressing factor, increased antitumor activities, as well as enhanced production of soluble cytotoxic factors in comparison with nonderivatized schizophyllan.[175] The oxidized polysaccharides from *P. cocos* possess the HO• scavenging activity, increasing the concentration to certain extent and then appearing to reach a plateau. A water-soluble oxidized derivative of (1–3)-β-D-glucan extracted from *P. cocos* Wolf sclerotium enhances bile acid binding capacity by using a TEMPO/NaBr/NaClO oxidation system. In addition, the derivative exhibits hydroxyl radical scavenging activity *in vitro*.[176] Moreover, new folate-connected schizophyllan exhibits specific affinity toward folate binding proteins and functions as a non-cytotoxic cancer-targeting antisense carrier that mediates effective antisense activity in cancer cells.[177] However, phosphated derivatives exhibited significantly stronger antitumor

activities relative to their unphosphated counterparts, suggesting that the effects of solubility and expanded chain conformation could improve antitumor activity.[178] Meanwhile, hydroxylated derivatives from schizophyllan could better stimulate the production of NO and TNF-α in macrophages than the native polysaccharides.[179]

4.2. Use with other medicines

Polysaccharides are considered as adjuvant medicines with conventional chemotherapy/radiotherapy to treat various cancers. Their incorporation into treatment regimens often reduce encountered side effects by patients.[180]

Mice were treated with polysaccharides from the fruit body of *G. lucidum* (GL-F), followed by administration of Con A, which would activate spleen lymphocytes to significantly express cytokines, such as IFN-γ, TNF-α and IL-2. In addition, the treatment of GL-F and Con A was suggested to enhance the function of T-helper cells. However, the production of IL-6 in spleen lymphocytes treated with GL-F could be activated by LPS and not by Con A. Another polysaccharide from the spore of *G. lucidum* (GL-S) group added with Con A could increase the release of IFN-γ, TNF-α, IL-4, and IL-6 from lymphocytes. The secretion of IL-6 in LPS-activated lymphocytes of the GL-S-treated group is significantly higher than those of the GL-P (polysaccharides from the pileus of *G. lucidum*) treated group. GL-F, GL-S, BS (polysaccharides from broken spore of *G. lucidum*), and US (polysaccharides from broken spore of *G. lucidum*) with ConA or LPS in the culture medium of lymphocytes all released cytokines, including IL-2, IL-4, IL-6, TNF-α and IFN-γ.[181]

The present study shows that 5-FU decreases ACF induced by AOM, and that the decrease combined with GLP was greater. GLP and 5-FU could induce a cumulative antitumor effect, suggesting that GLP in combination with other chemotherapeutic drugs may improve the effectiveness of cancer treatment.[182] In addition, another study reported that a GLP ameliorated nausea and vomiting induced by cisplatin (CDDP), as well as improved food intake, in a dose-dependent manner in a rat pica model based on measured kaolin intake.[183] Furthermore, GLP ameliorates small-intestine injury caused by 5-FU, CDDP, CPA, and gefitinib.[10] Mansour and coworkers suggested that schizophyllan alone or in combination with TAM decreases the incidence of DMBA-induced mammary carcinomas in mice.[23] Moreover, spleen weight was significantly increased by the treatment of α-(1→4)-glucan-β-(1→6)-glucan-protein complex polysaccharide from *A. blazei* Murill associated with 5-FU, thereby suggesting an immunomodulatory activity in tumor weigh reduction.[184]

Green tea extract in combination with GLP improved the antiproliferative effect, whereas sole administration of GLP only slightly decreased the colony formation of MDA-MB-231 cells. Furthermore, combination of green tea extract and GLP down-regulated the expression of c-myc in breast cancer cells and synergistically inhibited the migration of invasive breast cancer cells.[180] Urokinase-type plasminogen activator (uPA) is well-documented to perform a crucial function in cell adhesion, migration and invasion.[185] Combination of green tea and *G. lucidum* suppressed the secretion of uPA from breast cancer cells.[180]

Similarly, schizophyllan together with conventional chemotherapy drugs, like tegafur or mitomycin C and 5-FU could result in a significant increase in median survival in inoperable gastric cancer patients.[186] A polysaccharide from *H. erinaceus* acted as an enhancer to sensitize doxorubicin (Dox)-mediated apoptotic signaling. Caspase 3 is activated in the apoptotic process induced by the combination treatment of *H. erinaceus* polysaccharide and Dox. The combined use of *H. erinaceus* polysaccharide and Dox could reduce the expression of cellular Fas-associated death domain interleukin-1b-converting enzyme like inhibitory protein (c-FLIP). Expression through up-regulating the activity of c-Jun NH2-terminal kinase and reducing the ratio of Bcl-2/Bax protein expression by the phosphorylation of p53 and/or down-regulation of c-FLIP$_L$ (an inhibitor of a caspase cascade) induced the secretion of cytochrome c from the mitochondria to cytosol.[58] In addition, *H. erinaceus* polysaccharide in combination with LPS acts synergistically to induce the increase of NO in rat peritoneal macrophages.[137]

4.3. Polysaccharide–protein complexes

The immunostimulatory activity of medicinal mushrooms not only occurs because of bioactive polysaccharides but also through various conjugations of polysaccharide–protein complexes or glycol conjugates, such as glycopeptides, proteoglucans and glycoproteins.[30] Polysaccharides could bind other conjugate molecules such as polypeptides and proteins and reach a higher level of complexity. The polysaccharides are generally considered as biological response modifiers to restore or enhance a variety of immune responses *in vivo* and *in vitro*, although the antitumor mechanisms of polysaccharides or polysaccharide–protein complexes are not yet clear.

