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A Review Mushrooms: A Source of Immunomodulating and Antitumor Polysaccharides

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ABSTRACT

The number of mushrooms on Earth is estimated at 1,40,000 of which may be only 10% are known. Meanwhile, of those ~ 14,000 species that we known today, about 50% are considered to possess varying degrees of edibility, more than 2,000 are safe and about 700 species are known to possess. In modern medicine, they represent a source of polysaccharides with antitumor and immunostimulating properties.These polysaccharides are present mostly as glucans with different types of glycosidic linkages, such as (1→3),(1→6)-β-glucan, (1→3)-α-glucans, and some are true heteroglycans. Different chemical modification is often carried out to improve the antitumor activity of polysaccharides, clinical qualities and water solubility. Two main procedures for chemical improvement are: modification of mushroom polysaccharides by Smith degradation (oxydo-reductohydrolysis) and activation by the method of formolysis. Carboxymethylation is another chemical method that transforms β-glucans into a water-soluble form. The commercial polysaccharides Lentinan, Schizophyllan and PSK (krestin) are widely used as antitumor material. Human clinical studies proved the beneficial activity of treatment with some other medicinal mushroom species also. These polysaccharides prevent oncogenesis, show direct antitumor activity against various allogeneic and syngeneic tumors and prevent tumor metastasis. Polysaccharides from mushrooms do not attack cancer cell directly, but produce their anti-tumor effects by stimulating macrophages such as natural Killer cells (NK-cell), T-cell, B-cell and macrophage-dependent immune system responses. The present review analyses the different chemical composition, structural correlations, chemical modifications and mode of actions as antitumor polysaccharides derived from fruit bodies and cultured mycelium in selected examples of medicinal mushrooms.

Keywords: Immunostimulating, polysaccharides, glucans antitumor, oncogenesis, allogeneic and syngeneic tumors.

1. Introduction

Mushrooms have been used in folk medicine throughout the world since ancient times [1-4]. The multifunctionality of mushrooms has drawn attention of the chemists and immunobiologists. The antitumor activity of mushrooms was first demonstrated by Lucas et al [5]. For centuries, Chinese and other healthcare practitioners employed mushrooms

to treat various diseases. They valued the power of some mushrooms as divine. Reishi Mushroom (*Ganoderma lucidum*) is called the 'mushroom of immortality' in China, and has been used as a tonic and strengthening medicine for thousands of years. Mushroom polysaccharides have immense utility, but most important aspect of mushroom polysaccharides is their immunomodulatory, anti tumor and anti cancer [6] effect as well. Several mushroom polysaccharides are widely used and commercialized worldwide as anti cancer agents for therapeutic purpose. *Lentinus edodes* (Lentinan, Japan**)** [7] *Schizophyllan commune* (Schizophyllum*)* [7] *Agaricus blazei* (Agarican, USA) [7-8] *Ganoderma lucidum* (Lingzhi, China), *Grifola frondosa* (Maitake, Japan) have been commercialized and used clinically as anti-tumor agents. Mushrooms is useful [7] against cancers of the stomach, esophagus, lungs, etc. The PSK [9] (Commercial name Krestin) is widely used for cancer immunotherapy in Japan and other Asian countries. It is very remarkable how reliable the facts collected by traditional eastern medicine are in the study of medicinial mushrooms [10-11, 3, 12].

Ikekawn et, al. reported the antitumor activities of polysaccharides obtained from fruit bodies of mushrooms belonging to the family *Polyporaceae* and a few other families, manifested as host-mediated activity against grafted cancer-such as Sarcoma 180 in animals[13-14]. Generally, medicinal properties of mushroom polysaccharides have been identified in vivo and in vitro models systems [15-16, 7].

A number of bioactive molecules, including antitumor substances, have been identified in many higher Basidiomycetes mushrooms and most of them have unique structures in different species. Moreover, different strains of one Basidiomycetes species can produce polysaccharides with different properties. For example, PSK (protein bound β-glucan) and polysaccharopeptide (PSP) are both protein bound polysaccharides, which are derived from the CM-101and COV -1 strains of the fungus *Coriolus versicolor* by Japanese and Chinese researchers respectively. Both proteoglucans have same polysaccharide component but with different protein molecules bound to the polysaccharide [17].

In the present review, antitumor and immunomodulating properties of polysaccharides from higher Basidiomycetes mushrooms are analysed. It includes the chemical composition, structure of polysaccharides in connection with their antitumor activity, different chemical modifications, clinical use of antitumor polysaccharides and mechanism of biological actions of medicinal mushrooms from fruit bodies and cultured mycelium.

2. Procedures for purification of polysaccharide

For successful extraction and purification of polysaccharides from fruit bodies or culture mycelia, the process involves elimination of low molecular weight substances from mushroom material using 80% ethanol, followed by three successive extractions with water(100°C,3h), 2% ammonium oxalate (100°C,6h) and sodium hydroxide (80°C,6h) [15-16].For structural analysis the polysaccharide under examination should be homogeneous. Numerous methods have been employed for purification; they involve fractional precipitation, selective precipitation with detergents or metal ions, chromatographic techniques, electrophoresis and ionophoresis to determine the homogenecity of the polysaccharide isolated. The technique of column chromatography (size exclusion chromatography)[18] is widely used for resolving a mixture of polysaccharides (neutral and anionic polysaccharide) based on their molecular weight

hydrodynamic volume. Sephadex and Sepharose gel are extensively employed in case of size exclusion chromatography. Packing particles can be polymers of acrylmide or more hydrophilic agarose or dextran. The size exclusion chromatography experiment is carried out in low pressure system employing large capacity columns (i.e. 1.6 × 70 cm and even larger) to separate large sample volume and therefore, preparative column is often used in size exclusion chromatography. Eluent concentration in the collected test tubes is often monitored using phenol-sulfuric method [19] and a chromatogram of sample distribution is obtained by plotting the test tube number against the carbohydrate concentration. Eluent concentration is also monitored with a RI detector and a chromatogram of sample distribution is observed.

