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专论与综述

灰树花活性多糖构效关系研究进展

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摘　要：灰树花是一种珍贵的食药用菌，具有降血糖、抗肿瘤、免疫调节和抗病毒等多种生物活性。灰树花多糖是其主要的活性成分，多糖的生物活性与多糖的结构密切相关。本文综述了从20世纪80年代起国内外已报道的灰树花活性多糖的结构表征。部分研究认为多糖的降血糖活性可能与β-1,6-葡聚糖主链化学结构相关，而灰树花多糖结构为β-1,6主链或β-1,3主链葡聚糖时具有较好的抗肿瘤活性。然而多糖结构异常复杂，精细结构的解析困难，导致目前灰树花多糖结构表征一般止步于单糖组成、分子量、糖苷键类型、分支结构和粗略分子链构象，但二维核磁(two dimensional nuclear magnetic resonance，2D-NMR)和高分辨质谱联用等技术的发展将有助于解开灰树花多糖构效关系，并为灰树花活性多糖的开发利用提供理论依据。

关键词：灰树花；多糖；降血糖；抗肿瘤；结构表征；构效关系

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Advances in structure-activity relationship of polysaccharides from *Grifola frondosa*

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Abstract: *Grifola frondosa* is a precious edible and medicinal fungus with anti-tumor, immunomodulatory, hypoglycemic, and antiviral activities. Polysaccharides are the main active component of *G. frondosa*, with the biological activity associated with the structure. We reviewed the structural characterization of active polysaccharides from *G. frondosa*. Some studies suggest that the blood sugar-lowering activity might be related to the chemical structure with β-1,6-glucan as the main chain, and the polysaccharides have better antitumor activity when the main chain is β-1,6-glucan or β-1,3-glucan. However, the complex structure makes it difficult to dissect the fine structure of *G. frondosa* polysaccharides. As a result, the structural characterization of *G. frondosa* polysaccharides is generally limited to monosaccharide composition, molecular weight, glycosidic bond type, branching structure, and rough molecular chain conformation. The advancing 2D-NMR and high-resolution mass spectrometry will help to disclose the structure-activity relationship and provide a theoretical basis for the development and utilization of *G. frondosa* polysaccharides.

Keywords: *Grifola frondosa*; polysaccharides; hypoglycemic; anti-tumor; structural characterization; structure-activity relationship

灰树花(*Grifola frondosa*)隶属于担子菌门层菌纲非褶菌目多孔菌科树花菌属[1]，是一种珍贵的食药兼用蕈菌[2]。现代医学表明，灰树花多糖类物质(多糖、糖蛋白、蛋白多糖)具有显著的生理活性[3]，如抗肿瘤[4]、调节机体免疫力[5]、抗氧化[6]、降血糖降脂[7]、抗病毒[8]和抗辐射[9]等。

虽然多糖类活性物质的功能显著，但天然多糖本身分子量大且一级结构及空间结构复杂，尤其是天然多糖多和蛋白质以非共价键的形式结合在一起，使得多糖的结构解析异常困难，导致活性多糖的结构和活性关系不明晰。多糖的生物活性与其自身结构密切相关，多糖的结构在一定程度上决定了其生物活性。多糖的分子量、单糖组成[10]、糖苷键连接方式[4]、分支度、支链类型及位置和构象[11]等都会影响多糖的生物活性。活性多糖构效关系的不明晰严重制约其活性多糖的产业化进程。

从 20 世纪 80 年代开始国内外就开始了对灰树花多糖的探究。本文归纳了灰树花降血糖、抗肿瘤、免疫调节、抗病毒等活性多糖的一级结构及其作用机制，以期为进一步解开灰树花活性多糖构效关系提供参考。

1 灰树花降血糖活性多糖一级结构表征及其作用机制研究

降血糖灰树花多糖的分子质量、结构或组成见表 1。

表 1　降血糖灰树花多糖的分子质量、结构或组成

Table 1　Molecular weight, structure or composition of *G. frondosa* polysaccharides with hypoglycemic activities

名称 Name	提取部位 Extractive fraction	分子量 Mw (kDa)	糖:蛋白 Polysaccharides: proteins	单糖组成 Monosaccharide composition	结构 Structure or construction	实验动物模型 Experimental animal model	作用机制 Mechanism of action	血糖降低率 Hypoglycemic rate (%)	参考文献 References
X-组分 X-fraction	子实体 Fruiting bodies	200	65:35	不详 Uncertain	α-1,4 分支的 β-1-6-葡聚糖 β-1,6 main chain with α-1,4 branches	雌性自发高血压小鼠 (KK-A^{y}) female spontaneously hypertensive mice	不详 Uncertain	50.0	[12-13]
SX-组分 SX-fraction	子实体 Fruiting bodies	20	10:90−25:75	不详 Uncertain	不详 Uncertain	自发高血压大鼠 Spontaneously hypertensive rat	改善糖耐量，对外源性胰岛素的敏感性增强，改善胰岛素敏感性 Improvement of glucose tolerance and the sensitivity to exogenous insulin, and enhancement of insulin sensitivity	11.9	[14-15]
MT-α-glucan	子实体 Fruiting bodies	40−45	不详 Uncertain	葡萄糖 Glc	α-D-葡聚糖 α-D-glucan	雌性自发高血压小鼠 (KK-A^{y}) female spontaneously hypertensive mice	增加胰岛素敏感性，改善外周靶组织的胰岛素抵抗 Increasement of insulin sensitivity and amelioration of insulin resistance in peripheral tar-get tissues	22.0	[16-17]
GFP	子实体 Fruiting bodies	45.3	不详 Uncertain	葡萄糖:鼠李糖:阿拉伯糖:木糖:甘露糖:半乳糖=31.29:24.74:5:1:3.42:6.89 Glc:Rha:Ara:Xyl:Man:Gal=31.29:24.74:5:1:3.42:6.89	β-1,4 和 β-1-6-葡聚糖 β-1,6 main chain with α-1,4 branches	体外/人 HepG2 细胞 *In vitro*/Human liver cancer cells HepG2	通过 Akt/GSK-3 通路增加葡萄糖代谢，刺激细胞内糖原合成 Increasement of glucose metabolism of and stimulation of intracellular glycogen synthesis through the Akt/GSK-3 pathway	*	[18]
GFP	子实体 Fruiting bodies	18.18−15 850	不详 Uncertain	葡萄糖:甘露糖:半乳糖:鼠李糖:葡萄糖醛酸:半乳糖醛酸:岩藻糖=27.59:25.49:15.02:5.18:9.49:7.30:9.92 Glc:Man:Gal:Rha:GlcUA:GalUA:Fuc=27.59:25.49:15.02:5.18:9.49:7.30:9.92	不详 Uncertain	STZ 和 HFD 联合诱导的糖尿病小鼠 Diabetic mice induced by STZ and HFD	通过改变肠道菌群和调节肝脏糖脂代谢相关基因 Though modulation of gut microbiota and regulation of hepatic glycolipid metabolism related genes	*	[19]

(待续)

(续表 1)

GFWE	子实体 Fruiting bodies	不详	不详 Uncertain	半乳糖:鼠李糖:甘露糖:葡萄糖:岩藻糖=22.66:12.10:11.88:5.81:4.51 Gal:Rha:Man:Glc:Fuc=22.66:12.10:11.88:5.81:4.51	不详 Uncertain	STZ 和 HFD 联合诱导的糖尿病 Wistar 大鼠 Diabetic Wistar rats induced by STZ and HFD	上调 AMPK-α、PPAR-α 和 GK 的相对 mRNA 和蛋白表达水平，下调 SREBP-1c 和 ACC，提高盲肠总胆汁酸和短链脂肪酸水平 Up-regulation of the relative mRNA and protein expression levels of adenine monophosphate activated protein kinase-α, peroxisome proliferator-activated receptors-α, and glucokinase, while down-regulation of sterol regulatory element-binding transcription factor-1c and acetyl CoA carboxylase; enhancement of the levels of total bile acids and short-chain fatty acids in the cecum	*	[20]
GFP-N	子实体 Fruiting bodies	12 600	不详 Uncertain	葡萄糖:阿拉伯糖:甘露糖=49.70:3.79:1.00 Glc:Ara:Man=49.70:3.79:1.00	α-D-甘露糖残基、α-L-阿拉伯糖残基、β-D-葡萄糖残基 α-D-Manp, α-L-Araf and β-D-Glcp	STZ 和 HFD 联合诱导的糖尿病 ICR 小鼠 Diabetic ICR mice induced by STZ and HFD	激活 IRS1、PI3K 和 GLUT4，抑制 JNK，改善口服糖耐量，减轻胰岛素抵抗，保护免受肝肾损伤，减轻炎症反应并且调节 T2DM 小鼠肠道菌群 Activation of insulin receptor substrate 1, phosphatidylinositol-3-kinase, and glucose31 transporter 4 and inhibition of c-Jun N-terminal kinase 1/2 and improvement of oral glucose tolerance, alleviation of insulin resistance, and protection against liver and kidney injury with reduced inflammation and modulation of gut microbiota in diabetic mice	*	[7]
GFP-W	子实体 Fruiting bodies	66.1	不详 Uncertain	木糖:甘露糖:岩藻糖:葡萄糖:半乳糖=1.37:1.36:1.22:1.10:1.00 Xyl:Man:Fuc:Glc:Gal=1.37:1.36:1.22:1.10:1.00	β-D-葡萄糖、α-1,2,6-半乳糖、α-2-甘露糖、α-3-L-岩藻糖 4 种连接单元 Four kinds of linkage type units as β-D-	人 HepG2 细胞 Human liver cancer cells HepG2	改善 IRS1、PI3K 的 mRNA 和蛋白表达，上调 GLUT4、下调 JNK1，显著增加地塞米松诱导的胰岛素抵抗 HepG2 细胞对葡萄糖的摄取 Increasement of the uptake of glucose in dexamethasone induced insulin resistant HepG2 cells by improving the mRNA and protein expression of insulin receptor	*	[21]

