Anti-tumor and Immuno-stimulating Activity of Fungal Polysaccharides

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Introduction

Enormous effort has been made to treat tumor. There are three kinds of methods for treatment of tumor. Chemotherapy, radiotherapy, and surgical operation has been main trend. Those methods, however, have some undesirable side effects. One of most fateful problems of cancer is recurrence after surgical resection of the primary tumor, which is caused by the presence of micrometastasis. And postoperative chemotherapy has the disadvantage such as insufficient effectiveness and a high level of toxic side effects. Recently, biological therapy become a new approach to overcome these side effect. In this method, one way is to stimulate or modulate immune function of human by immuno-stimulating or immuno-modulating molecules. The other way is through molecule with anti-tumor compounds. Characteristic property of those compound lies in natural products and its low toxic potential.

Until now, low molecular weight molecules and high molecular weight substances were found to have anti-tumor and immuno-modulating activity. Previously polysaccharides have been received much attention because of adhesives, food additives or animal foods (Whistler et al., 1976). In effort of developing new anti-tumor substances with low toxicity, numerous polysaccharides from yeast, algae, bacteria, higher plants and especially fungi have been investigated for anti-tumor and immuno-modulating activities. Thus the high molecular weight molecule was reported to have anti-tumor activity through host mediated immunity. In this brief article, attention will be paid to polysaccharides which is especially fungal origin.

Fungal Polysaccharides with Anti-tumor and Immuno-modulating Activities

Most of anti-tumor active and immuno-modulating polysaccharides have been isolated from Basidiomycetes (Table 1). Besides Basidiomycetes, fungi belonging to the Ascomycetes and Oomycetes has been also reported to have polysaccharides with anti-tumor activity against various animal tumors and even human xenografts. The source of polysaccharides are various from fruiting body, mycelium to culture filtrate. Most of anti-tumor polysaccharides are β-glucan. These polysaccharides consist of β-1,3 glucosidic linkages with β-1,6 branched chains. Even though the average structure are similar, the anti-tumor activities of these β-D-glucan are different. Now, lentinan from *Lentinus edodes* (Chihara et al., 1969) and *Schizophyllan* from *Schizophyllum commume* (Tabata et al., 1981), are in clinical use. Recently, chitin, chitosan, and chitooligosaccharides also has been reported to have anti-tumor activities (Suzuki et al., 1986). In next section, a general discussion will be given on what factoris important in anti-tumor activity of polysaccharides.

Table 1. Some immuno-modulating anti-tumor polydaccharides from fungi

Fungus	*Source*	Structure	M.W.
Basidiomycetes			
Araricus bisporus	Fruiting body	β-glucan	
Coriolus versicolor	Fruiting body	β-glucan	
	Mycelium	β-glucan-protein(PSK, Krestin)	2.0×106
Lentinus edodes	Fruiting body	β-glucan	1.0×106
	Mycelium	β-mannan-peptide	7.8×104
	Medium	Hetero-glucan-protein	
Pholiota nameko	Fruiting body	Galacto-β-glucan	
Schizophyllum commune	Medium	β-glucan(Schizophyllan)	4.4×106
Vovariella volvacea	Fruiting body	α-Manno-β-glucan	4.0×105
Ascomycetes			
Sclerotium glucanicum	Fruiting body	β-glucan(Scleroglucan)	
Cordyceps ophioglossoides	Medium	β-glucan	2.0×106
Oomycetes			
Phytophthora parasitica	Mycelium	β-glucan	2.0×105

Structural Factors which have an Effect on Anti-tumor Activity of Polysaccharides

Triple Helix Conformation

It was reported that the anti-tumor activity of lentinan or pachyman is lost by denaturation with urea or dimethyl sulfoxide (DMSO), but the activity is regained by removal of these substances (Chihara et al., 1970). Also, Misaki et al (1986) showed DMSO-extracted glucan showed a relatively low activity. Although the glucan is structurally similar to anti-tumor active lentinan and schizophyllan, the low activity might be attributed to the incomplete formation of triple strands due to the DMSO-extraction. Schizophyllan dissolves as a rigid triple helix in pure water at 25℃ and as a single randomly coiled chain in dimethyl sulfoxide (Yanaki et al., 1986) (Fig. 2). Schizophyllan in a single chain did not show anti-tumor activity. Those facts supported Chihara's hypothesis that the ordered structure of a glucan is responsible for its antitumor activity (Chihara, 1977).