The ion exchange chromatography using the diethylaminoethyl (DEAE)-cellulose is especially suitable for separating neutral and acidic polysaccharides.

Finally, the purity of the polysaccharide fractions is determined from the chromatogram (number of peaks, size and symmetry of peaks) of size exclusion or ion exchange chromatography.

3. Chemical composition of Mushrooms

Mushrooms, the popularly called miracle food, are being used extensively in many countries for food and fodder since the 17th century [20]. These are used as tasty, healthy, nutritional foods through the world.

Mushrooms are ~90 % water by its weight. The remaining 10 % consists of 10-40 % protein, 2-8 % fat, 3-28 % carbohydrate, 3-32 % fiber, 8-10 % ash and minerals, potassium, calcium, phosphorous, magnesium, iron, zinc, copper [21]. Some vitamins, particularly niacin, thiamine, riboflavin, biotin and vitamin C are present in mushrooms. Most mushrooms contain a variety of bioactive molecules including nucleotides, terpenoids, glycoproteins and polysaccharides.The approximate composition [22] of this mushroom (*volvariella sp.*) has been reported in **Table 1.**

Constituents	Percent
Moisture	92.41
Carbohydrate	2.19
Protein	2.94
Crude fat	0.40
Crude fiber	1.15
Ash	0.99
Nitrogen	0.80

The mycelium of mushroom contains essential amino acids which are needed to build the proteins that make our bodies function. On nutritional value studies of few species of mushrooms have shown that the proteins of some mushrooms are equal to muscle protein [23-24]. On the basis of Robinson and Davidson's investigation [25], the efficiency of protein production from a given quantity of carbohydrates in mushrooms and other higher

fungi is about 65 % compared with about 20 % for pork, 15 % for milk, 5 % for poultry and 4 % for beef. Mushrooms are good sources of dietary supplements, functional foods, phyto-chemicals and nutraceuticals [26-28]. These dietary supplements are used for the enhancement of health, fitness and prevention of various human diseases.

4. Structural composition of some important antitumor and immunomodulator agents

Three polysaccharide based carcinostatic [29](immunotherapeutic) agents, Krestin, Lentinan and Sonifilan, have already been developed from mushroom. These are used currently in the treatment of cancer of the digestive organs, lung and breast, as well as cancer of the stomach and cervical cancer respectively. Mushroom polysaccharides are also used as multipurpose medicines that are not only carcinostatic but also anti-inflammatory, antiviral (against AIDS), hypoglycaemic and antithrombotic[29].

Lentinan, produced from Shiitake mushroom, *Lentinus edodes*, is a β-(1→3), β-(1→6) glucan. There is an immense literature related to the anticancer effect of lentinan on animal and human carcinomas. It was first isolated and studied by Chihara et al. who demonstrated that its antitumor effects were greater than other mushroom polysaccharides[30].There have been numerous clinical trials of lentinan in Japan, and the drug is now manufactured and sold by several pharmaceutical companies. Lentinan has been successfully used in prolonging the overall survival of cancer patients, especially those with gastric and colorectal carcinomas [31-33].

Schizophyllan is the polysaccharide derived from the mushroom *Schizophyllum commune*. It has been shown to be cytostatic in sarcoma-180 tumor xenographs[7]. Various clinical trials have been carried out in Japan. Early clinical studies with schizophyllan in combination with conventional chemotherapy (tegafur or mitomycin C and 5-fluorouracil) in a randomized controlled study of 367 patients with gastric cancer showed significant increase in median survival[34]. Recently schizophyllan has also been shown to increase overall survival of patients with head and neck cancers [35]. In a randomized controlled study of schizophyllan in combination with radiotherapy,showed that it significantly prolonged the overall survival of stage II cervical cancer patients [36-37]. Schizophyllan is currently produced commercially by several Japanese pharmaceutical companies.

Several studies have shown that β-D-glucan derived from another mushroom *Grifola frondosa* (also known as Maitake) have strong antitumor activity in xenographs [38]. More recently, a highly purified extract, β-(1→3),(1→6)glucan (Grifron-D, GD) has become available. GD has considerable immunomodulating and antitumor activities in animal models, and is orally bioavailable [39].Maitake D-fraction and crude Maitake powders have demonstrated remarkable inhibition of metastasis in a mouse model, especially in the prevention of hepatic metastases. GD has been shown to have a cytotoxic affect on human prostate cancer cells (PC9) in vitro, possibly acting through oxidative stress, and causing 95% cell death by an apoptosis [40].

