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Review

Molecular mechanisms of bioactive polysaccharides from *Ganoderma lucidum* (Lingzhi), a review

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ABSTRACT

Ganoderma lucidum, commonly known as "*Lingzhi*" in Chinese, are well-known medicinal mushrooms. Lingzhi has been used in traditional Chinese herbal medicines for more than two thousand years. *G. lucidum* polysaccharides (GLPs) are present at high levels in *G. lucidum* cells and GLPs have molecular weights ranging from thousands to millions. GLPs have been widely studied for their various biological activities, such as antioxidant, antitumor, anti-inflammatory, antiviral, anti-diabetes, and immunomodulatory activities. The methods for GLPs extraction and characterization are mature, but the comprehensive research on the relationship between GLPs structure (*i.e.*, molecular weight, tertiary structure, branching, substituents, and monosaccharide composition) and function is still quite limited. The aim of this review is to update and summarize the mechanisms of the various bioactive polysaccharides extracted from *G. lucidum*. The information presented on these biomechanisms should be valuable in the research and development of GLPs-derived therapeutics.

Contents

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1. Introduction

Ganoderma lucidum, commonly referred to as "*Lingzhi*" in Chinese, is one of the most well-known medicinal mushrooms, and has been used in herbal medicines and as a functional food in China and many other Asian counties for more than two thousand years. In recent decades, *G. lucidum* related biological and pharmacological research has been focused on the bioactivity compounds extracted from its fruiting bodies and these include polysaccharides, triterpenoids, sterols, proteins and peptides. Polysaccharides represent one of the most abundant components in *G. lucidum*, contributing to a major group of bioactive constituents with numerous biological activities, including antioxidant [1], antifatigue [2], anti-decrepitude (prolonging life) [3], antitumor (anticancer) [4,5], anti-inflammatory [6,7], immunomodulation [8], hypoglycemic [9] and hypolipidemic [10] activities. The aim of this review is to summarize the currently established mechanisms of the bioactive polysaccharides extracted from *G. lucidum*. This should lead to a better understanding of the relationships between the structural features and biological activities of *G. lucidum* polysaccharides (GLPs). Moreover, the mechanisms of GLPs' bioactivities need to be summarized for the better and precise usage.

2. Overview of GLPs purification

GLPs are biomacromolecules with a molecular weight (M_W) distribution of 10^3–10^6 Da. Most are β-glucans with some strands of monosaccharide residues as their side chains. Many bioactivities of the GLPs result from their 3D structure, a helical conformation. GLPs are water-soluble but insoluble in alcohol, therefore the method of GLPs isolation normally involves water extraction and alcohol precipitation. The water-extraction product is generally low in viscosity, so that crude GLPs precipitation is easily obtained by filtration [11]. In addition, a few acidic GLPs can be extracted using 5%–15% (w/w) NaOH solution or Na_2CO_3 solution at the temperature below 10 °C after hot water extraction [12]. Other methods have been applied to the isolation of GLPs including enzymes (cellulase and pectinase) to degrade cellulose and pectin in cell walls or ultrasonic/microwave assistant extraction [13]. The crude GLPs isolated by alcohol precipitation usually contain some impurities, such as pigment, proteins, monosaccharides, and oligosaccharides. Pigments can be removed using activated carbon during water extraction. Proteins impurities can be removed by enzymatic digestion (*e.g.*, pronase digestion treatment for 2–4 h at 37 °C), Sevag method, trichlorotrifluoroethane (CCl_2FCClF_2) method, and trichloroacetic acid (TCA) method. These impurities can also be removed by column chromatography.

Column chromatography is a common purification process used to obtain pure and homogeneous polysaccharides. There are several column chromatography methods available for GLPs purification, including anion exchange column chromatography, gel permeation chromatography, and affinity chromatography. The purity of product isolated by cellulose column chromatography is high, but the flow rates are low and this method is not suitable for high viscosity polysaccharides [14]. DEAE-Sepharose Fast Flow anion exchange column chromatography provides a high flow rate and good chemical stability [15]. Further purification through gel permeation chromatography is usually needed to obtain a homogeneous polysaccharide [15].

3. The relationships between chemical structure and bioactivity

There is currently no general agreement on the relationship of structure to the bioactivity of GLPs [16]. Instead the bioactivity of individual GLPs must be considered on a case-by-case basis.

GLPs also belong to a major group of physiologically active features, and they are often called biological response modifiers (BRMs) [17]. Nowadays, the study between structure and bioactivity β-D-glucans these mainly focuses on their BRM activity [18]. Principle among these structural features is the M_W, monosaccharide compositions, contents of glycosidic bonds, configurations of main and branch chains, and types and numbers of substituents. The glycosidic bonds of main chain can be a single type or a mixture of α-(1→3) glucan, α-(1→6) glucan, mannan, and galactosan, with α, β-glucans or other linkages. The most common GLPs are listed in Table 1. This table showed that the main chains of GLPs mostly consist of β-D-Glc*p* and some α-D-Gal*p*, and it will have antioxidant activity if the side chain has β-D-Glc*p*, and it will have immunomodulation activity if the side chain has α-L-Fuc*p*.