(待续)

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(续表 1)

				Glcp-(1→, →2, 6)-α-Galp-(1→, →2)-α-Manp-(1→, and→3)-α-L-Fucp-(1→		substrate 1, phosphatidylino-sitol-3-kinase, and upregulation of glucose transporter 4 and downregulation of c-Jun N-terminal kinase 1		
F2	子实体 Fruiting bodies	452	62.5:37.5	葡萄糖:甘露糖:半乳糖:木糖:阿拉伯糖:鼠李糖:核糖=26.74:22.79:16.76:16.02:14.29:2.05:1.35 Glc:Man:Gal:Xyl:Ara:Rha:Rib=26.74:22.79:16.76:16.02:14.29:2.05:1.35	含 β 糖苷键相连的吡喃环 Pyran rings linked by β glycosidic bonds	STZ 和 HFD 联合诱导的糖尿病 SD 大鼠 STZ and HFD induced diabetes in SD rats	上调 IR (Try1361)蛋白磷酸化水平和下调 IRS1 (Ser307)蛋白磷酸化水平，改善胰岛素抵抗 Improvement of insulin sensitivity as a result of the increased protein levels of phospho-IR (Try 1361) and decreased levels of phospho-IRS-1 (Ser307)	*	[22-23]
F3			78.3:21.7	核糖:阿拉伯糖:木糖=74.73:14.20:11.08 Rib:Ara:Xyl=74.73:14.20:11.08				
GF5000	子实体 Fruiting bodies	5	38.2:37.8	葡萄糖:阿拉伯糖:甘露糖:核糖:鼠李糖:木糖:半乳糖=47.06:30.05:18.89:1.93:1.65:0.26:0.16 Glc:Ara:Man:Rib:Rha:Xyl:Gal=47.06:30.05:18.89:1.93:1.65:0.26:0.16	不详 Uncertain	ALX 和 HFD 联合诱导的糖尿病雄性 SD 大鼠 Diabetic male SD rats induced by ALX and HFD	通过调节肠道菌群的组成来抑制 TLR4/MyD88/NF-κB 途径进而缓解炎症水平，改善胰岛素抵抗 Improvement of insulin resistance is relation of modulating composition of gut microbiota, which may help to alleviate inflammation by inhibition of TLR4/MyD88/NF-κB pathway	*	[24]
GFP-2	菌丝体 Mycelia	45.3	不含蛋白、核酸 Contains no protein or nucleic acid	不详 Uncertain	含 β 型糖苷键和吡喃糖环 Contain β glycosidic bonds and pyranose ring	人 HepG2 细胞 Human liver cancer cells HepG2	提高糖原代谢能力，活化胰岛素抵抗信号传导途径，促进机体糖代谢能力 Improvement of glycogen metabolism, activation of insulin resistance signal transduction pathway, and promotion of glucose metabolism	*	[25]

注：*：文献中未标明具体数值，但与糖尿病模型组相比具有统计学差异；STZ：链脲佐菌素；HFD：高脂饮食；ALX：四氧嘧啶；JNK：c-Jun 氨基末端激酶；PI3K：磷脂酰肌醇-3-激酶；GLUT4：葡萄糖转运体 4；AMPK-α：腺嘌呤单磷酸激活蛋白激酶-α；PPAR-α：过氧化物酶体增殖物激活受体-α；GK：葡萄糖激酶；SREBP-1c：甾醇调控元素结合转录因子-1c；ACC：乙酰辅酶 A 羧化酶

Note: *: Specific values are not indicated, but there are statistical differences compared with the diabetes model group; STZ: Streptozocin; HFD: High fat diet; ALX: Alloxan; JNK: c-Jun N-terminal kinase; PI3K: Phosphatidylin-ositol-3-kinase; GLUT4: Glucose transporters 4; AMPK-α: Adenine monophosphate activated protein kinase-α; PPAR-α: Peroxisome proliferator-activated receptors-α; GK: Glucokinase; SREBP-1c: Sterol regulatory element-binding transcription factor-1c; ACC: Acetyl CoA carboxylase.

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1.1 灰树花降血糖活性多糖的单糖组成及其分子量

灰树花降血糖多糖既可以来源于子实体，也可以从发酵的菌丝体(孢内)或发酵液(孢外)中获取，但绝大多数是从灰树花子实体中分离提取获得。除了 MT-α-glucan[16-17]外，灰树花降血糖活性多糖均为杂多糖，其单糖组成多为葡萄糖、甘露糖、葡萄糖、半乳糖、阿拉伯糖和木糖。几乎所有的降血糖活性多糖都含有葡萄糖，仅有本团队从灰树花子实体获得的活性多糖 F3 的单糖组成为核糖、阿拉伯糖和木糖[22-23]。

灰树花活性多糖的降血糖活性与其分子量也有一定的关系。研究表明，多糖分子量较低则无法形成多糖产生活性的聚合结构，但分子质量太大则不利于多糖穿过多重细胞膜进入生物体内而发挥生物学活性[26]。一般而言，把较高分子量的多糖降解为较低的分子量能显著提高其生物活性[27]。然而从表 1 可以看出，灰树花降血糖活性多糖的分子量范围跨度较大，从 20 kDa 的灰树花 SX-组分[14]到 12 600 kDa 的 GFP-N[7]，都表现出较好的降血糖活性。

1.2 灰树花降血糖活性多糖的糖苷键连接方式

多糖通常是由 10 个以上单糖通过糖苷键连接而成的生物大分子物质，糖苷键的连接方式对多糖的生物活性有重要的影响[28]。1994 年，Kubo 等[12]水提醇沉得到的灰树花子实体糖蛋白聚合物 X-组分(糖:蛋白=65:35)，其结构为具有 α-1,4 支链的 β-1,6-葡聚糖主链，对自发性糖尿病 KK-A^y 小鼠口服给药，具有显著的降血糖活性，推测其高活性可能与 β-1,6-葡聚糖主链化学结构相关。2014 年 Ma 等[18]提取得到的灰树花子实体多糖 GFP (分子量为 45.3 kDa)主链结构主要由 β-1,4 和 β-1,6-葡聚糖组成。

1.3 灰树花降血糖活性多糖的作用机制

1.3.1 调节胰岛素信号转导通路，增强胰岛素受体(insulin receptor，IR)和胰岛素受体底物 1 (insulin receptor substrate 1，IRS1)的表达

IR 酪氨酸磷酸化水平的降低和 IRS1 丝氨酸磷酸化水平的增加会导致胰岛素信号通路被阻断，引发胰岛素抵抗，进而造成 2 型糖尿病(diabetes mellitus type 2，T2DM)。研究者[22-23]通过阴离子交换层析从灰树花子实体中分离纯化获得降血糖活性多糖 F2 和 F3，发现 F2 和 F3 均可上调 IR (Try1361)蛋白磷酸化水平并下调 IRS1 (Ser307)蛋白磷酸化水平，从而改善胰岛素抵抗，进而降低 T2DM 大鼠的空腹血糖水平。在体外高糖刺激培养的骨骼肌 L6 细胞中，灰树花 SX-组分(分子量为 20 kDa)也可通过增加 IR (y)的磷酸化水平、降低 IRS1 (s)的磷酸化、激活受损的胰岛素信号转导通路、增强胰岛素敏感性，从而增加葡萄糖摄取[14,29]。

灰树花多糖 GFP-N 能通过激活 IRS1、phosphatidylin-ositol-3-kinase (PI3K)和 glucose transporters (GLUT4)等，调节 IRS1/PI3K 和 c-Jun N-terminal kinase (JNK)信号通路，改善肝脏胰岛素抵抗，发挥其降血糖作用[7]。灰树花子实体多糖 GFP 能激活 HepG2 细胞膜 IRS1 信号，上调 Akt (蛋白激酶) (Ser473)的蛋白磷酸化表达水平并抑制糖原合成酶激酶(GSK-3)的表达水平，通过 Akt/GSK-3 途径促进葡萄糖代谢，刺激细胞内糖原合成[18]。灰树花多糖 GFP-W 通过改善 IRS1、PI3K、GLUT4 的 mRNA 和蛋白表达上调，以及 JNK 的 mRNA 和蛋白表达下调来增加葡萄糖摄入，从而改善胰岛素抵抗和血糖升高[21]。