Polysaccharide isolated from *Agaricus blazei* was shown to be an immune system stimulant, promoting body's natural defense mechanisms to fight a variety of infectious agents and conditions, including cancer. The immunostimulating activity and antitumor action of *Agaricus blazei* extracts were investigated in different laboratory models, including Sarcoma 180 and fibrosarcoma tumor-bearing mice[8,41].Seven polysaccharide fractions obtained from *A. blazei* fruit bodies were demonstrated to have antitumor

activity [42]. Analyses of physico-chemical properties of water-soluble polysaccharide fractions having high antitumor activity showed that their main components were β-(1→6), β-(1→3)-glucan, acidic β-(1→6), α-(1→4)- glucan, and acidic β-(1→6), α-(1→3)-glucan[42].A new antitumor polysaccharide, β-(1→2); β-(1→3)-glucomannan [43] active against Sarcoma 180 was recently separated from liquid cultured mycelium of *Agaricus blazei*.

An antitumor glucan (HA β-glucan) was isolated from the neutral polysaccharide fraction of a hot-water extraction of the edible mushroom *Pleurotus ostreatus* [44].
A few number of antitumor or immunomodulating polysaccharides of different chemical structure from higher Basidiomycetes mushrooms has been identified and main types are presented in Table 2.

5. Some protein containing antitumor polysaccharides

Not only polysaccharides, but also several protein-polysaccharide complexes and protein containing polysaccharides from different mushrooms have been found to show immunomodulatory and anti-cancer effects.

The protein bound polysaccharide (PSK) from turkey tail mushroom (*Trametes versicolor*) prevents liver cancer [45] and also useful for hepatitis B [46-47] and chronic active hepatitis[12]. The PSK [9] (commercial name Krestin) is widely used for cancer immunotherapy in Japan and other Asian countries, and it is considered that its antitumor effect is derived from its immunomodulating activity on the tumor-bearing host.
Another protein-polysaccharide complex, 0041, was obtained from submerged culture mycelium of *Agaricus blazei*; the main components of this polysaccharide are glucose and mannose [48].

Strong anti tumor activity has also been found in protein containing polysaccharide isolated from *Tricholoma species*[49]. Protein-containing polysaccharides extracted from fruiting bodies of a Chinese mushroom named *Pleurotus sajor-caju* [50]were fractionated and purified. 16 polysaccharide fractions were extracted and they were found to show antitumor activities (Table 3).The most antitumor-active water soluble fractions from *Pleurotus sajor-caju* are P-I protein-containing xyloglucan with Man:Gal:Xyl:Glc in the polysaccharide at a molar ratio 2:12:42:42,and P-III-2 protein-containing mannogalactan consisting of Xyl:Man:Gal (9:35:56 molar ratio).The most antitumor-active water -insoluble polysaccharides are P-IV-1 protein-containing xylan; P-V-1a protein-containing glucoxylan consisting of Glc:Xyl (40:44 molar ratio) and P-V-2a protein-containing xyloglucan consisting of Xyl:Glc (36:62 molar ratio).

The most antitumor-active water soluble fractions from *Pleurotus citrinopileatus* mushrooms [51] are a protein-containing heteropolysaccharide composed of glucose, mannose, arabinose, and galactose; a glycoprotein consisting of glycan:protein = 40:60 (w/w), with the glyco-chain composed of glucose, xylose, mannose, galactose, and fucose; another glycoprotein consisting of glycan:protein = 50:50 (w/w), and the glycan moiety consisting of glucose, galactose, xylose, mannose, and fucose. The most antitumor-active two water-insoluble polysaccharides are protein-containing β-D-glucans, composed of glucan : protein = 80:20, and 68:32 (w/w), respectively. The glucan moieties of both were almost all (1→3)-β-D-glucan, and their molecular weights were 68×10^4 and 40×10^4

Species	Polysaccharide
Agaricus blazei	α-(1→4)-: β-(1→6)- glucan α-(1→6)-: α-(1→4)- glucan β-(1→6)-: β-(1→3)- glucan β-(1→6)-: α-(1→3)- glucan β-(1→2); β-(1→3)-glucomannan
Ganoderma lucidum	β-(1→3)-glucuronoglucan Mannogalactoglucan
Grifola frondosa	β-(1→3): β (1→6)glucan Mannoxyloglucan Xyloglucan Mannogalactofucan
Lentinus edodes	β-(1→3): β-(1→6) glucan. Galactoglucomannan
Schizophyllum commune	β-(1→3)-glucan
Pleurotus sajor-caju	β-(1→3)-glucan Xyloglucan Mannogalactoglucan Mannogalactan Glucoxylan
Pleurotus citrinopileatus	Arabinogalactan (1→3)-β-D-glucan.

Table 2: Chemical structure of antitumor and immunomodulating polysaccharides of higher Basidiomycetes.

and another two water insoluble β-D-glucans with molecular weight 190×10^4 and 120×10^4 respectively. Both are composed of glucan:protein = 87:13 (w/w). Both glucan moieties are mainly (1→3)-β-D-glucan.

6. Structural features of polysaccharides exhibiting immunomodulation and antitumor properties

Anti-tumor activity of polysaccharide greatly depends of their chemical composition, configuration and physical properties. Antitumor activity is exhibited by a wide range of glycans extending from homopolymers to highly complex heteropolymers [52]. Differences in activity can be correlated with solubility in water, size of the molecules, branching rate and form. Although it is difficult to correlate the structure and antitumor activity of complex polysaccharides, some relationships can be drawn. It is obvious that structural features such as β-(1→3) linkages in the main chain of the glucan and additional β-(1→6) branch points are needed for antitumor action. β-glucans containing mainly (1→6)-linkages have less activity. High molecular weight glucans appear to be more effective than those of low molecular weight [16, 15, 53] However, obvious variations in antitumor polysaccharides have also been noted.