3.1. Tertiary structure and bioactivities

GLPs also exhibit a tertiary structure (triple helix), which is formed between the hydrogen bonds in the second carbon position and are stabilized through side chains. Hence, the bioactivities of GLPs are often associated with the tertiary structure (the β-structure), like the biopolymers DNA and RNA.

Synytsya et al. [35] demonstrated that a triple helix tertiary structure is present in high M_W (2.5×10^4 - 4×10^4 Da) β-D-glucans. Once exposed to harsh conditions, such as alkaline, carbamide and dimethyl sulfoxide, this structure can be disrupted resulting in a random coil, reduce polysaccharide bioactivity. These polysaccharides exhibit a conformational transition from ordered structures to random coils in basic solutions, when the concentration of NaOH is 0.19 to 0.24 M. One chain will dissociate from the triple helix, eventually leading to a random coil at NaOH concentrations of >0.24 M [36]. A conformation transition occurs at NaOH concentrations higher than 0.15 M or at temperatures above 135 °C. Moreover, the bioactivities will disappear when the triple helix tertiary structure changing into a random coil.

3.2. Branching, substituents and bioactivities

These active branched backbones of GLPs are a key factor in their BRM activities and the hydrogen bonds between these backbones maintain GLPs structure. Once the branched backbone of polysaccharides has more than four monosaccharide units, it shows no antitumor activity [37]. Moreover, when the branching (degree of substitution) is between 20% - 33%, GLPs bioactivity is quite high [38,39]. A GLPs having a regular repeating unit with a degree of branching of 1/3, displays activity for stimulating macrophages to release nitric oxide (NO) on cell level [33]. Lo et al. [40] found that if a side-chain has an arabinose 1→4 and a mannose 1→2 linkage, it will display higher reducing power ability.

Often functional substituents can be changed through chemical modifications, such as acetylation, sulfation, sulfonylation, alkylation, and carboxymethylation. For example, Xu et al. [41] used carboxymethylation to increase the water solubility of a GLPs and this increased antioxidant activity by almost 40%. GLPs spatial configuration, M_W, type, number, and location of substituents can all be changed through chemical modification. Acetylation can change the directionality and exposure of hydroxyl groups enhancing the water solubility of a GLPs. Bioactivity can be influenced by the number and position of acetyl groups [42]. Bioactivity can also be improved by controlled sulfation when each sugar residue has a D.S. (1.0–1.6) sulfate groups [43]. Alkylation can also afford a polysaccharide having surface active properties capable of destroying the HIV envelope protein [44]. Water solubility of a polysaccharide can be enhanced by carboxymethylation. Zhang et al. [6] used chlorosulfonic acid/pyridine to obtain a sulfated GLPs derivative (GLPss58) with potent anti-inflammatory activity. GLPss58 can inhibit the binding of L-selectin to its ligand with an IC_{50} of 13.5 μg/ml, resulting in the inhibition of the SLC-induced chemotaxis of L-selectin$^+$ HPBLs and homing of lymphocytes from peripheral blood to secondary lymphoid tissues. This GLPss58 also interacted with and interfered with major pro-inflammatory cytokines (TNF-α and IFN-γ), hence inhibiting the activation of the complement system. At the same time GLPss58 does not influence the activation of immune cells.

A proteoglycan is a hyperbranched heteropolysaccharide covalently bound to a protein. Serine and threonine residues are linked to GLPs through *O*-glycosidic linkages, but these linkages can be easily broken

Table 1
The structures and bioactivities of GLPs.