1.3.2 抑制 α-葡萄糖苷酶活性

α-葡萄糖苷酶可分解二糖生成葡萄糖，从而升高血液的血糖值。通过抑制 α-葡萄糖苷酶

可减少葡萄糖生成量，从而抑制血糖升高。

MT-α-葡聚糖和 GF-H 具有明显的降血糖功能，其药效作用发挥与抑制 α-葡萄糖苷酶活性有关[16-17,30-31]。

1.3.3 调节肠道菌群

对链脲佐菌素(streptozocin，STZ)、高脂饮食(high fat diet，HFD)联合诱导的糖尿病小鼠，HFD 诱导的 T2DM 小鼠灌胃给药不同的灰树花多糖 GFP，发现其均可通过调节肠道菌群、调节肝脏糖脂代谢相关基因的 mRNA 表达以及与胆固醇代谢相关基因的表达水平，增强肝内胆汁酸(bile acid，BA)合成和排泄，达到降血糖的效果[19,32]。对 STZ、HFD 联合诱导 T2DM 小鼠灌胃给药灰树花多糖 GFP-N 发现，实验小鼠肠道菌群中 *Bacteroidetes* 丰度升高，而 *Firmicutes* 和 *Proteobacteria* 丰度降低，通过调节菌群丰度和细菌结构从而降低小鼠的血糖水平[7]。

Xiao 等[24]采用超滤法截留灰树花子实体水提物中分子量大于 5 kDa 的大分子组分 GF 5000，通过测定灰树花 GF 5000 干预的 T2DM 大鼠肠道内容物的细菌 16S rRNA 基因的 V3−V4 区间序列并进行生物信息学分析，推测灰树花改善胰岛素抵抗的作用，与其通过改善肠道菌群的组分来抑制 toll 样受体 4/骨髓分化原发性反应 88/活化 B 细胞的核因子-轻链增强子(toll-like receptors，TLR4/myeloid differentiation primary response gene 88，MyD88/nuclearfactorkappa-B，NF-κB)途径中关键因子的表达进而抑制靶器官的炎症水平密切相关。

2 灰树花抗肿瘤活性多糖一级结构表征及其作用机制研究

抗肿瘤灰树花多糖的分子质量、结构或组成见表 2。

2.1 灰树花抗肿瘤活性多糖的单糖组成及其分子量

与灰树花降血糖活性多糖一样，子实体也是灰树花抗肿瘤活性多糖的主要提取部位。抗肿瘤的灰树花活性多糖以杂多糖居多，其单糖组成主要为葡萄糖、半乳糖、甘露糖和木糖等(表 2)。除了灰树花多糖 GFPW (分子量为 15.7 kDa)的单糖组成不含葡萄糖外，其余的抗肿瘤活性多糖均含有葡萄糖[48]。

多糖抗肿瘤活性多糖分子量分布范围尚无定论，灰树花抗肿瘤活性多糖的分子量范围广泛(1.79−12 600 kDa)，而且大部分为较高分子量的多糖。有研究指出，相对分子质量太低的多糖生物活性较低甚至无活性，较高分子量的多糖才能够维持其空间构象，但陈向东等[55]提取获得的灰树花菌丝体多糖 GFP 2 分子量仅为 2.6 kDa，荷瘤小鼠肿瘤生长抑制实验结果表明，其仍具有良好的抗肿瘤活性。

2.2 灰树花抗肿瘤活性多糖的糖苷键连接方式

灰树花多糖抗肿瘤活性如表 2 所示，灰树花多糖的结构为 β-1,6 主链或 β-1,3 主链葡聚糖时具有较好的抗肿瘤活性。灰树花菌丝体多糖 Grifolan (GRN)的结构为具有 β-1,6 分支的 β-1,3-葡聚糖，通过体外对 RAW 264.7 巨噬细胞的抑制实验表明，其能够激活巨噬细胞促进细胞因子的产生，增强免疫系统对肿瘤细胞的免疫应答[54]。1987 年，日本的 Kodama 等从灰树花子实体里获得了一种不溶于酸、可溶于碱的蛋白多糖聚合物 D-组分(蛋白含量约 30%，分子量为 1 200−2 000 kDa)，结构为具有 β-1,3 分支的 β-1,6-葡聚糖[57]，经腹腔注射和口服给药均能对荷瘤小鼠具有很强抗肿瘤作用[38]。

另外，从表 2 还可以看出，多数活性多糖具有支链结构，因此可以推测多糖的支化度对

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表 2　抗肿瘤灰树花多糖的分子质量、结构或组成

Table 2　Molecular weight, structure or composition of *G. frondosa* polysaccharides with anti-tumor activities

名称 Name	提取部位 Extractive fraction	分子量 Mw (kDa)	糖:蛋白 Polysaccharides: proteins	单糖组成 Monosaccharide composition	多糖分子结构 Structure or construction	动物/细胞类型 Animal/Cell type	作用机制 Mechanism of action	抑瘤率 Anti-tumor rate (%)	参考文献 References
Grifolan-7N	子实体 Fruiting bodies	1 200	不详 Uncertain	葡萄糖 Glc	β-1,3-葡聚糖 β-1,3-glucan	雄性 ICR 小鼠/180 肉瘤腹水细胞肿瘤 Male ICR mice/Sarcoma 180 tumor ascites cells	不详 Uncertain	97.00	[33]
AP-组分 AP-component	子实体 Fruiting bodies	不详 Uncertain	不详 Uncertain	不详 Uncertain	带分支的 β-1,3-葡聚糖 β-1,3-glucan branched	雄性 ICR 小鼠/180 肉瘤腹水细胞肿瘤 Male ICR mice/Sarcoma 180 tumor ascites cells	不详 Uncertain	95.00	[34]
AS-组分 AS-component			糖:蛋白质:糖醛酸=0.75:0.05:0.10 Sugar:protein: uronic acid= 0.75:0.05:0.10	不详 Uncertain				59.00	
CF-1	子实体 Fruiting bodies	不详 Uncertain	不详 Uncertain	葡萄糖、木糖和甘露糖 Glc, Xyl and Man	β-1,6 分支的 β-1,3-葡聚糖 β-1,6-branched β-1,3-glucan	雄性 ICR 小鼠/180 肉瘤腹水细胞肿瘤 Male ICR mice/Sarcoma 180 tumor ascites cells	不详 Uncertain	98.80	[35]
GRN-LE	子实体 Fruiting bodies	5	91.0:0.3−84.0:1.8	不详 Uncertain	β-1,6 分支的 β-1,3-葡聚糖 β-1,6-branched β-1,3-glucan	雄性 ICR 小鼠/180 肉瘤腹水细胞肿瘤 Male ICR mice/Sarcoma 180 tumor ascites cells	不详 Uncertain	99.00	[36-37]
D-组分 D-component	子实体 Fruiting bodies	1 200−2 000	70:30	半乳糖、甘露糖、葡萄糖和 N-乙酰氨基葡萄糖和岩藻糖 Gal, Man, Glc and N-acetylglucosamine and Fuc	β-1,3 分支的 β-1,6-葡聚糖 β-1,3 branched β-1,6-glucan	雄性 ICR 小鼠/180 肉瘤腹水细胞肿瘤 Male ICR mice/ Sarcoma 180 tumor ascites cells	通过激活细胞免疫抑制小鼠异体肿瘤的生长 Immunosuppression of allogeneic tumor growth in mice by activating cells	>69.10	[38-41]

(待续)

(续表 2)