Polysaccharide	Total Sugar(%)	Protein (%)	MWx10^3	Component sugar (molar%)				Tumor inhibition ratio at 3 weeks(%)
				Glc	Xyl	Man	Gal	
P-I	75.6	24.1	278	43.7	42.3	1.9	11.8	84.8
P-I-α	69.5	23.5	420	24.1	72.5	2.7	0.7	53.1
P-I-β	67.0	26.3	68	53.5	27.2	1.6	17.7	49.8
P-II-α	52.6	42.1	10	56.0	40.7	3.3	-	59.4
P-II-β	84.6	6.9	24	-	16.2	-	83.8	31.7
P-III-1	67.7	27.5	11	71.6	5.5	-	22.9	48.7
P-III-2	76.1	16.2	15	-	9.4	34.6	56.0	74.6
P-III-3	22.5	75.3	10	50.0	14.9	13.1	22.0	34.5
P-IV-1	52.2	20.5	19	5.2	91.2	-	3.6	90.8
P-IV-2	50.5	44.1	17	9.2	86.2	-	4.6	8.0
P-IV-3	50.1	49.0	13	2.9	80.5	-	16.6	8.4
P-V-1a	15.4	70.5	87	39.8	43.7	7.8	8.7	76.9
P-V-1b	3.0	96.8	24	-	97.9	-	2.1	51.6
P-V-2	69.6	2.8	627	33.9	40.3	-	1.9	84.5
P-V-2a	68.8	2.5	700	62.2	35.5	-	2.3	11.0
P-V-2b	74.8	4.5	190	30.9	69.1	-	-	84.6

Table 3: Stucture and antitumor activity of *Pleurotus sajor-caju* fruit bodies polysaccharides against Sarcoma 180 in mice. (P-I to P-III, Water-soluble; P-IV to P-V, Water-insoluble)

Unlike β-(1→3)-glucans, α-(1→3)-glucuronoxylomannans, which are characteristic of jelly mushrooms are notstrongly dependent on molecular weight.

Antitumor polysaccharides may have other chemical structures, such as hetero-β-glucans[54] heteroglycans [55], β-glucan-protein [56], α-manno-β-glucan[54], α-glucan-protein and heteroglycan-protein complexes[57]. Linear low molecular weight α-(1→4)-glucans can also exhibit immunomodulatory and anticancer properties [57].Triple-helical conformation of β-(1→3)-glucan is known to be important for their immune-stimulating activity. When lentinan was denatured with dimethyl sulfoxide, urea, or sodium hydroxide, helical structure was lost while primary structure was not affected, but tumor inhibition properties were lowered with progressive denaturation[58].The investigation of schizophyllan [59] also gave same result which confirm the correlation between antitumor activity and triple helix structure.

7. Structural modification for improvement of antitumor and anti cancer properties

Different approaches exist for improvement of the antitumor activity of mushroom polysaccharides by chemical modification, which is also necessary to improve their clinical qualities, water solubility and ability to permeate stomach walls after oral intake. Two main procedures for chemical improvement are: modification of mushroom polysaccharides by Smith degradation (oxydo-reductohydrolysis) and activation by the method of formolysis[60-61].The most successful schemes for such methods have been developed for *Ganoderma lucidum*, *Grifola frondosa* and *Leucopaxillus giganteus*. Carboxymethylation is another chemical method that transforms β-glucans into a water-soluble form. Carboxymethylated glucan from Pleurotus ostreatus exhibited immunomodulatory effects, mainly increase phagocytic activity(paulik). A water-insoluble, alkali-soluble linear α-(1→3)-glucan obtained from fruiting bodies of *Amanita muscaria* and *Agrocybe aegerita* had little or no antitumor effect, while their carboxymethylated products showed potent antitumor activity [62-63].

Mushroom polysaccharides have also been used as immunoceuticals. Immunoceuticals are substances having immunotherapeutic effectiveness when administered orally. More than 50 mushroom species have yielded potential immunoceuticals that exhibit anticancer activity in vitro or in animal models. Six of these polysaccharides that have been investigated in human cancers include Lentinan, Schizophyllan, Active hexose correlated compounds (AHCC), Maitake D-fraction, polysaccharide-K and Polysaccharide-P[30].

8. Mechanisms of antitumor and immunomodulating action by mushroom polysaccharides

The biochemical mechanisms that initiate biological activity of mushroom polysaccharides are still not clearly understood. The antitumor action of polysaccharides requires an intact T-cell component; their activity is mediated through a thymus-dependent immune mechanism [64].

The polysaccharides from mushrooms do not attack cancer cells directly, but produce their antitumour effects by activating immune response through stimulation of NK-cells, T-cells, B-cells etc. in the host[65] Mushrooms are usually used as adaptogens and immunostimulants. An adaptogens is defined first by Brekhman as any substance that meets specific criteria for the category of natural derived “biological response modifier” (BRM) [7] or immunopotentiators. The term “biological response modifiers” have been defined as those agents that modify the host’s biological response by stimulation of the immune system, which may result in various therapeutic effects. The criteria for biological response modifiers are (1) cause no harm and place no additional stress on the body,(2)the body to adapt to various environmental and biological stresses,(3) exert a nonspecific action on the body, supporting some or all of the major systems, including nervous, hormonal, and immune systems, as well as regulatory functions[66].