No	Name of GLPs	Monosaccharide composition	Molecule weight (Da)	Structure unit	Bioactivities	References
1	LZ-C-1	L-Fuc, D-Glc and D-Gal	7.00×10^3	β-D-Glc*p* (1→4) and α-L-Fuc*p* (1→2) branches on [→6)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1]$_2$→[6)-α-D-Gal*p*-(1]$_m$→6)-α-D-Gal*p*-(1→[6)-α-D-Gal*p*-(1]$_n$→	Antioxidant	[19]
2	LZ-D-1	L-Fuc, D-Glc and D-Gal	2.80×10^4	m+n=4; α-L-Fuc*p* (1→2) branch on [→6)-α-D-Gal*p*-(1]$_2$→ 6)-α-D-Gal*p*-(1→[6)-α-D-Gal*p*-(1→]$_2$ with a →6)-β-D-Glc*p*-(1→ residue	Stimulate proliferation of mouse spleen lymphocytes *in vitro*	[20]
3	LZ-B-1	L-Fuc, D-Glc and D-Gal, small amounts of L-Ara and D-Man	1.12×10^4	β-D-Glc*p* (1→4) and α-L-Fuc*p* (1→2) branches on [→6)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1]$_{(0.5\text{-}1.0)}$→[6)-α-D-Gal*p*-(1]$_m$→6)-α-D-Gal*p*-(1→[6)-α-D-Gal*p*-(1]$_n$→; m+n=4; with →m,n,x,y)-β-D-Hex*p*-(1→ residue (m,n,x,y might be any one of 0,2,3,4,6)	Stimulate proliferation of mouse spleen lymphocytes *in vitro*	[21]
4	LMG	D-Glc	3.98×10^3	β-D-Glc*p* (1→6) branches on β-D-Glc*p*-(1→{3)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→[3)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1]$_n$→}$_m$3)-β-D-Glc*p*	Antioxidant	[22]
5	GLP-1	D-Glc, D-Gal, and L-Fuc	1.07×10^5	Mainly composed of →6)-β-D-Glcp-(1→, →3)-β-DGlcp-(1→, with →6)-α-D-Galp-(1→ and few L-Fucp-(1→ residues	Protecting the spleen and thymus, promoting hematopoiesis and improving Immunoglobulin A levels in serum	[23]
6	GLP-2	D-Glc	1.95×10^4	→6)-β-D-Glcp-(1→, with →3)-β-D-Glcp-(1→ residues	Protecting the spleen and thymus, promoting hematopoiesis and improving Immunoglobulin A levels in serum	[23]
7	PGL	D-Glc	1.26×10^5	β-D-Glc*p* (1→3) branch; β-D-Glc*p* (1→4) and β-D-Glc*p* (1→3) branches; β-D-Glc*p* (1→4) and β-D-Glc*p* (1→3) and β-D-Glc*p* (1→3) branches on →6)-β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→	Unknown	[24]
8	SP	D-Glc	1.00×10^4	R (1→6) branches on →3)-β-D-Glc*p*-(1→[3)-β-D-Glc*p*-(1→]$_m$3)-β-D-Glc*p*-(1→[3)-β-D-Glc*p*-(1→]$_m$; R= β-D-Glc*p*-(1→; R= β-D-Glc*p*-(1→[3)-β-D-Glc*p*-(1→]$_n$6)-β-D-Glc*p*-(1→; R= β-D-Glc*p*-(1→[3)-β-D-Glc*p*-(1→]$_n$6)-β-D-Glc*p*-(1→ [3)-β-D-Glc*p*-(1→]$_n$; n= 0, 1 or 2 2(n+n′)+m+m′=7	Stimulate proliferation of mouse spleen lymphocytes *in vitro*	[25]

(continued on next page)

Table 1 (*continued*)

No	Name of GLPs	Monosaccharide composition	Molecule weight (Da)	Structure unit	Bioactivities	References
9	GLSWA-I	D-Glc	1.57×10^5	β-D-Glc*p* 1 ↓ 4 β-D-Glc*p* β-D-Glc*p* β-D-Glc*p* 1 1 1 ↓ ↓ ↓ 6 6 4 →[3)-β-D-Glc*p*-(1]$_4$→3)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→[6)-β-D-Glc*p*-(1→]$_3$	Promote DNCB induced delayed-type ear swelling in mice	[26]
10	GLP-2	D-Gal, D-Man, D-Glc, L-Ara and L-Rha	2.20×10^4	→6)-α-D-Man*p*-1→ →4)-α-D-Glc*p*-1→ L-Ara L-Rha 1 1 1 1 ↓ ↓ ↓ ↓ 6 6 6 6 …→4)-α-D-Gal*p*-(1→…→4)-α-D-Gal*p*-(1→…→4)-α-D-Gal*p*-(1→…→4)-α-D-Gal*p*-(1→…	Stimulate lymphocyte proliferation (T and B cells) and the production of antibodies against sheep red blood cells (SRBC) in mice	[27]
11	GLPCW-II	D-Glc, L-Fuc and D-Gal	1.20×10^4	α-L-Fuc*p* 1 ↓ 6 →6)-α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→6)-α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→	Unknown	[28]
12	LZ-D-4	L-Fuc, D-Ara, D-Gal, D-Glc, D-Xyl, D-Man, D-GalA and D-GlcA (1: 0.24: 5.70: 16.01: 0.58: 6.29: 1.71: 6.67)	1.56×10^4	Unknown	Inhibition to the proliferation of L1210 cell lines *in vitro*	[29]
13	LZ-D-9	L-Fuc, D-Gal, D-Glc, D-Man, D-GalA and D-GlcA (1: 7.09: 28.58: 8.96: 1.24: 5.22)	1.30×10^4	Unknown	Inhibition to the proliferation of L1210 cell lines *in vitro*	[29]
14	GLP-F1-1	D-Glc and D-GalA (34:1)	2.50×10^6	Backbone chain: →4)-D-β-Glc*p*-(1→ and →4,6)-β-D-Glc*p*-(1→ Branched chains: →6)-β-D-Glc*p*-(1→ and →4)-β-D-Gal*p*-(1→	Unknown	[15]
15	GLP_{UAE}	D-Man, L-Rha, D-Glc, D-Gal and L-Ara (2.58: 1.25: 11.17: 2.5: 1)	4.66×10^5	Unknown	Scavenging the DPPH and hydroxyl radicals, reducing power and cellular protective effect on yeast cells from UV damage	[30]
16	GLP_{HWE}	D-Man, L-Rha, D-Glc, D-Gal and D-Ara (3.11: 1.11: 19.44: 2.33: 1)	7.03×10^5	Unknown	Scavenging the DPPH and hydroxyl radicals, reducing power and cellular protective effect on yeast cells from UV damage	[30]
17	GLP_L1	D-Glc	5.20×10^3	→3)-D-Glc*p*-(1→, →4)-D-Glc*p*-(1→, →6)-D-Glc*p*-(1→, →3,6)-D-Glc*p*-(1→ and →4,6)-D-Glc*p*-(1→ (21.9: 20.3: 23.7: 24.0: 3.7)	Antioxidant	[31]
18	GLP_L2	D-Glc, D-Gal and D-Man (29: 1.8: 1.0)	1.54×10^4	→3)-D-Glc*p*-(1→, →4)-D-Glc*p*-(1→, →6)-D-Glc*p*-(1→, →3,6)-D-Glc*p*-(1→ and →4,6)-D-Glc*p*-(1→ (23.0: 34.6: 7.0: 14.1: 3.0) A few →6)-D-Gal*p*-(1→	Antioxidant	[31]
19	*G. lucidum* glucans	D-Glc	Unknown	β-D-Glc*p* 1 ↓ 6 β-D-Glc*p*-(1→{3)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→[3)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1]$_n$→}$_m$3)-β-D-Glc*p*	Unknown	[32]
20	GLP20	D-Glc	3.75×10^6	β-D-Glc*p* 1 ↓ 6 3)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→	Stimulating macrophage cells	[33]
21	GLSA50-1B	D-Glc	1.03×10^5	β-D-Glc*p* 1 ↓ 4 [β-D-Glc*p*]$_n$ 1 ↓ 4 → 6)-β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→ n=0-7	Unknown	[34]