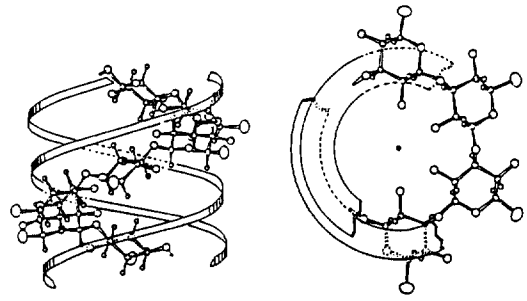

Fig. 1. Triple helix conformation of β-(1-3)-D-glucan.

Degree of Branching and Branching Component

Anti-tumor activity of OL-3 from *Omphalia lapidescence* against the solid form of sarcoma 180 in ICR mice was almost negative, which seemed to arise from its highly branched structure (Saito et al., 1990). On the contrast, linear β-(1,3)-D-glucan also had essentially no activity against the tumor. Buta component from *Pleurotus ostreatus* (Fr) Quel showed anti-tumoractivity. The polysaccharide is consisted of a skeleton of β-(1,3)-linked glucan with galactose, mannose as branches (Yoshioka, 1975). Among the branched (13)-b-glucan, glucans with D.B. (degree of branching) 1:5, 1:2, and 1:3.4 shows 97%, 34%, and 48.6% anti-tumor activities, respectively. There-

fore branched glucose might not be a require ment for anti-tumor activity of β-glucan. Instead whatever residues branches are, the degree of branching is more important to the activity.

The effect of branching residue(s) on anti-tumor activity was further investigated by chemical modification of the residues. When D-glucose residues of the branched glucan were modified to the 3,6-anhydro D-glucose residue by partial sulfation and then alkali treatment, the resulting modified polysaccharide showed essentially no anti-tumor activity. Also, introduction of hydrophilic polyol or glyceryl groups give enhancement effects, whereas introduction of hydrophobic groups as epoxy groups may result in the opposite effect (Kishida et al., 1992). Those results suggest that substituted groups located outside the backbone chains must play an important role in exhibiting anti-tumor action somehow. In addition, it is not ruled out the possibility that such effect might be partly due to increase in the solubility.

Polyhydroxyl Groups

Pestalotan from *Pestalotia* sp. 815 shows moderate growth-inhibitory activities against mouse-implanted tumors. However, when the b-glucosyl groups of the side chains were modified by periodate oxidation and borohydride reduction, the resulting water-insoluble b-glucan polyol exhibited potent anti-tumor activities (Misaki et al., 1984). Misaki et al (1981) also showed that the anti-tumor activity decreased with reduction in the polyol content, and its complete removal resulted in elimination of the activity. Those facts confirm that the attachment of many polyhydroxyl groups to the β-(1,3)-linked glucan backbone gives a remarkable enhancement effect on the anti-tumor activity of the branched b-glucan. It is thus most feasible that the distribution of numerous polyhydroxyl groups outside of the b-(13)-D-glucans may afford augmentation of the immuno-stimulating potency of the host.

Molecular Weight

When native schizophyllan (M.W. 4.4×10^6) from *Schizophyllum commune* was depolymerized by ultrasonic irradiation, two sonic-degraded schizophyllans with molecular weight 5.6×10^5, and 2.3×105 were obtained, respectively. The sonic-degraded schizophyllan has the same chemical structure as native schizophyllan, except for its lower molecular weight. There is no difference between these polysaccharides preparations in their activities against tumor (Tabata et al., 1981). When ithas a molecular weight lower than 5×10^4 however, the tumor inhibition activity was lost. Also, it was reported that aproportion of triple helices decreases monotonically with decreasing molecular weight. Thus in case of schizophyllan, higher molecular weight than 5×10^4 and helix conformation are required for showing anti-tumor activity. Moreover, reduction in size by acid-degradation drastically reduced the anti-tumor activity.