Lentinan appears to act as a host defence potentiator (HDPs) which is able to restore or augment the responsiveness of host cells to lymphocytokynes, hormones, and other biologically active substances by stimulating maturation, differentiation or proliferation of cells involved in host defence mechanism [67]. HDP s are functionally different from BRMs. Lentinan is thus able to increase host resistance against various kinds of infectious disease including AIDS.Lentinan is known to be able to restore the suppressed activity of helper T-cells in the tumor-bearing host to their normal state, leading to

complete restoration of humoral immune responses [53]. The same effect is true for PSK, while it has no substantial effect on immune responses of the host under normal conditions. The biological action of Lentinan has been demonstrated by Chihara et al, and Possible mode of action of β-D-glucan as biological response modifier (BRM) was established by Mizuno T. [68] and is shown in the schematic diagram Fig. 1.

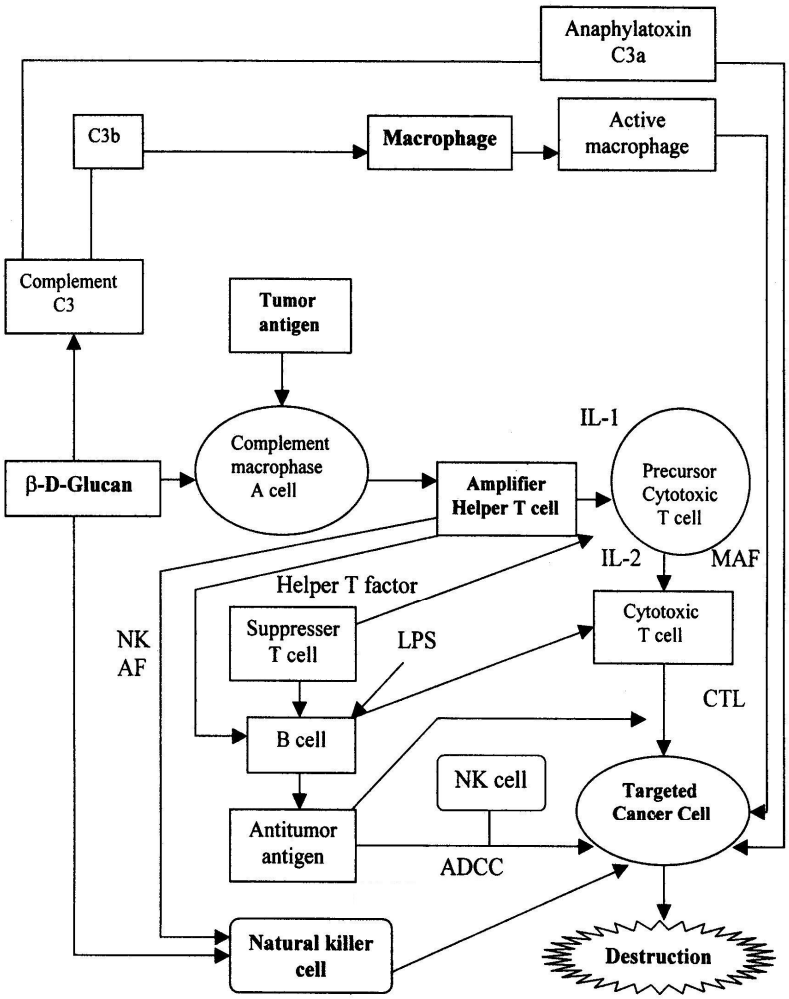

Figure 1: Schematic representation showing the mode of action of polysaccharide, β-glucan as biological response modifier to target cancer cell. NK: Natural Killer cell; AF: Antibody Formation; LPS: Liver Protein Serum; ADCC: Antibody Dependent Cell mediated Cytotoxicity; CTL: Cytotoxic T-Lymphocyte; MAF: Macrophage Activating Factor; IL-1: Interlukine 1; IL-2: Interlukine 2.

9. Conclusion

Higher Basidiomycetes mushrooms contain biologically active polysaccharides in their fruit bodies, culture mycelia or culture broth. Mushroom polysaccharides are of different chemical composition, mainly belonging to the group of β-glucans The antitumor activity of different mushroom polysaccharides are characterized by their molecular weight, degree of branching and higher structure. The main structural feature of polysaccharide is β-(1→3) linkages in the main chain of the glucan with additional β-(1→6) branches. The β-glucans containing (1→6) linkages have less activity. The glucans of high molecular weight is more effective than those of low molecular weight. Triple-helical conformation of β-(1→3)-glucan is known to be important for their immune-stimulating activity. The chemical modification is necessary in many cases to improve not only the antitumor activity of mushroom polysaccharides, but also their clinical qualities, water solubility and the ability to permeate stomach walls after oral ingestion. The mechanism of anti–tumor action of polysaccharide is still not completely clear. The polysaccharides do not attack cancer cells directly, but produce their anti-tumor effects by activating macrophages such as NK-cells,T-cells,B-cell etc in the host. The immunomodulating action of mushroom polysaccharides is especially valuable as a biological response modifier (BRM), prevention of metastatic tumors and as a co-treatment with chemotherapy.