MD-组分 MD-component	子实体 Fruiting bodies	1 000	80:20−99:1	葡萄糖 Glc	β-1,3 分支的 β-1,6-葡聚糖 β-1,3 branched β-1,6-glucan	雄性 C3H/HeJ 小鼠/MM-46 肿瘤细胞 Male C3H/HeJ mice/MM-46 carcinoma cells	增强免疫激活 Enhancement of immune activation	54.60	[41]
L-MD-组分 L-MD-component		250	不详 Uncertain					67.90	[42]
MZ-组分 MZ-component	子实体 Fruiting bodies	20	84:16	葡萄糖 Glc	β-1,6 和 β-1,3 分支的 β-D-葡聚糖 β-1,6 and β-1,3 branches of β-D-glucan	雄性 C3H/HeJ 小鼠/小鼠巨噬细胞细胞系 J774.1 Male C3H/HeJ mice/Murine macrophage cell line J774.1	提高 TNF-α 和 IL-12 的生产和小鼠巨噬细胞 J774.1 的抗原呈递 Increasement of TNF-α and IL-12 production and antigen presentation of mouse macrophage J774.1	*	[43]
F Ⅰ, F Ⅱ, FⅢ	子实体 Fruiting bodies	500−250	不详 Uncertain	葡萄糖、木糖、岩藻糖、甘露糖、半乳糖 Glc, Xyl, Fuc, Man, and Gal	每隔 5 个葡萄糖以 β-1,6 连接的 β-1,3-葡聚糖 β-1,3-glucan branched with β-1,6 linkage and average chain length of 5 residues	雌性 ICR/JCL 小鼠/180 肉瘤腹水细胞肿瘤 Female ICR/JCL mice/Sarcoma 180 tumor ascites cells	不详 Uncertain	50.00	[44]
MZF	子实体 Fruiting bodies	23	46:54	半乳糖:甘露糖:岩藻糖:葡萄糖=1.24:1.00:0.95:0.88 Gal:Man:Fuc:Glc=1.24:1.00:0.95:0.88	α-1,6-葡萄糖，α-l,3-岩藻糖，α-1,6-甘露糖，β-1,3-葡萄糖，β-1,3,6-葡萄糖组成 α-1,6-glucose, α-l,3-fucose, α-1,6-mannose, β-1,3-glucose, β-1,3,6-glucose	雌性 BALB/cA 小鼠/Colon-26 癌细胞 Female BALB/cA mice/Colon-26 carcinoma cells	增加肿瘤部位 T 细胞的积累，诱导细胞免疫和肿瘤消退 Increasement of T cell accumulation in tumor site, inducement of cellular immunity and tumor regression	47.60	[45]
LMw-GFP	子实体 Fruiting bodies	1.79	糖:蛋白质:糖醛酸=98.83:0.34:0.21 Carbohydrates:proteins:uronic acid=98.83:0.34:0.21	葡萄糖 Glc	α-D-葡萄糖、α-1,4-葡萄糖和 α-1,3,-葡萄糖组成 α-D-glucose, α-1,4-glucose and α-1,3,-glucose composition	雌性 BALB/c 小鼠/H22 肝癌细胞 Female BALB/c mice/H22 heptoma cells	保护免疫器官，增强 NK 细胞、巨噬细胞和淋巴细胞的活性，通过线粒体凋亡途径诱导 H22 实体瘤细胞凋亡 Protection of immune organs, and enhancement	40.10	[46]

(待续)

(续表 2)

						of the killing activity of NK cells, the phagocytosis of peritoneal macrophages, inducing the tumor cells apoptosis *in vivo* via mitochondrial apoptotic pathway		
GFAP	子实体 Fruiting bodies	644.9	糖含量=94.28% Carbohydrates content= 94.28%	葡萄糖:半乳糖:甘露糖=2.18:0.23:1.00 Glc:Gal:Man= 2.18:0.23:1.00	β-1,3-葡萄糖和α-1,3-D-甘露糖组成 β-1,3-glucose and α-1,3-D-mannose	雌性 BALB/c 小鼠/小鼠 H22 肝癌细胞 Female BALB/c mice/Murine H22 hepatocarcinoma cells	显著提高 NK 细胞、巨噬细胞、$CD19^+$ B 细胞和 $CD4^+$ T 细胞的活性，导致 H22 细胞的凋亡通过 G0/G1 受阻 Improvement of the activities of NK cells, macrophages, $CD19^+$ B cells and $CD4^+$ T cells, leading to the apoptosis of H22 cells via G0/G1 phase arrested	36.72	[47]
GP11	子实体 Fruiting bodies	6.9−33.0	不详 Uncertain	葡萄糖:半乳糖:甘露糖=5.04:2.61:1.00 Glc:Gal:Man= 5.04.2.61:1.00	1,6-D-甘露糖，1,4-葡萄糖等 1,6-D-mannose, 1,4-glucose and so on	雌性 ICR 小鼠/Anti-mouse TLR2，TLR4/Heps 肿瘤细胞 Female ICR mice/Anti-mouse TLR2, TLR4/Heps tumor cells	增加胸腺和脾脏的相对重量以及血清 TNF-α 和 IL-12 水平，通过 TLR4 介导 NO 和 TNF-α 的上调而改善免疫功能 Increasement of the relative thymus and spleen weights as well as the serum levels of TNF-α and IL-2, and increasement of NO and TNF-αsecretion through the TLR4 signaling pathway and improvement of immune function	>15.75	[5]
GFPW	子实体 Fruiting bodies	15.7	不含蛋白 No protein	半乳糖:岩藻糖:甘露糖=1.00:0.44:0.41	O-2 位连接支链的α-(1,6)-半乳糖 O-2-α-(1,6) galactose	体外/人脑微血管内皮细胞 *In vitro*/Human	显著降低人脑微血管内皮细胞的增殖(迁移和成管)且呈剂量和时间依赖性	*	[48]

(待续)

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(续表 2)

				Gal:Fuc:Man=1.00:0.44:0.41	linked to the branched chain	microvascular endothelial cells	Inhibition of endothelial cell proliferation in a dose- and time-dependent manner, and reduce of endothelial cell migration and tube formation as well	
GFP-22	子实体 Fruiting bodies	27.2	不详 Uncertain	葡萄糖:甘露糖:半乳糖=15.20:2.80:1.00 Glc:Man:Gal=15.20:2.80:1.00	β-1,4-D-葡萄糖、β-1,3-D-葡萄糖、α-1,6-D-葡萄糖、α-1,6-D-半乳糖、α-1,4,6-D-甘露糖和α-1,3,6-D-甘露糖组成 β-1,4-D-glucose, β-1,3-D-glucose, α-1,6-D-glucose, α-1,6-D-galactose, α-1,4,6-mannose and α-1,3,6-D-mannose	Balb/c strain 小鼠/腹腔注射 CTX 建立免疫抑制动物模型 Balb/c strain mice/Animal model of immunosuppression was established by intraperitoneal injection of CTX	改善或逆转 CTX 诱导的免疫抑制，显著提高脾、胸腺指数、脾淋巴细胞增殖和脾细胞细胞因子的产生；一种免疫调节剂 Improvement or reversion of the CTX-induced immunosuppression, significantly enhancement of the spleen and thymus indices, spleen lymphocyte proliferation and cytokines production in splenocytes; an immunomodulator	*	[49]
GF70-F1	子实体 Fruiting bodies	1 260	92.6:2.2	葡萄糖:半乳糖:甘露糖=56.00:1.24:1.00 Glc:Gal:Man=56.00:1.24:1.00	β-1,6 分支的 β-1,4 主链 β-1,6-branched β-1,4-backbone	体外/Parental RAW 264.7 murine 巨噬细胞 *In vitro*/Parental RAW 264.7 murine macrophages	抑制脂多糖诱导巨噬细胞中 TNF-α 和 IL-6 的产生以及 NF-κB 的活化 Inhibition of TNF-α and IL-6 production as well as NF-κB activation in lipopolysaccharide-induced macrophages	*	[50]
PGF-2	子实体 Fruiting bodies	118.803	95.40:2.25	甘露糖:半乳糖:葡萄糖=2.35:1.22:1.00 Man:Gal:Glc=2.35:1.22:1.00	含 α-糖苷键，-O-Ser 连接多糖与氨基酸 Containing α-glycosidic bond, -O-Ser connects polysaccharides to amino acid	不详 Uncertain	不详 Uncertain	*	[51]

(待续)

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(续表 2)

LELFD 组分	菌丝体 Mycelia	不详 Uncertain	不详 Uncertain	不详 Uncertain	β-1,3-葡聚糖主链 β-1,3-glucan backbone	雌性 ICR/Slc 小鼠/180 肉瘤腹水细胞肿瘤 Female ICR/Slc mice/Sarcoma 180 tumor ascites cells	明显提高(肉瘤 180/小鼠)抗肿瘤活性，且对巨噬细胞具有补体 C3 活化作用 Increasement of the antitumor activity of (sarcoma 180 mouse) and activation of macrophages with complement C3	*	[52]
GFPS1b	菌丝体 Mycelia	21	糖:蛋白质:糖醛酸=81.32:16.60:4.25 Carbohydrates: proteins:uronic acid=81.32:16.60:4.25	葡萄糖:半乳糖:阿拉伯糖=4.00:2.00:1.00 Glc:Gal:Ara=4.00:2.00:1.00	O-6 位 α-1,4-D-葡萄糖残基分支的 α-1,4-D-半乳糖、α-1,3-D-葡萄糖主链 O-6 with glycosyl residues composed of α-L-arabinose-1,4-α-D-glucose (1→linked residues	体外/乳腺肿瘤 MCF-7 细胞 *In vitro*/mammary tumor MCF-7 cells	对乳腺肿瘤 MCF-7 细胞的增殖具有明显的抑制作用 Inhibition of the proliferation of mammary tumor MCF-7 cells	*	[53]
GRN	菌丝体 Mycelia	500	不详 Uncertain	葡萄糖 Glc	β-1,6 分支的 β-1,3-葡聚糖 β-1,6-branched β-1,3-glucan	体外/RAW264.7 细胞巨噬细胞 *In vitro*/RAW264.7 macrophages cells	激活巨噬细胞产生 IL-6、IL-12 和 TNF-α，促进细胞因子的产生 Activation of macrophages to produce IL-6, IL-12 and TNF-α, and promotion of the production of cytokines	*	[54]
GFP2	菌丝体 Mycelia	2.6	糖含量=98.4% Carbohydrates content=98.4%	葡萄糖 Glc	α-1,3，α-1,6，α-1,4-糖苷键 α-1,3, α-1,6, α-1,4-glycosidic bond	ICR 小鼠/S180, Heps, EACICR mice/细胞 S180、Heps、EAC cells	不详 Uncertain	17.00−26.56	[55]
GFG-3a	菌丝体 Mycelia	88.01	糖含量=6.20% Carbohydrates content=6.20%	甘露糖:葡萄糖:果糖:阿拉伯糖=4.510:2.460:1.331 Man:Glc:Fru:Ara=4.510:2.460:1.331	β-1,4-半乳糖苷键，β-D-葡萄糖苷键 β-1,4-galactoside bond, β-D-glucoside bond	体外/小鼠肉瘤细胞 S-180 和人肝癌细胞 *In vitro*/Bel-7402mouse sarcoma cell line S-180 and human hepatoma cell line Bel-7402	不详 Uncertain	*	[56]