Notes: Man (mannose), Rha (rhamnose), GalA (galacturonic acid), GlcA (glucuronic acid), Glc (glucose), Gal (galactose), Fuc (fucose), Ara (arabinose), Xyl (xylose).

through β-elimination. Following β-elimination the GLPs bioactivities of protein tyrosine phosphatase 1B (PTP1B) inhibition and antihyperglycemic potency decreases, *i.e.*, protein branching enhances these GLPs' bioactivities [45]. Zeng et al. [1] used three typical deproteinization methods (neutral protease method, TCA precipitation, and $CaCl_2$ salting out) to remove ~87% of the proteins with a loss of ~11% polysaccharide and found that the M_W and major functional groups (antioxidant activities) remained unchanged after deproteinization.

3.3. Molecular weight and bioactivities

Generally, the larger the M_W of GLPs, the higher will be its bioactivity. For example, if two different extraction methods (ultrasound-assisted extraction and hot water extraction) are used to obtain GLPs having different M_W, the larger GLPs has higher activity of scavenging DPPH and hydroxyl radicals, better-reducing power, and better ability to protect yeast cells from UV damage [30]. Ultrasonic waves degrade high M_W GLPs enhancing their antioxidant activities [46]. Sui et al. [47] first reported that the submerge-cultured intracellular GLPs (GLPs-In) had a high M_W fruiting body GLPs (GLPs-Fr) (>10^6 Da). In contrast, a submerge-cultured extracellular GLP (GLP-Ex) had a low M_W (~10^3 Da). These results indicate that GLPs with a high M_W had enhanced bioactivity for the stimulation of macrophage RAW264.7 cell viability and NO release level, and inhibitory activity towards HepG2 cells.

Some reports hold the opposite view. For example, Xu et al. [48] first reported that the hypolipidemic and antioxidant activities of GLPs improved with reduced M_W after ultrasonic degradation, despite its primary structure remaining unchanged. Other researchers [49] used dialysis to produce two crude polysaccharide fractions (M_W <1.2×10^4 Da and >1.2×10^4 Da), and found that the production of cytokines by splenocytes and macrophages for the low M_W fraction was much higher than for the high M_W fraction.

3.4. Monosaccharide units and bioactivities

In general, the monosaccharide composition of GLPs is relatively constant. And GLPs mostly consist of D-glucose, D-fructose, D-galactose, D-mannose, D-xylose, L-fucose, L-rhamnose and L-arabinose. Moreover, the difference in monosaccharide composition between fruiting bodies, mycelia, and spores are mostly reflected in their compositional ratio.