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REFERENCES

1. C. Hobbs, *Medicinal mushroom*, Botinica Press, Santa Cruz CA 251 p, 1957.
2. P.V. Wasson and R.G. Wasson, *Pantheon Books*, New York, 433p. 1957.
3. S.P.Wasser, A.LWeis and E.Nevo, Shiitake mushrooms [*Lentinus edodes* (Berk) sing] *Medicinal mushrooms*, Peledfus, Haifa, 39p, 1997.
4. S.P.Wasser, A.L.Weis and E.Nevo, Reishi mushroom [*Ganaoderma lucidum* (Curt.:Fr.) P.Krst] *Medicinal mushroom*, Peledfus, Haifa, , 96p. 1997.
5. E.H. Lucas, R.U. Byerrum, D.A. Clarke,R.L. Ringler, J.A. Stevens and C.C. Stock, Tumor inhibition in Boletus edulis and other Holobasidiomycetes, *Antibiotic Chemotherapy*, 7 (1957) 1-4.
6. V.E.Ooi and F.Liu, Immunomodulation and anti cancer activity of polysaccharide -protein complexes, *Current Medicinal Chem*istry, *7* (2000) 715-729.
7. S.P.Wasser and L.A.Weis, Medicinal prpperties of substances occurring in Higher Basidiomycetes mushrooms:current perspectives, *International Journal of Medicinal Mushrooms*, 1(1999) 31-62.
8. H.Kawagishi, R.Inagaki, T.Kanao and T.Mizuno, Fractionation and antitumor activity of the water-insoluble residue of *Agaricus blazei* fruiting bodies, *Carbohydrate Research*, 186 (1989) 267.
9. C.Iguchi, Y.Nio, H.Takeda, K.Yamasawa, N.Hirahara, T.Toga, M.Itakuru and K.Tamura, Plant polysaccharide PSK: cytostatic effects on growth and invasion;

modulating effect on the expression of HLA and adhesion molecules on human gastric and colonic tumor cell surface, *Anticancer Research*, 21 (2001) 1007-1013.
10. J.Ying, X.Mao, Q.Ma, Y.Zong and H.Wen, Icons of medicinal fungi from China, Science Press, Beijing, 1987.
11. C.Hobbs, *Medicinal mushrooms: an exploration of tradition, healing and culture*, Botinica Press, Santa Cruz, Calif, 1995.
12. P.Stamets, *Growing gourmet and medicinal mushrooms*, 3rd edn, Ten Speed Press, Berkeley, Calif, 2000.
13. T.Ikekawa, H.Saitoh, W.Feng, H. Zhang, L.Li and T. Matsuzawa, Antitumor activity of extracts and polysaccharides, *Chemical and pharmaceutical bulletin*, 40 (1992) 1954-1957.
14. T.Ikekawa,Beneficial effects of edible and medicinal mushrooms in health care, *International Journal of Medicinal Mushrooms*, 3 (2001) 291-298.
15. T.Mizuno, Development of antitumor polysaccharides from mushroom fungi,Foods *Food Ingred J. Jpn*,167 (1996) 69-85.
16. T.Mizuno, The extraction and development of antitumor active polysaccharides from medicinal mushrooms in Japan, *International Journal of Medicinal Mushrooms*, 1 (1999) 9-29.
17. S.Hiroshi and M.Takeda, Diverse biological activity of PSK (Krestin),a protein-bound polysaccharide from *Coriolus versicolor* (Fr.) Quel. The Chinese University Press, Hong Kong, (1993) 237-245.
18. T.I. Williams and H. Weil, The Historical phases of chromatography, *Arkiv. Kemi., 5*, (1953) 283.
19. S.W. York, K.A. Darvill, M. McNeil, T. T. Stevenson and P. Albersheim, Isolation and characterization of a plant cell walls and cell wall components, *Methods in Enzymology*, 118 (1985) 33-40.
20. F.A. Gilbert and R.F. Robinson, Food from Fungi, *Economic Botany* 11 (1957) 126-145.
21. W.M. Breene, Nutritional and medicinal value of specialty mushroom, *Journal of Food Protection*, 53 (1990) 883-894.
22. K. Ghosh, K. Chandra, S.K. Roy, S. Mondal, D. Maiti, D. Das, A.K. Ojha and S.S. Islam, Structural investigation of a polysaccharide (Fr. I) isolated from aqueous extract of an edible mushroom *Volvariella diplasia*, *Carbohydrate Research*,343(2008) 1071-1078.
23. W. Lintzel, The Nutritional value of edible mushroom proteins, *Biochemistry* 308 (1941) 413-419.
24. H. Fitzpatrick, B. Esselen and E. Weir, Composition and nutritive value of mushroom protein, *J. Am. Dietet. Assoc*., 22 (1946) 318-323.
25. R.F. Robinson and R.S. Davidson, The large scale growth of higher fungi, *Advanced Applied microbiology*, 1(1959) 261-278.
26. V. Brower, Nutraceuticals: Poised for a healthy slice of the health care market? *Nature Biotechnology*, 16 (1988) 728-731.
27. S.H. Zeisel, Regulation of nutraceuticals, *Science*, 285 (1999) 1853-1855.
28. S.T. Chang and J.A. Buswell, Mushroom nutriceuticals, *World Journal of Microbiology and Biotechnology*, 12 (1996) 473-476.
29. A.S. Daba and O.U. Ezeronye, Anti-Cancer effect of polysaccharides isolated from