注：*：文献中未标明具体数值，但多糖剂量组的肿瘤抑制结果较肿瘤模型组有显著性差异；TNF-α：肿瘤坏死因子；IL：白细胞介素；NK 细胞：自然杀伤细胞；CTX：环磷酰胺

Note: *: Specific values are not indicated, but the tumor inhibition results of polysaccharide dose group were significantly different from that of tumor model group; TNF-α: Tumor necrosis factor-α; IL: Interleukin; NK cell: Natural killer cell; CTX: Cyclophosphamide.

于多糖的抗肿瘤活性很重要。Li 等[58]对具有抗肿瘤活性的 GFP 多糖进行热处理降低其支化度后，发现其体外抗肿瘤作用也随之降低。

2.3 灰树花抗肿瘤活性多糖的作用机制

2.3.1 对肿瘤细胞的直接抑制作用

灰树花多糖具有显著的抗肿瘤功效，可直接抑制肿瘤细胞。灰树花多糖 GFP 对细胞体外增殖抑制实验[3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide，MTT]表明，其可以抑制人类肝癌细胞 HepG2 的生长，而且有明显的浓度依赖关系[59]。灰树花菌丝体多糖 GFPS1b 对乳腺肿瘤 MCF-7 细胞的增殖具有明显的抑制作用[53]。

2.3.2 诱导细胞凋亡

细胞凋亡调控发生异常是导致恶性肿瘤发生的关键原因之一。Konno 等[60]通过观察体外培养的人类前列腺癌 PC-3 细胞加入灰树花 D 组分的实验，发现 24 h 内有超过 95%的细胞死亡，推断很大可能是发生了细胞凋亡。

2.3.3 增强免疫功能

灰树花多糖的抗肿瘤效果显著，其主要是通过增强机体免疫力，包括提高对肿瘤细胞的杀伤能力。通过雌性 ICR 小鼠实验，Mao 等[5]发现提取得到的灰树花子实体多糖 GP11 (分子量为 6.9−33.0 kDa)能通过 TLR4 介导 NO 和 TNF-α (tumor necrosis factor，TNF)上调，改善免疫应答而间接参与抗肿瘤活性。此外，Matsui 等[61]认为，D 组分除了激活细胞免疫能力细胞外还具有抗肿瘤作用。王莉蕊[62]分离提取得到的 GFP-A 可以显著增强 RAW 264.7 细胞的吞噬活性，并且在一定浓度范围内提高其 NO 的释放量，上调细胞内 TNF-α、IL-1β、IL-6、IL-12、IFN-γ 细胞因子及细胞中诱导型一氧化氮合酶的 mRNA 水平的表达，实现免疫调节作用。本团队前期研究也发现灵芝孢子粉多糖能够增强免疫功能，显著降低脾脏及肿瘤微环境中某些淋巴细胞的表达，能够恢复肿瘤微环境中肿瘤浸润淋巴细胞的特异性识别和杀伤功能[63-64]。

2.3.4 抗肿瘤转移

肿瘤转移是指恶性肿瘤细胞离开原发肿瘤，通过各种途径到达远处组织或器官并继续增殖生长而形成转移灶的过程。因此，治愈恶性肿瘤的主要方法有抗肿瘤转移。Masuda 等[65]对灰树花 MD 组分进行研究发现，腹腔给药的时间会影响抗癌细胞转移的效果，表明 MD 组分能够抑制肿瘤细胞转移。

3 灰树花免疫调节活性多糖及其一级结构表征

研究认为，灰树花多糖免疫调节活性是其重要的生物活性，能够增强免疫细胞的活性。灰树花子实体多糖 GFPBW1 的分子量为 300 kDa，结构为 β-1,6-分支的 β-1,3-葡聚糖，其能通过 Dectin-1/Syk/NF-κB 信号通路激活巨噬细胞[66]。灰树花子实体多糖 GFP-A 的分子量为 848 kDa，其具有 TLR4 和丝裂原活化蛋白激酶介导的免疫活性[67]。灰树花子实体多糖 GFPBW 2 的分子量为 26.2 kDa，在 O-6 位有 β-1,3 分支的 β-1,3 和 β-1,4 葡萄糖，雌性 ICR 小鼠实验表明，其通过触发细胞因子分泌而激活巨噬细胞[68]。Meng 等[69]提取得到的灰树花子实体多糖 GFP，其分子量为 155 kDa，结构为 1,3 和 1,3,4-葡聚糖主链，在体外 RAW 264.7 巨噬细胞实验中能够提高细胞增殖，促进细胞因子或趋化因子的产生，从而发挥免疫刺激活性。

4 灰树花其他生物活性多糖及其一级结构表征

灰树花多糖除了具有降血糖、抗肿瘤、免

疫调节活性等功能外，还有抗病毒、抗氧化、抗辐射等生物活性。在多糖的单糖组成成分中，葡萄糖仍然是最主要的成分；多糖的结构分析表明，最主要的糖苷键是 α-1,4、β-1,3 和 β-1,6 连接键型(表 3)。

5 展望

综上所述，灰树花多糖的生理功能活性可能与 β-1,6-葡聚糖主链化学结构相关(图 1)，但灰树花活性多糖构效尚未完全明晰，首要原因是灰树花活性多糖的低得率。比如，已被开发成食品补充剂的灰树花 D 组分和 X 组分，其得率分别为 0.30%和 0.05%[72-73]。活性多糖的低得率限制了活性研究和结构分析对原料的需要。食用菌子实体细胞壁组织含有丰富的几丁质结构，致使多糖难以被高效提取[3]，因此亟须采用新的辅助提取技术，如微波、超声波、生物酶、超高压、亚临界和脉冲电场等创新技术应用于多糖辅助提取，实现多糖的高效制备[74]。

大部分食用菌多糖以糖苷键相互交联，作为细胞壁的主要成分，也通过糖肽键与蛋白质结合[75]，越来越多的研究表明，蛋白质对多糖生理功能活性起着至关重要的作用，当 β 葡聚糖与蛋白质以复合物形态存在时，其生物效率得到提升[76]，然而这些结合蛋白的存在更增加了多糖精细结构解析的困难。因此，理清结构蛋白和活性多糖的相互作用，对于进一步解开灰树花活性多糖的构效关系至关重要。

灰树花多糖经化学修饰如磷酸化[77]、羧甲基化和硒化[78]等能增加多糖的生物活性。同时，灰树花多糖主要活性成分 β-1,3/1,6-葡聚糖呈高度分支并呈三股螺旋结构，因此未来的研究需要进一步确定灰树花多糖的三维结构和功能之间的关系[2]。灰树花功能多糖构效关系的探究将有助于设计出更多潜在的促进健康的药物和基于化学修饰的功能性食品。

表 3 其他生物活性灰树花多糖的分子质量、结构或组成

Table 3 Molecular weight, structure or composition of *G. frondosa* polysaccharides with other activities

名称 Name	提取部位 Extractive fraction	分子量 Mw (kDa)	糖:蛋白 Polysaccharides: proteins	单糖组成 Monosaccharide/ Composition	结构 Structure/Construction	生理活性 Physiological activity	参考文献 References
GFP30-2-a	子实体 Fruiting bodies	2 040.0	不详 Uncertain	葡萄糖:半乳糖=1.000:0.098 Glc:Gal=1.000:0.098	α-D-1,4-葡萄糖 α-D-1,4-glucose	不详 Uncertain	[70]
Se-GFP-22	子实体 Fruiting bodies	4 130.0	糖含量=97% Carbohydrates content=97%	甘露糖:葡萄糖:半乳糖=13.30:23.30:1.00 Man:Glc:Gal=13.30:23.30:1.00	β-1,3,6-D-甘露糖、α-1,4,6-D-半乳糖分支的 α-1,4-D-葡聚糖 α-1,4-D-glucan main chain with β-1,3,6-D-mannose and α-1,4,6-D-galactose branched	抗氧化 Antioxidative	[71]
GFP1	菌丝体 Mycelia	40.5	不详 Uncertain	葡萄糖:岩藻糖=2.3:0.5 Glc:Fuc=2.3:0.5	α-1,3-D-岩藻糖分支 β-1,6-D-葡聚糖 β-1,6-D-glucan main chain with α-1,3-D-fucose branched	抗病毒 Antiviral	[8]