Different monosaccharide contents can exhibit different bioactivities. The monosaccharide contents of exopolysaccharides (EPS) influence their anti-inflammatory activity towards macrophages, with high galactose > high rhamnose > high glucosamine [50]. Other reports show that the rhamnose content can enhance the antioxidant ability through radical scavenging [51], moreover, GLP-2 has a higher ability to regulate Immunoglobulin G levels with a higher proportion of glucose [23]. The more types of monosaccharides present, the higher the antioxidant and the immunostimulating activities of GLPs [52]. Si et al. [53] used response surface methodology (RSM) and Plackett-Burman design (PBD) to optimize the medium composition for producing *G. lucidum* mycelium exopolysaccharide (GLEPS). They concluded that the GLEPS with higher uronic acid content showed stronger *in vitro* antioxidant activity through reductive capacity, radical scavenging, and chelation of transition metal catalysts. Moreover, polysaccharide containing a main chain of 1→6 linked glucose and 1→4 linked arabinose showed an improved ability to scavenge $DPPH^-$ radicals [40].

Monosaccharide composition is regulated by enzymes expressed during the growth period of *G. lucidum*. Peng et al. [54] concluded that the synthesis of three GLEPS monosaccharides (glucose, galactose, and mannose) were effected by the activities of α-phosphoglucomutase (PGM), phosphoglucose isomerase (PGI) and phosphomannose isomerase (PMI) at the mycelium stage. These enzymes were also impacted by temperature and pH, the optimum temperature and pH were 30 °C and 4.0, respectively. They also showed different monosaccharide compositions (glucose, galactose and mannose contents) in crude GLEPS to study activity on anti-Hepa 1-6 (human hepatoma cell line) and MDA-MB-231 (human breast cancer cell line) tumor cell lines level. The results indicated that the EPS with high galactose and mannose contents had a strong antitumor activity. Overview, these major enzymes impact the major monosaccharides and these monosaccharides influence GLEPS' antitumor activity.

4. The mechanisms of GLPs bioactivities

4.1. Antioxidant mechanism

This bioactivity has been widely studied since the end of the 20th century. One of the mechanisms suggested is the termination of radical chain reactions through the combination of hydrogen and electron from GLPs or between GLPs and radicals. Shi et al. [52] found that the antioxidant mechanism of GLPs was due to the supply of hydrogen from the polysaccharide, which could combine with radicals, and then form a stable radical to end the radical chain reaction. This group also suggested that the radical chain reaction would terminate through the combination of radical ions with the polysaccharide. GLPs can also exert beneficial antioxidant effects by reducing lipid peroxidation (LPO) and malondialdehyde (MDA) levels [55]. Moreover, GLPs can donate electrons to react with free radicals *in vitro*, to stop the radical chain reactions. GLPs can also enhance antioxidant activity by enhancing catalase (CAT) activity, which catalyzes the breakdown of H_2O_2 [51].

It is widely reported that GLPs can prevent glutathione peroxidase (GSH-Px) depletion and LPO, and increase superoxide dismutase (SOD) activity. In addition, GLPs can stimulate the synthesis of enzymes involved in free radical production, establishing a redox cycle to protect cells from oxidative stress [55–58].

4.2. Immunomodulation mechanism

The most accepted immunomodulation mechanisms of GLPs is through the promotion of macrophage proliferation, the activation of T- and B-lymphocytes and natural killer cells (NK), lymphokine-activated killer cells (LAK) and other immune cells, accelerating the proliferation of spleen cells, and the production of cytokines and antibodies.

Macrophages are a key target for controlling non-specific immunity. Some GLPs can activate macrophages proliferation, with a stimulatory effect reaching 171% at a concentration of 20 μg/mL [59]. GLPs can also stimulate macrophages to produce the secondary mediator nitric oxide (NO), which is an important mediator of the nonspecific host defense against invaders, and a quantitative index of macrophage activation [52]. In the research examining nonspecific immunity through carbon clearance test, the immune activity of macrophages and the reticular endothelial cell (REC) in spleen, liver or other organs showed decreased concentration in plasma [60].

The immunomodulatory mechanism of GLPs increases the phagocytic activity of macrophages and the protection of macrophages viability. GLPs (from mycelia) were cultured on a special solid medium, soybean curd residue (SCR) [60]. Phagocytic activity, at a concentration of 1.25 μg/mL, was greater than the positive control (LPS, 1 μg/mL). Moreover, it also increased the survival level of the macrophages from 58% (negative control, 5.00 μg/mL doxorubicin) to 97% (40.00 μg/mL GLPs).

Thus, an immunomodulation mechanism has become the premise of other bioactivities, including anti-diabetes, antitumor, anticancer and anti-inflammatory activities.

4.3. Hypoglycemic mechanism

Controlling hypoglycernia is becoming a hotspot of Chinese herbal medicine research on anti-diabetes polysaccharides. There are four categories of anti-diabetes GLPs [61]: 1. improving β-cell dysfunction and enhancing insulin action; 2. improving glucose metabolism; 3. regulating the MAPK pathway; and 4. delaying the progression of diabetic

complications. The main hypoglycemic mechanism of GLPs is detailed below [62]. One involves the promoting of insulin secretion and the repair of β-islet cells by regulating hormone levels and the second involves regulating the activities of the enzymes that control the glucose metabolism.