higher basidiomycetes, *African Journal of Biotechnology,* 2 (2003) 672-678.
30. G. Chihara, J. Hamuro, Y.Y. Maeda, Y. Arai and F. Fukuoka, Fractionation and purification of the polysaccharides with marked antitumor activity, especially lentinan, *Lentinus edodes*, *Cancer Research,* 30 (1970) 2776-2781.
31. H. Furue and I. Kitoh, Phase-III Study of lentian, *Japanese Journal of Cancer and Chemotherapy*, 8 (1981) 944-960.
32. T. Taguchi, H. Furue, H, T. Kimura, T. Kondo, T. Hattori, T. Itoh and N. Osawa, End Point results of phase-III study of lentinan, *Japanese Journal of Cancer and Chemotherapy*, 12 (1985) 366-380.
33. T. Taguchi, H. Furue, H, T. Kimura, T. Kondo, T. Hattori, T. Itoh and N. Osawa, End point result of a randomized controlled study on the treatment of gastrointestinal cancer with a combination of lentinan and chemotherapeutic agents, *Excerpta. Medical,* 6 (1985) 151-165.
34. H. Furue, Clinical evaluation of schizophyllan(SPG) in gastric cancer-randomised controlled studies, International Journal of Immunopharmacology, 7 (1985) 333-336.
35. Y. Kimura, H. Mizuno, K. Satake, H. Tahara and M, Tsukuda, Clinical evaluation of sizofilan as assistant immunotherapy in treatment of head and neck cancer, *Acta Otolargynzol*. 511 (1994) 192-195.
36. K. Okamura, T. Kinukawa, Y. Tsumura, T. Otani, T. Itoh, H. Kobayashi, O. Matsuura, M. Kobayashi, T. Fukutsu and S. Ohshima, Clinical evaluation of schizophyllan, *Cancer,* 58 *(*1986) 865-872.
37. K. Okamura, T. Kinukawa, Y. Tsumura, T. Otani, T. Itoh, H. Kobayashi, O. Matsuura, M. Kobayashi, T. Fukutsu and S. Ohshima, Adguvant immunochemotherapy: two randomized controlled studies of patients with carnivals cancer, *Biomedical Pharmacotherapy*, 43 (1989) 170-181.
38. S. Kurashiga, Y. Akuzawa and F. Eudo, Effects of lentinus Edodes, Grifola frondosa and Pleuratus Ostreatus administration on cancer out break and activities of Macrophages and Lymphocytes in mice treated with a carcinogen, N-butyl-N-butanoinitrosoamine, *Immunopharmacology and Immunotoxicology,* 19 (1997) 175-185.
39. I. Nishida, H. Nanba and H. Kuroda, Antitumor activity exhibited by orally administered extracts from fluid body of Grifola Frondosa(Maitake), *Chemical and pharmaceutical bulletin,* 36 (1988) 1819-1827
40. S.A. Fulleroton and A.A. Samadi, Induction of apoptosis in human prostatic cancer cells with beta glucan(Maitake mushroom polysaccharide), *Mol. Urol.*, 4 (2000) 7-13.
41. T. Mizuno, R. Inagaki, T. Kanao, T. Hagiwara, T. Nakamura, H. Ito, K. Shimura, T. Sumiya and A. Asakura, Antitumor activity and some properties of water insoluble hetero-glycans from “Himematsutake”, the fruiting body of *Agaricus blazei* Murrill, *Agric. Biol. Chem.* 54 (1990) 2897-2906.
42. T. Mizuno, T. Hagiwara, T. Nakamura, H. Ito, K. Shimura, T. Sumiya and A. Asakura, Antitumor activity and some properties of water-soluble polysaccharides from” Himematsutake”, the fruiting body of *Agaricus blazei* Murrill, *Agri. Biol. Chem.* 54 (1990) 2897-2906.
43. H. Tsuchida, M. Mizuno, Y. Taniguchi, H. Ito, M. Kawade and K. Akasaka, Glucomannan separated from *Agaricus blazei* mushroom culture and antitumoragent