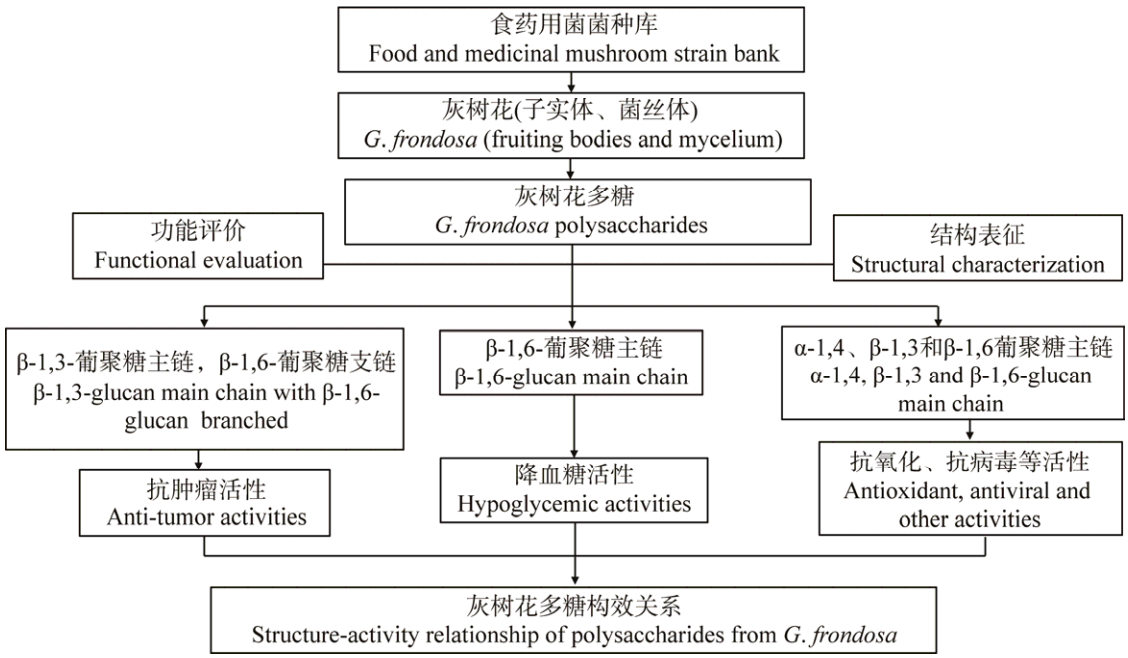

图 1　灰树花多糖构效关系研究

Figure 1　Study on structure-activity relationship of *G. frondosa* polysaccharides.

REFERENCES

[1] 戴玉成, 杨祝良. 中国药用真菌名录及部分名称的修订[J]. 菌物学报, 2008, 27(6): 801-824
Dai YC, Yang ZL. A revised checklist of medicinal fungi in China[J]. Mycosystema, 2008, 27(6): 801-824 (in Chinese)

[2] He XR, Wang XX, Fang JC, Chang Y, Ning N, Guo H, Huang LH, Huang XQ, Zhao ZF. Polysaccharides in *Grifola frondosa* mushroom and their health promoting properties: a review[J]. International Journal of Biological Macromolecules, 2017, 101: 910-921

[3] Wu JY, Siu KC, Geng P. Bioactive ingredients and medicinal values of *Grifola frondosa* (Maitake)[J]. Foods: Basel, Switzerland, 2021, 10(1): 95

[4] Meng X, Liang HB, Luo LX. Antitumor polysaccharides from mushrooms: a review on the structural characteristics, antitumor mechanisms and immunomodulating activities[J]. Carbohydrate Research, 2016, 424: 30-41

[5] Mao GH, Ren Y, Feng WW, Li Q, Wu HY, Dun J, Zhao T, Xu CQ, Yang LQ, Wu XY. Antitumor and immunomodulatory activity of a water-soluble polysaccharide from *Grifola frondosa*[J]. Carbohydrate Polymers, 2015, 134: 406-412

[6] Yeh JY, Hsieh LH, Wu KT, Tsai CF. Antioxidant properties and antioxidant compounds of various extracts from the edible Basidiomycete *Grifola frondosa* (Maitake)[J]. Molecules: Basel, Switzerland, 2011, 16(4): 3197-3211

[7] Chen YQ, Liu D, Wang DY, Lai SS, Zhong RT, Liu YY, Yang CF, Liu B, Sarker MR, Zhao C. Hypoglycemic activity and gut microbiota regulation of a novel polysaccharide from *Grifola frondosa* in type 2 diabetic mice[J]. Food and Chemical Toxicology, 2019, 126: 295-302

[8] Zhao C, Gao LY, Wang CY, Liu B, Jin Y, Xing Z. Structural characterization and antiviral activity of a novel heteropolysaccharide isolated from *Grifola frondosa* against enterovirus 71[J]. Carbohydrate Polymers, 2016, 144: 382-389

[9] 汪维云. 灰树花多糖的抗辐射作用研究[J]. 安徽农业大学学报, 2003, 30(2): 210-212
Wang WY. Anti-radiation effect of *Grifola frondosa* polysaccharide[J]. Journal of Anhui Agricultural University, 2003, 30(2): 210-212 (in Chinese)

[10] 倪力军, 王媛媛, 何婉瑛, 张立国. 8 种多糖的单糖组成、活性及其相关性分析[J]. 天津大学学报(自然科学与工程技术版), 2014, 47(4): 326-330
Ni LJ, Wang YY, He WY, Zhang LG. Monosaccharide

composition, activity and their correlation analysis in eight polysaccharides[J]. Journal of Tianjin University: Science and Technology, 2014, 47(4): 326-330 (in Chinese)

[11] Ferreira SS, Passos CP, Madureira P, Vilanova M, Coimbra MA. Structure-function relationships of immunostimulatory polysaccharides: a review[J]. Carbohydrate Polymers, 2015, 132: 378-396

[12] Kubo K, Aoki H, Nanba H. Anti-diabetic activity present in the fruit body of *Grifola frondosa* (Maitake). Ⅰ[J]. Biological & Pharmaceutical Bulletin, 1994, 17(8): 1106-1110

[13] Nanba H. Proteoglucan and antidiabetic drug thereof: US5773426[P]. 1998-06-30

[14] Preuss HG, Echard B, Bagchi D, Perricone NV, Zhuang C. Enhanced insulin-hypoglycemic activity in rats consuming a specific glycoprotein extracted from Maitake mushroom[J]. Molecular and Cellular Biochemistry, 2007, 306(1/2): 105-113

[15] Preuss HG, Echard B, Fu J, Perricone NV, Bagchi D, Kaylor M, Zhuang C. Fraction SX of Maitake mushroom favorably influences blood glucose levels and blood pressure in streptozotocin-induced diabetic rats[J]. Journal of Medicinal Food, 2012, 15(10): 901-908

[16] Hong L, Xun M, Wutong W. Anti-diabetic effect of an alpha-glucan from fruit body of Maitake (*Grifola frondosa*) on KK-Ay mice[J]. The Journal of Pharmacy and Pharmacology, 2007, 59(4): 575-582

[17] Lei H, Guo SZ, Han JC, Wang Q, Zhang XX, Wu WT. Hypoglycemic and hypolipidemic activities of MT-α-glucan and its effect on immune function of diabetic mice[J]. Carbohydrate Polymers, 2012, 89(1): 245-250

[18] Ma XL, Zhou FC, Chen YY, Zhang YY, Hou LH, Cao XH, Wang CL. A polysaccharide from *Grifola frondosa* relieves insulin resistance of HepG2 cell by Akt-GSK-3 pathway[J]. Glycoconjugate Journal, 2014, 31(5): 355-363

[19] Guo WL, Deng JC, Pan YY, Xu JX, Hong JL, Shi FF, Liu GL, Qian M, Bai WD, Zhang W, et al. Hypoglycemic and hypolipidemic activities of *Grifola frondosa* polysaccharides and their relationships with the modulation of intestinal microflora in diabetic mice induced by high-fat diet and streptozotocin[J]. International Journal of Biological Macromolecules, 2020, 153: 1231-1240

[20] Pan YY, Wan XZ, Zeng F, Zhong RT, Guo WL, Lv XC, Zhao C, Liu B. Regulatory effect of *Grifola frondosa* extract rich in polysaccharides and organic acids on glycolipid metabolism and gut microbiota in rats[J]. International Journal of Biological Macromolecules, 2020, 155: 1030-1039