Another hypoglycemic mechanism is treating insulin injury. GLPs could decrease the concentration of IL-1β, IL-6, and TNF-α in plasma [63]. These proinflammatory cytokines are secreted due to the development of inflammation-induced adipocytes and macrophages, and result in lipolysis, lipid transport and exacerbate lipotoxicity, damaging insulin signaling in peripheral tissues through endocrine effects. GLPs reduce insulin concentrations and insulin-regulated lipogenesis in the liver. GLPs can also prevent the development type 2 diabetic mellitus (T2DM), nonalcoholic fatty liver disease (NAFLD), and cardiovascular disease (CAD).

Liu et al. [64] used inulin and GLPs to cure T2DM rats. This combination drug not only significantly ameliorated hyperinsulinemia but also reduced the homeostasis model assessment-insulin resistance (HOMA-IR) values. GLPs also enhanced glycogenesis and improved lipid metabolism to some extent, increasing the expressions of glycogen synthase (GS) and glucose transporter 4 (GLUT4) in the liver and skeletal muscles. In addition, the expression of p85 protein and high-density lipoprotein cholesterol (HDL-C) levels increased, and the expression of glycogen synthase kinase 3β (GSK-3β), total cholesterol (TC), total triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) levels were decreased. Researchers also studied the key genes and proteins of the PI3K/Akt pathway, and found that these were significantly enhanced and the phosphorylation of protein kinase B (Akt) was promoted after treatment. However, this drug did not promote islet cells to secrete insulin.

One final mechanism involves an influence on the MAPK pathway [65]. GLPs activate AMP-activated protein kinase (AMPK) to reduce the mRNA levels of glucose regulatory enzymes and inhibit hepatic glucose production for decreasing fasting serum glucose (FSG) levels, increase insulin resistance by enhancing GLUT4 expression and downregulating resistance of expression, and reduce epididymal fat/body weight (BW) ratios by decreased mRNA levels of lipogenic genes fatty acidy synthase (FAS), acetyl-CoA carboxylase 1 (ACC1).

4.4. Antitumor mechanism

The enhancement of the host's immune system is a main antitumor mechanism of GLPs, rather than direct cytotoxicity towards tumor cells. Just like their immunomodulating mechanism, GLPs activate macrophages, NKs, neutrophils, T-lymphocytes, B-lymphocytes and cytotoxic T-lymphocytes (CTL), and promote the expression of cytokines, such as TNF-α, IFN-c, and IL-1β. Some common cancers which GLPs can target and their main antitumor mechanisms are shown in Fig. 1 [66].

The antitumor mechanisms of macrophages have been described by De et al. [67], Feng et al. [68] and Chan et al. [69] speculated that β-glucans, like GLPs, are first absorbed in proximal small intestine, then decompose into micromolecular fragments (may be monosaccharides or oligosaccharides) and are carried to the bone marrow and endothelial reticular system, to finally be captured by macrophages. These micromolecular fragments are then released by the activated macrophages and received by circulating granulocytes, monocytes and dendritic cells (DCs). In summary, the innate immune response starts with the help of GLPs. For instance, NKs secrete chemical substances that destroy tumor cells by destroying cell membranes, and neutrophils effectively burst targeted cells through cell-mediated phagocytosis. In addition, immunological memory is triggered, and T-lymphocytes, B-lymphocytes and CTL begin to work.

Another antitumor mechanism of GLPs involves restricting the proliferation and the induction of apoptosis of tumor cells. In addition, GLPs also reduce the expression of integrin to inhibit tumor cell adhesion. GLPs have the ability to reactivate mutant p53 in colorectal cancer cells, and p53 is an effective tumor suppressor that can stem the reproduction of cells containing oncogenic lesions through many pathways (growth inhibition, apoptosis or senescence, tumor stroma modulation, angiogenesis and metabolism and interrupt the invasion) [70]. B-cell lymphoma 2 (Bcl-2) family, death associated protein kinase 1 (DAPK1) and caspase-3 also play important roles in the process of tumor inhibition [66]. By directly adding GLPs into tumor cell medium, proliferation is restricted and the induction of apoptosis of S-180 and HL-60 tumor cells is restricted. Therapy with the serum from GLPs treated mice, or the splenocyte and peritoneal macrophages treated with GLPs are both effective. Thus, this mechanism of GLPs clearly involves other factors.