containing as active ingredient, *Japanese Patent*, 11-080206, 26th March 2001.
44. Y. Yoshioka, R. Tabeta, H. Satio, N. Uehara and F. Fukuoka,Antitumor polysaccharides from *P. Ostreatus*(Fr.) Quel.: Isolation and structure of a [beta]-glucan, *Carbohydrate Research*., 140 (1985) 93-100.
45. N. Wang, Carcinogenic course of rat liver cancer induced by a flaxtoxin B 1 and effect of *Polyporus versicolor* polysaccharide on carcinogenic action, *Tianjin Yiyao*, 17 (1989) 534-536.
46. Z. Lin and Y. Huang, Protective action of Lentinan against experimental liver injuries, *Journal of Beijing Medical University*, 19 (1987) 93-95.
47. Y. Mizoguchi, H. Katoh, K.Kobayashi, S.Yamamoto and S.Morisawa, Protection of Liver cells against experimental damage by extract of cultured *Lentinus edodes* mycelia (LEM), *Gastroenterology Japan*, 22 (1987) 459-464.
48. M. Hikichi, E. Hiroe and S. Okubo, Protein polysaccharide 0041, *European Patent 0939082*, 9 January 1999.
49. H.X. Wang, W.K. Liu, V.E. Ooi and S.T. Chang, Immunomodulatory and antitumor activities of a polysaccharide-peptide complex from a mycelia culture of Tricholoma sp., a local edible mushroom, *Life Science*, 57 (1995) 269-281.
50. C. Zhuang, T. Mizuno, A. Shimada, H. Ito, C. Suzuki, Y. Mayuzumi, H. Okamoto, Y. Ma and H. Li, Antitumor protein containing polysaccharide from a Chinese mushroom Fengweigu or Houbitake, *Pleurotus sajor-caju* (Fr.) Sing, *Biosci. Biotechnol. Biochem*., 57 (1993) 901-906.
51. J. Zhang, G. Wang, H. Li, C. Zhuang, T. Mizuno, H. Ito, C. Suzuki, H. Okamoto and J. Li, Antitumor polysaccharides from Chinese mushroom, "Yuhuahgmo", the fruiting body of *Pleurotus citrinopileatus*, *Biosci. Biotechnol. Biochem*. 58 (1994) 1195-1201.
52. V.E.C. Ooi and F. Liu, A review of pharmacological activities of mushroom polysaccharides, *International Journal of Medicinal Mushrooms*, 1 (1999) 195-206.
53. T. Mizuno, Bioactive substances in *Hericium erinaceus* (Bull.: Fr.) Pers.(Yamabushitake),and its medicinal utilization, *International Journal of Medicinal Mushrooms*, 1 (1999) 105-119.
54. T. Mizuno, H. Saito, T. Nishitoba and H. Kawagashi, Antitumor active substances from mushrooms, *Food Rev. Int.*, 11 (1995) 23-61.
55. Q.P. Gao, R.Seljelid, H.Q. Chen and R. Jiang,Characterization of acidic heteroglycans from *Tremella fuciformis* Berk, with cytokine stimulating activity, *Carbohydr. Research*, 288 (1996) 135-142.
56. H. Kawagishi, T. Kanao, R. Inagaki, T. Mizuno, K. Shimura, H. Ito, T. Hagiwara and T.Hakamura, Formulation of a potent antitumor (1→6)-beta-D-glucan-protein complex from Agaricus blazei fruiting bodies and antitumor activity of the resulting products, *Carbohydr.ate Polymer*, 12 (1990) 393-404.
57. K.Matsushita, Y.Kuramitsu, M.Obara, M.Kobayashi and Y.Q.Li, Combination therapy of active hexose correlated compound plus UFT significantly reduces the metastasis of rat mammary adenocarcinoma, *Anti Cancer Drugs*, 9 (1998) 343-350.
58. Y.Y.Maeda, S.T.Watanabe, C.Chihara and M.Rokutanda, Denaturation and renaturation of a β-1,6:1,3-glucan,lentinan, associated with expression of T-cell-mediated responses, *Cancer Research*, 48 (1988) 671–675.

59. T.Yanaki, W.Ito and K.Tabata, Correlation between antitumor activity of schizophyllan and its triple helix, *Agric. Biol. Chem*., 509 (1986) 2415-2416.
60. T. Mizuno, P. Yeohlui T. Kinoshita, C. Zhuang, H. Ito and Y. Mayuzumi,Antitumor activity and chemical modification of polysaccharides from Niohshimeji mushroom, *Tricholoma giganteum*, *Biosci. Biotechnol. Biochem.*, 60 (1996) 30-33.
61. T.Mizuno, C.Zhuang, K.Abe, H.Okamoto, T.Kiho, S.Ukai, S.Leclerc and L.Meijer, Antitumor and hypoglycemic activities of polysaccharides from the sclerotia and mycelia of *Inonotus obliquus*, *International Journal of Medicinal Mushrooms*, 1 (1999) 301-316.
62. T. Kiho, I.. Yoshida, M. Katsuragawa, M. Sakushima, S. Usui and S. Ukai, Polysaccharides in fungi: XXXIV. A polysaccharide from the fruiting bodies of *Amanita muscaria* and the antitumor activity of its carboxymethylated product, *Biol. Pharm.Bull*., 17 (1994) 1460-1462.
63. I. Yoshida, T. Kiho, S. Usui, M. Sakushima and S. Ukai, Polysaccharides in fungi: XXXVII.Immunomodulating activities of carboxymethylated derivatives of linear (1→3)-alpha-D-glucans extracted from the fruiting bodies of *Agrocybe cylindracea* and *Amanita muscaria, Biol. Pharm.Bull*., 19 (1996) 114-121.
64. A.T. Borchers, J.S. Stern, R.M. Hackman, C.L. Keen and E.M. Gershwin, Mushrooms, tumors and immunity *Soc. Exp. Biol. Med.*, 221 (1999) 281–293.
65. S.P. Wasser, Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides, *Appied Microbiology and Biotechnology*, 60 (2002) 258-274.
66. Brekhman II, *Man and biologically active substances.* Pergamon Press, New York, 1980.
67. G. Chihara, J. Hamura,Y.Y. Maeda,T. Shilo, T. Suga, N. Takasuka and T. Sasaki, Anti tumor and metastasis inhibitory activities of lentinan as an immunomodulator : an over view, *Cancer Detect. Prev* (Supl)., 1 (1987) 423-443.
68. T. Mizuno, Medicinal properties and clinical effects on *Agaricus blazei* Murr., *International Journal of Medicinal Mushrooms*, 4 (2002) 267-290.