[21] Chen YQ, Liu YY, Sarker MMR, Yan X, Yang CF, Zhao LN, Lv XC, Liu B, Zhao C. Structural characterization and antidiabetic potential of a novel heteropolysaccharide from *Grifola frondosa* via IRS1/PI3K-JNK signaling pathways[J]. Carbohydrate Polymers, 2018, 198: 452-461

[22] Xiao C, Wu QP, Xie YZ, Zhang JM, Tan JB. Hypoglycemic effects of *Grifola frondosa* (Maitake) polysaccharides F2 and F3 through improvement of insulin resistance in diabetic rats[J]. Food & Function, 2015, 6(11): 3567-3575

[23] 吴清平, 肖春, 杨小兵, 谢意珍, 李森柱. 灰树花多糖 F2 的制备方法及其降血糖功能: CN103724445A[P]. 2014-04-16
Wu QP, Xiao C, Yang XB, Xie YZ, Li SZ. Preparation method and blood sugar lowering function of *Grifola frondosa* polysaccharide F2: CN103724445A[P]. 2014-04-16 (in Chinese)

[24] Xiao C, Jiao CW, Xie YZ, Ye LH, Li QQ, Wu QP. *Grifola frondosa* GF5000 improves insulin resistance by modulation the composition of gut microbiota in diabetic rats[J]. Journal of Functional Foods, 2021, 77: 104313

[25] 周富川. 灰树花多糖降血糖活性及作用机理的研究[D]. 天津: 天津科技大学硕士学位论文, 2013
Zhou FC. The molecular mechanism and hypoglycemic activity of exo-polysaccharide produced by *Grifola frondosa*[D]. Tianjin: Master's Thesis of Tianjin University of Science & Technology, 2013 (in Chinese)

[26] 边亮, 陈华国, 周欣. 植物多糖的抗肿瘤活性研究进展[J]. 食品科学, 2020, 41(7): 275-282
Bian L, Chen HG, Zhou X. Recent advances in understanding the antitumor activity of polysaccharides from plants[J]. Food Science, 2020, 41(7): 275-282 (in Chinese)

[27] 刘淑贞, 周文果, 叶伟建, 陈江平, 巫朝华, 陈东清, 黄建联. 活性多糖的生物活性及构效关系研究进展[J]. 食品研究与开发, 2017, 38(18): 211-218
Liu SZ, Zhou WG, Ye WJ, Chen JP, Wu CH, Chen DQ, Huang JL. Research advance on biological activity and structure-activity relationships of bioactive polysaccharide[J]. Food Research and Development, 2017, 38(18): 211-218 (in Chinese)

[28] Mansel BW, Ryan TM, Chen HL, Lundin L, Williams MAK. Polysaccharide conformations measured by solution state X-ray scattering[J]. Chemical Physics Letters, 2020, 739: 136951

[29] Konno S, Alexander B, Zade J, Choudhury M. Possible hypoglycemic action of SX-fraction targeting insulin signal transduction pathway[J]. International Journal of General Medicine, 2013, 6: 181-187

[30] Hong L, Zhang MM, Wang Q, Guo SZ, Han JC, Sun HJ, Wu WT. MT-α-glucan from the fruit body of the Maitake medicinal mushroom *Grifola frondosa* (higher Basidiomyetes) shows protective effects for hypoglycemic pancreatic β-cells[J]. International Journal of Medicinal Mushrooms, 2013, 15(4): 373-381

[31] Su CH, Lu TM, Lai MN, Ng LT. Inhibitory potential of *Grifola frondosa* bioactive fractions on α-amylase and α-glucosidase for management of hyperglycemia[J]. Biotechnology and Applied Biochemistry, 2013, 60(4): 446-452

[32] Li L, Guo WL, Zhang W, Xu JX, Qian M, Bai WD, Zhang YY, Rao PF, Ni L, Lv XC. *Grifola frondosa* polysaccharides ameliorate lipid metabolic disorders and gut microbiota dysbiosis in high-fat diet fed rats[J]. Food & Function, 2019, 10(5): 2560-2572

[33] Iino K, Ohno N, Suzuki I, Miyazaki T, Yadomae T, Oikawa S, Sato K. Structural characterisation of a neutral antitumour β-D-glucan extracted with hot sodium hydroxide from cultured fruit bodies of *Grifola frondosa*[J]. Carbohydrate Research, 1985, 141(1): 111-119

[34] Ohno N, Iino K, Oikawa S, Sato K, Ohsawa M, Yadomae T. Fractionation of acidic antitumor beta-glucan of *Grifola frondosa* by anion-exchange chromatography using urea solutions of low and high ionic strengths[J]. Chemical & Pharmaceutical Bulletin, 1986, 34(8): 3328-3332

[35] Ohno N, Suzuki I, Oikawa S, Sato K, Miyazaki T, Yadomae T. Antitumor activity and structural characterization of glucans extracted from cultured fruit bodies of *Grifola frondosa*[J]. Chemical & Pharmaceutical Bulletin, 1984, 32(3): 1142-1151

[36] Rui TD, Adachi Y, Ishibashi KI, Ohno N. An unambiguous structural elucidation of a 1, 3-β-D-glucan obtained from liquid-cultured *Grifola frondosa* by solution NMR experiments[J]. Carbohydrate Research, 2009, 344(3): 400-404

[37] Adachi Y, Ohno N, Ohsawa M, Sato K, Oikawa S, Yadomae T. Physiochemical properties and antitumor activities of chemically modified derivatives of antitumor glucan “grifolan LE” from *Grifola frondosa*[J]. Chemical & Pharmaceutical Bulletin, 1989, 37(7): 1838-1843

[38] Nanba H, Hamaguchi A, Kuroda H. The chemical structure of an antitumor polysaccharide in fruit bodies of *Grifola frondosa* (Maitake)[J]. Chemical & Pharmaceutical Bulletin, 1987, 35(3): 1162-1168

[39] Mizuno T, Zhuang C. Maitake, *Grifola frondosa*: pharmacological effects[J]. Food Reviews International, 1995, 11(1): 135-149

[40] Hishida I, Nanba H, Kuroda H. Antitumor activity exhibited by orally administered extract from fruit body of *Grifola frondosa* (Maitake)[J]. Chemical & Pharmaceutical Bulletin, 1988, 36(5): 1819-1827

[41] Nanba H, Kubo K. Antitumor substance extracted from *Grifola*: US5854404[P]. 1998-12-29

[42] Miyake N, Masuda Y, Munechika Inoue YM, Matuda K, Okamoto K, Nanba H, Kodama N. Low molecular weight Maitake MD-fraction (klasma-MD) hydrolyzed with endo-β-1,6-glucanase of *Trichoderma harzianum* induces antitumor activities[J]. Mycoscience, 2006, 47(2): 78-83

[43] Masuda Y, Kodama N, Nanba H. Macrophage J774.1 cell is activated by MZ-fraction (klasma-MZ) polysaccharide in *Grifola frondosa*[J]. Mycoscience, 2006, 47(6): 360-366

[44] Mizuno T, Ohsawa K, Hagiwara N, Kuboyama R. Fractionation and characterization of antitumor polysaccharides from Maitake, *Grifola frondosa*[J]. Agricultural and Biological Chemistry, 1986, 50(7): 1679-1688

[45] Masuda Y, Matsumoto A, Toida T, Oikawa T, Ito K, Nanba H. Characterization and antitumor effect of a novel polysaccharide from *Grifola frondosa*[J]. Journal of Agricultural and Food Chemistry, 2009, 57(21): 10143-10149

[46] Ji HY, Yu J, Liu AJ. Structural characterization of a low molecular weight polysaccharide from *Grifola frondosa* and its antitumor activity in H22 tumor-bearing mice[J]. Journal of Functional Foods, 2019, 61: 103472

[47] Yu J, Ji HY, Liu C, Liu AJ. The structural characteristics of an acid-soluble polysaccharide from *Grifola frondosa* and its antitumor effects on H22-bearing mice[J]. International Journal of Biological Macromolecules, 2020, 158: 1288-1298

[48] Wang Y, Shen XK, Liao WF, Fang JP, Chen X, Dong Q,

Ding K. A heteropolysaccharide, L-fuco-D-manno-1, 6-α-D-galactan extracted from *Grifola frondosa* and antiangiogenic activity of its sulfated derivative[J]. Carbohydrate Polymers, 2014, 101: 631-641

[49] Li Q, Zhang FM, Chen GY, Chen Y, Zhang WJ, Mao GH, Zhao T, Zhang M, Yang LQ, Wu XY. Purification, characterization and immunomodulatory activity of a novel polysaccharide from *Grifola frondosa*[J]. International Journal of Biological Macromolecules, 2018, 111: 1293-1303