Actually, anti-angiogenesis belongs to part of anti-proliferation and pro-apoptotic effects [71]. There are two kinds of angiogenesis inhibitors, direct and indirect [72]. One prevents vascular endothelial cells from proliferating and migrating, while the other prevents the expression of, or blocks the activity of, a tumor protein that activates angiogenesis, or blocks the expression of its receptor on endothelial cells. GLPs suppress the development of human umbilical cord vascular endothelial cells (HUVEC) by increasing the expression of Bax and decreasing the expression of Bcl-2, which will result in apoptosis. GLPs can adjust MAPK and Protein Kinase B signaling to inhibit cancer angiogenesis. Previous research had found that GLPs prevent the release of angiogenic factors (VEGF and TGF-β 1) to inhibit the morphogenesis of capillary around tumor cells [73,74].

One last mechanism is cell cycle arrest modulation. Generally, GLPs can induce cell cycle arrest in the G_1-phase by restricting the ERK1/2 or ERK5 pathway, and stay at the G_2/M phase. Hence, the growth of tumor cells is in stasis. Moreover, eukaryotic cell cycle is regulated by cyclins and cyclin-dependent kinases (CDKs), and CDK1 is an important regulator bound to cyclin B. GLPs can also increase tyrosine15 (Y15) phosphorylation through deactivating CDK1, which might be a major mechanism inducing a stasis of cells in the G_2/M cell cycle phase [66].

4.5. Protection of the intestinal mucosal barrier mechanism

GLPs are also widely used to regulate intestinal flora. GLPs on oral administration have the ability to regulate the intestinal biological barrier functions by increasing microbiota richness, decreasing the *Firmicutes*-to-*Bacteroidetes* ratio, and inducing the changes of the certain intestinal bacteria (*S24-7*, *SMB53*, *Rikenellaceae*, *Allobaculum*, *Rc4-4*, and *Ruminococcaceae*) [75]. GLPs significantly increase the relative abundance of *Firmicutes* and decrease that of *Bacteroidetes* at the phylum level. GLPs greatly increase the relative abundance of *Lachnospiraceae*, *Roseburia*, and *Lactobacillus*, revealing a potential therapy mechanism for treating chronic pancreatitis by modulating the intestinal microbiota [76]. GLPs also stimulate peritoneal macrophages (PMs) to prevent indomethacin-induced small intestinal injury through granulocyte-macrophage colony-stimulating factor (GM-CSF), eliciting anti-inflammatory effect [77].

4.6. Hypolipidaemic and lipid antioxidant mechanism

A rats study showed that given twice daily with average 0.1 mg/g crude GLPs could contribute to hypolipidaemic and lipid antioxidant. The effects was through decreasing the body weight gain (WG), food efficiency ratio (FER), levels of plasma triacylglycerol (TG), plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), liver weight, malondialdehyde (MDA) values, increasing the level of faecal fat, cholesterol and high-density lipoprotein cholesterol (HDL-C) and serum SOD and glutathione peroxidase (GPx) activities [78]. Another research showed that GLPs were good for attenuating antioxidant status in diabetic pancreas [79].

4.7. Anti-inflammatory mechanism

Another rats study showed that given daily with 0.5125–2.0500 mg/g crude GLPs could calmed the inflammation element expression,