Glycopeptides are structurally similar to glycoproteins but possess a smaller chain of amino acids. Polysaccharopeptide (PSP) and PSK belong to this class of compounds with antitumor activities, and are both isolated from CM-101 and Cov-1 strains of *Trametes versicolorare*, respectively.[187] PSP and PSK contain a D-glucose backbone, as well as α-(1→4) and β-(1→3) glycosidic linkages in the polysaccharide chain.[188] PSK inhibits the development of cancer metastasis via preventing adhesion, invasion, motility, and metastatic growth of tumor cells, as well as performs apoptotic the activity in lymphoma, leukemia and pancreatic cells.[189] PSK suppresses TGF-β1 and MMPs to inhibit tumor invasiveness *in vitro* and may serve as an anti-metastatic drug. However, these conclusions should be researched more in the future.[190] Furthermore, numerous clinical studies have shown the potential use of PSK as an adjuvant for other conventional cancer therapies.[191] For instance, Oba et al showed that, as an adjuvant immune chemotherapy, PSK increased the survival rate of patients after curative gastric cancer resection.[192] Meanwhile, immunochemotherapy treatment with PSK of colon cancer patients would increase the presence of active diffuse nuclear accumulation type β-catenin.[193] In addition, another study found that PSK improves the gene expression of several cytokines such as TNF-α, IL-1, IL-8 and IL-6 *in vivo* or *in vitro*. These cytokines were produced by monocytes, macrophages, and other cell types, mediate multiple biological effects by directly stimulating the cytotoxic T-cells against tumors, and enhances antibody production by B lymphocytes and IL-2 receptor expression on T-lymphocyte.[122] Most recent studies have shown that constituents from PSK served as ligands for toll-like receptors-4, which lead to the induction of TNF-α and interleukin (IL-6) inflammatory cytokines.[194] Similarly, PSP could inhibit the development of a variety of human cancer cell lines and increase the levels of IgG and C3 complement to reverse tumor-induced immunodeficiencies in mice.[195] Moreover, a polysaccharide–peptide complex extracted from *Pleurotus abalones* exhibit antioxidant effects.[196]

Proteoglycans belong to glycoproteins but are heavily glycosylated. These polysaccharides include a core protein with one or more covalently attached glycosaminoglycan chains. GLIS, a B-cell stimulating factor that is isolated from *G. lucidum*, contains seven different monosaccharides, mainly D-glucose, D-galactose, and D-mannose in the molar ratio of 3:1:1.[30] GLIS directly stimulates the activation of B lymphocytes, bone marrow-derived macrophages. In addition, the release of immunomodulatory substances, such as IL-1β, TNF-α and NO, also exhibit a high capacity to increase both humoral and cellular immune response of tumor-bearing mice and displays significant activity to increase macrophage-mediated tumor cytotoxicity *in vitro* and inhibit tumor growth *in vivo*.[49] In addition, proteoglycans from fruiting bodies or mycelium of *P. linteus* improve

hormonal and cell-mediated immune functions as well as inhibit tumor growth and metastasis.[197]

Glycoproteins are proteins that contain oligosaccharide chains that are covalently bound to polypeptide side chains. The proteins possess a protein core that is surrounded by numerous glucan chains that are attached to protein moiety by O- or N-glycosidation.[32] Glycoproteins in mushrooms contain β-glucan–protein, α-glucan–protein and heteroglycan–protein complexes.[1] *Agaricus subrufescens* (*A. subrufescens*) contained several bioactive metabolites, including polysaccharides and glycoproteins, which could induce immunostimulant and antitumor activities.[198] An α-(1→4)-glucan-β-(1→6)-glucan-protein of *A. subrufescens* possesses tumor growth inhibition activity via host-mediated mechanisms. Further clinical trials have demonstrated pharmacological benefits on cancer treatment and immunostimulation.[184] Polysaccharide–protein complex (PSPC), which is isolated from *Tricholoma lobayense*, could restore and improve the phagocytic function of macrophages of the tumor-bearing mice[30] and the mitogenic activity of T cells of tumor-bearing mice. The development of S-180 implanted in mice intraperitoneally or subcutaneously was inhibited by PSPC, without toxicity *in vivo*.[61] PSPC also activates peritoneal exudate cells (PEC) to secrete reactive nitrogen intermediates (RNI) and TNF-α, increasing significantly after PSPC treatment in tumor-bearing mice, and exerts indirect cytotoxic activity against P815 mastocytoma cells and L929 mouse fibroblast cells.[199] In addition, FIII-2b (from *H. erinaceus*) is a galactoxyloglucan–protein complex that shows antitumor activity.

5. Future perspectives

This review mainly describes the current knowledge on the mechanisms of mushroom polysaccharides. To elaborate the anticancer mechanisms of polysaccharides and polysaccharide–protein complexes, we need to obtain a comprehensive understanding at the cellular and molecular levels. Future research should focus on the relationship between the structure and antitumor activity, clarify their anticancer mechanisms at the molecular level, and improve the different biological activities by means of chemical modification. In addition, antitumor polysaccharides differ considerably in chemical structure and physical properties, as well as possess the highest potential structural variability. The largest structural variability can induce the polysaccharide highest capacity for carrying biological information. Future research involves determining the relationship between the three-dimensional structure and structure function of polysaccharides. This knowledge will help scientists to design high potential antitumor drugs based on 3D structures. In the future, more research should focus on the basic structure parameters of polysaccharides by means of high resolution instrumental methods. This structure will help us understand how the structural characteristics well, such as degrees of branches or stiffness. On the other hand, in using chemical method or molecular biology technology to control the structural characteristics, scientists can create a polysaccharide, for which a significant scope of properties can be predicted.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.carres.2016.02.008. These data include MOL files and InChiKeys of the most important compounds described in this article.

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