[50] Su CH, Lu MK, Lu TJ, Lai MN, Ng LT. A (1→6)-branched (1→4)-β-D-glucan from *Grifola frondosa* inhibits lipopolysaccharide-induced cytokine production in RAW264.7 macrophages by binding to TLR2 rather than dectin-1 or CR3 receptors[J]. Journal of Natural Products, 2020, 83(2): 231-242

[51] 李小定, 欧阳天贽, 荣建华, 吴谋成. 灰树花多糖PGF-2 的理化性质及化学结构的初步表征[J]. 菌物学报, 2005, 24(2): 245-250
Li XD, Ouyang TZ, Rong JH, Wu MC. Physical and chemical properties and chemical structure of polysaccharide fraction *pgf*-2 from *Grifola frondosa*[J]. Mycosystema, 2005, 24(2): 245-250 (in Chinese)

[52] Zhuang C, Mizuno T, Ito H, Shimura K. Chemical modification and antitumor activity of polysaccharides from the mycelium of liquid-cultured *Grifola frondosa*[J]. Nippon Shokuhin Kogyo Gakkaishi, 1994, 41(10): 733-740

[53] Cui FJ, Tao WY, Xu ZH, Guo WJ, Xu HY, Ao ZH, Jin J, Wei YQ. Structural analysis of anti-tumor heteropolysaccharide GFPS1b from the cultured mycelia of *Grifola frondosa* GF9801[J]. Bioresource Technology, 2007, 98(2): 395-401

[54] Adachi Y, Okazaki M, Ohno N, Yadomae T. Enhancement of cytokine production by macrophages stimulated with (1.RAR.3)-β-D-glucan, grifolan (GRN), isolated from *Grifola frondosa*[J]. Biological and Pharmaceutical Bulletin, 1994, 17(12): 1554-1560

[55] 陈向东, 刘晓雯, 吴梧桐. 低分子量灰树花多糖的分离、纯化和抗肿瘤活性[J]. 中国天然药物, 2006, 4(1): 77-80
Chen XD, Liu XW, Wu WT. Separation, purification and antitumor activities of low molecular weight polysaccharide from *Grifola frondosa*[J]. Chinese Journal of Natural Medicines, 2006, 4(1): 77-80 (in Chinese)

[56] Cui FJ, Zan XY, Li YH, Yang Y, Sun WJ, Zhou Q, Yu SL, Dong Y. Purification and partial characterization of a novel anti-tumor glycoprotein from cultured mycelia of *Grifola frondosa*[J]. International Journal of Biological Macromolecules, 2013, 62: 684-690

[57] Kodama N, Komuta K, Sakai N, Nanba H. Effects of D-fraction, a polysaccharide from *Grifola frondosa* on tumor growth involve activation of NK cells[J]. Biological & Pharmaceutical Bulletin, 2002, 25(12): 1647-1650

[58] Li XQ, Liu AJ. Relationship between heat treatment on structural properties and antitumor activity of the cold-water soluble polysaccharides from *Grifola frondosa*[J]. Glycoconjugate Journal, 2020, 37(1): 107-117

[59] 孟梦. 灰树花多糖的分离纯化及其抗肿瘤作用机理的研究[D]. 天津: 天津科技大学硕士学位论文, 2013
Meng M. Research on the purification and the anti-tumor mechanism of an polysaccharide from *Grifola frondosa*[D]. Tianjin: Master's Thesis of Tianjin University of Science & Technology, 2013 (in Chinese)

[60] Konno S. Maitake D-fraction: apoptosis inducer and immune enhancer[J]. Alternative and Complementary Therapies, 2001, 7(2): 102-107

[61] Matsui K, Kodama N, Nanba H. Effects of Maitake (*Grifola frondosa*) D-fraction on the carcinoma angiogenesis[J]. Cancer Letters, 2001, 172(2): 193-198

[62] 王莉蕊. 灰树花胞外多糖的分离纯化及免疫调节作用研究[D]. 天津: 天津科技大学硕士学位论文, 2014
Wang LR. Studies on the isolation, purification and immunomodulatory activity of extracellular polysaccharide from *Grifola frondosa*[D]. Tianjin: Master's Thesis of Tianjin University of Science & Technology, 2014 (in Chinese)

[63] Su JY, Su L, Li D, Shuai O, Zhang YF, Liang HJ, Jiao CW, Xu ZC, Lai Y, Xie YZ. Antitumor activity of extract from the sporoderm-breaking spore of *Ganoderma lucidum*: restoration on exhausted cytotoxic T cell with gut microbiota remodeling[J]. Frontiers in Immunology, 2018, 9: 1765

[64] Su JY, Li D, Chen QJ, Li MX, Su L, Luo T, Liang DL, Lai GX, Shuai O, Jiao CW, et al. Anti-breast cancer enhancement of a polysaccharide from spore of *Ganoderma lucidum* with paclitaxel: suppression on tumor metabolism with gut microbiota reshaping[J]. Frontiers in Microbiology, 2018, 9: 3099

[65] Masuda Y, Murata Y, Hayashi M, Nanba H. Inhibitory effect of MD-fraction on tumor metastasis: involvement of NK cell activation and suppression of intercellular

adhesion molecule (ICAM)-1 expression in lung vascular endothelial cells[J]. Biological & Pharmaceutical Bulletin, 2008, 31(6): 1104-1108

[66] Fang JP, Wang Y, Lv XF, Shen XK, Ni XY, Ding K. Structure of a β-glucan from *Grifola frondosa* and its antitumor effect by activating Dectin-1/Syk/NF-κB signaling[J]. Glycoconjugate Journal, 2012, 29(5/6): 365-377

[67] Ma XL, Meng M, Han LR, Cheng D, Cao XH, Wang CL. Structural characterization and immunomodulatory activity of *Grifola frondosa* polysaccharide via toll-like receptor 4-mitogen-activated protein kinases-nuclear factor κB pathways[J]. Food & Function, 2016, 7(6): 2763-2772

[68] Wang Y, Fang JP, Ni XY, Li J, Liu Q, Dong Q, Duan JY, Ding K. Inducement of cytokine release by GFPBW2, a novel polysaccharide from fruit bodies of *Grifola frondosa*, through dectin-1 in macrophages[J]. Journal of Agricultural and Food Chemistry, 2013, 61(47): 11400-11409

[69] Meng M, Cheng D, Han LR, Chen YY, Wang CL. Isolation, purification, structural analysis and immunostimulatory activity of water-soluble polysaccharides from *Grifola frondosa* fruiting body[J]. Carbohydrate Polymers, 2017, 157: 1134-1143

[70] Zhang AQ, Deng JY, Yu SY, Zhang FM, Linhardt RJ, Sun PL. Purification and structural elucidation of a water-soluble polysaccharide from the fruiting bodies of the *Grifola frondosa*[J]. International Journal of Biological Macromolecules, 2018, 115: 221-226

[71] Li Q, Wang W, Zhu Y, Chen Y, Zhang WJ, Yu P, Mao GH, Zhao T, Feng WW, Yang LQ, et al. Structural elucidation and antioxidant activity a novel Se-polysaccharide from Se-enriched *Grifola frondosa*[J]. Carbohydrate Polymers, 2017, 161: 42-52

[72] Shigesue K, Kodama N, Nanba H. Effects of Maitake (*Grifola frondosa*) polysaccharide on collagen-induced arthritis in mice[J]. Japanese Journal of Pharmacology, 2000, 84(3): 293-300

[73] Kurushima H, Kodama N, Nanba H. Activities of polysaccharides obtained from *Grifola frondosa* on insulin-dependent diabetes mellitus induced by streptozotocin in mice[J]. Mycoscience, 2000, 41(5): 473-480

[74] Yin CM, Noratto GD, Fan XZ, Chen ZY, Yao F, Shi DF, Gao H. The impact of mushroom polysaccharides on gut microbiota and its beneficial effects to host: a review[J]. Carbohydrate Polymers, 2020, 250: 116942

[75] Zhang C, Li SS, Zhang JJ, Hu CL, Che G, Zhou M, Jia L. Antioxidant and hepatoprotective activities of intracellular polysaccharide from *Pleurotus eryngii* SI-04[J]. International Journal of Biological Macromolecules, 2016, 91: 568-577

[76] Rop O, Mlcek J, Jurikova T. Beta-glucans in higher fungi and their health effects[J]. Nutrition Reviews, 2009, 67(11): 624-631

[77] Chan JYY, Chan E, Chan SW, Sze SY, Chan MF, Tsui SH, Leung KY, Chan RYK, Chung IYM. Enhancement of *in vitro* and *in vivo* anticancer activities of polysaccharide peptide from *Grifola frondosa* by chemical modifications[J]. Pharmaceutical Biology, 2011, 49(11): 1114-1120

[78] Zhang WN, Lu YM, Zhang YP, Ding QY, Hussain S, Wu QX, Pan WJ, Chen Y. Antioxidant and antitumour activities of exopolysaccharide from liquid-cultured *Grifola frondosaby* chemical modification[J]. International Journal of Food Science & Technology, 2016, 51(4): 1055-1061