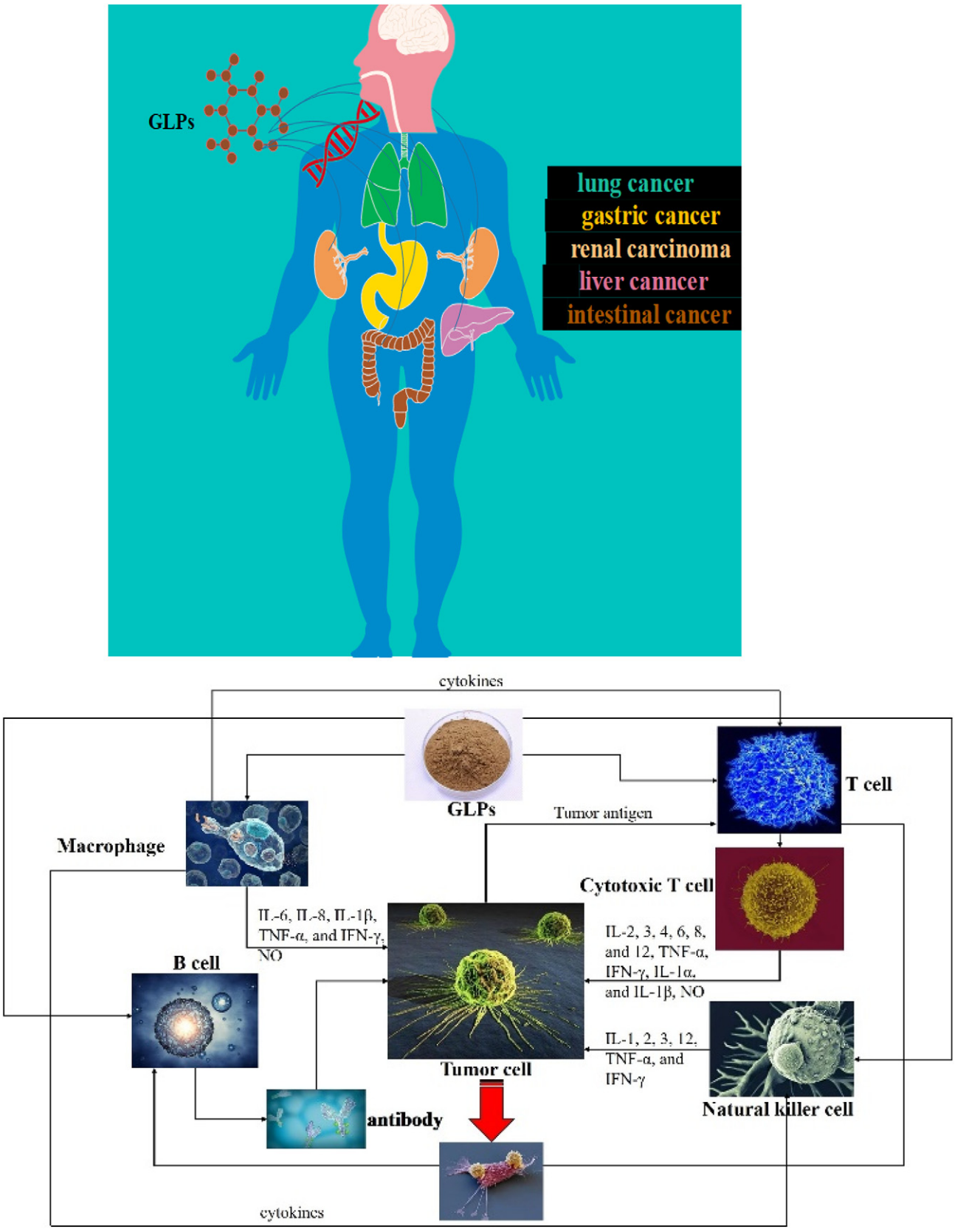

Fig. 1. The GLPs antitumor activities to common tumors (top) and the general antitumor mechanism of GLPs (bottom).

including NO synthase (iNOS), cyclooxygenase 2 (COX2), TNF-α, nuclear factor-κB (NF-κB) and IL-6 [7]. A research found a favorable potential anti-inflammatory agent, a sulfated polysaccharide from *G. lucidum* (GLPss58). It could inhibit not only the L-selectin-mediated inflammation, but also the complement system- and cytokines mediated-inflammation [6].

5. GLPs in combination with other compounds

GLPs can also be used as an ancillary drug in treating cancer. GLPs are usually combined with inulin [64], 5-fluorouracil (5FU) [70,80] and doxorubicin (DOX) [81]. GLPs not only enhanced the toxic or hypoglycemic effects of these drugs but also reduce their toxicity and protect non-malignant cells from the accumulation of reactive oxygen species (ROS).

GLPs can be combined with selenium nanoparticles and equipped with the ability to inhibit inflammation induced by LPS [82]. In other research GLPs have been formulated into gold nanocomposites for cancer treatment through DCs induced T cell response [83]. Liu et al. [84] used soybean phosphatide, tween 80 and cholesterol to encapsulate GLPs in liposomes, resulting in improved stimulation of splenic lymphocyte proliferation. These researchers also encapsulated GLPs and ovalbumin (OVA) in liposomes [85] and this GLPL/OVA enhanced humoral and cellular immune response through activating and maturing of DCs in draining lymph nodes. In some studies, GLPs have been loaded into sodium alginate (NaAlg) through an electrospray (ES) process and these micro-particles could protect GLPs from oxidative degradation during storage [86]. More complicated Yolk—shell particles (YSPs) have been fabricated by tri-needle coaxial electrospraying and used for wound healing. GLPs were encapsulated into the outer shell of the YSPs as the major antioxidant components [87]. A combination of GLPs and triterpenoids from *G. lucidum* afforded a multi-compound drug with cytotoxicity and immunomodulatory activities, enhancing the release of cytokines and activating the immune cells [88].

6. Conclusions

There are diverse extraction methods used to prepare GLPs during different growth periods (spore powers, mycelium and fruiting body), which afford diverse GLPs with various structural features and bioactivities. In this review, the relationships between structural features and bioactivities have been summarized. In addition, several bioactivity mechanisms have been discussed in detail, involving antioxidant, immunomodulation, hypoglycemic, antitumor, hypolipidaemic and lipid antioxidant, and anti-inflammatory activities. New types of drugs containing GLPs are being invented, for example, nanocomposites and liposomes containing anticancer drugs with improved performance. In conclusion, we demonstrate that the investigation of GLPs is a very active field warranting further investigation.

7. Future perspectives

Antitumor is still a wide applied filed for GLPs, because of its nontoxicity. Now, tumor-targeted drug delivery and the development of antitumor drug targeting carrier system are research hotspots. GLPs have natural advantage of hydrophilicity during absorbtion when used as a part of multiple compound antitumor drug. Moreover, modified polysaccharides of *G. lucidum* will have better anti-tumor activity and tumor targeting.

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