Most polysaccharides with anti-tumor activity is high molecule above 100,000 of molecular weight. It has been known that for a long time molecular weight is a critical parameter in the antigenicity of a molecule. Thus, proteins with a molecular weight of less than 10,000 do not stimulate the formation of antibody. Kabat and Bezer (1958) showed that dextrans with an average molecular wight of 90,000 or higher were immunogenic inman; dextrans with an average molecular weight of 50,000 or lower were not immunogenic. The accumulated evidence points to the generality that $45{,}000\pm5{,}000$ is the molecular weight above which polysaccharides are immunogenic and below which their immunogenicity falls off rapidly (Bishop and Jennings, 19 82).

Therefore, Host-mediated anti-tumor and immuno-stimulating activities of fungal polysaccharide seem to require molecular weight-limit. Also the limit of a polysaccharide appears to relate to its structure. For polysaccharides above molecular weight limit, structure plays more important

Table 2. Effects of anomeric configuration type and monosaccharide sugar in some polysaccharides on anti-tumor activies against sarcoma 180 solid tumor. (modified from Misaki et al, 1981)

Polysaccharide	Glycosidic linkage		Dose (mg/kg×days)	Inhibition ratio (%)
	Back bone	Side chain		
Schizophyllum commune				
Schizophyllan	β-(1-3)-Glc	β-(1-6)-Glc	1×10	95.1
Auricularia auricula-judae				
β-D-glucan	β-(1-3)-Glc	β-(1-6)-Glc	8×10	96.6
Acidic polydaccharide	β-(1-3)-Man	β-(1-2)-Xyl	10×10	18.5
Streptococcus salivarius				
α-D-glucan	α-(1-3)-Glc	α-(1-6)-Glc	30×10	18.8
Brasenia schreberi				
Acidic polysaccharide	β-(1-3)-Gal β-(1-3)-Man	(1-3)-Rha (1-3)-Fuc	30×10	−26.4

role in anti-tumor activity rather than its size. However, in case of polysaccharides below molecular-weight limit, both structure and size should be considered as factors that affect the activities.

Anomeric Configuration Type and Monosaccharide Components

α-glucan, fucogalactan, mannofucogalactan from fruiting body of *Ganoderma lucidum* were anti-tumor inactive polysaccharides (Mizuno et al., 1984). Also mannogalatan did not inhibit tumor growth. Thus glucan with configuration and other polysaccharides with b-configuration such as mannan and galactan seems to not have anti-tumor activity (Table 2).

But the tendency is not true for peptidomannan (KS-2) from mycelia of *Lentinus edodes*. It has also linked mannose and its molecular weight is $6.0 \sim 7.5 \pm 10^4$. When administered orally, KS-2 showed an effective suppression of tumor growth as dose levels between 1 and 100 (mg/kg). Therefore, KS-2 is unique in its chemical structure because it is primarily a peptide containing ma nnan and has a relatively small molecular size. On the other hand, it was reported that neither lentinan nor schizophyllan administered orally suppressed tumor growth. Thus KS-2 seems again to be unique in its biological activity. These data indicated that KS-2 is a novel class of anti-tumor substances (Fuji et al., 1978).

Linkage Type

Chemically synthesized branched β-(13)-glucans with D-arabinofuranosyl or D-mannosyl side chains exhibited strong anti-tumor activity, as expected. But branched β-(14)-D-glucans having similar side chains had no anti-tumor effect (Matsuzaki et al., 1986). And a polysaccharide from fruiting body of *Grifola frondosa* inhibits the growth of an allogeneic tumor in mice through the activation of cellular immunity (Ohno et al., 1984). Its structure is β-(16)-linked glucan main chain with 1,3-linked branches. Those facts indicated that (13)- and (16)-linkage types are also important in anti-tumor activity.

Protein Moiety

Protein component of Krestin or PSK is reportedly essential to anti-tumor property (Hirase et al, 1976). But most of anti-tumor polysaccharide is glucan rather than a glycoprotein or a peptidoglycan. Thus sugar moiety seems to be primarily responsible for anti-tumor activity rather than protein and other moiety.

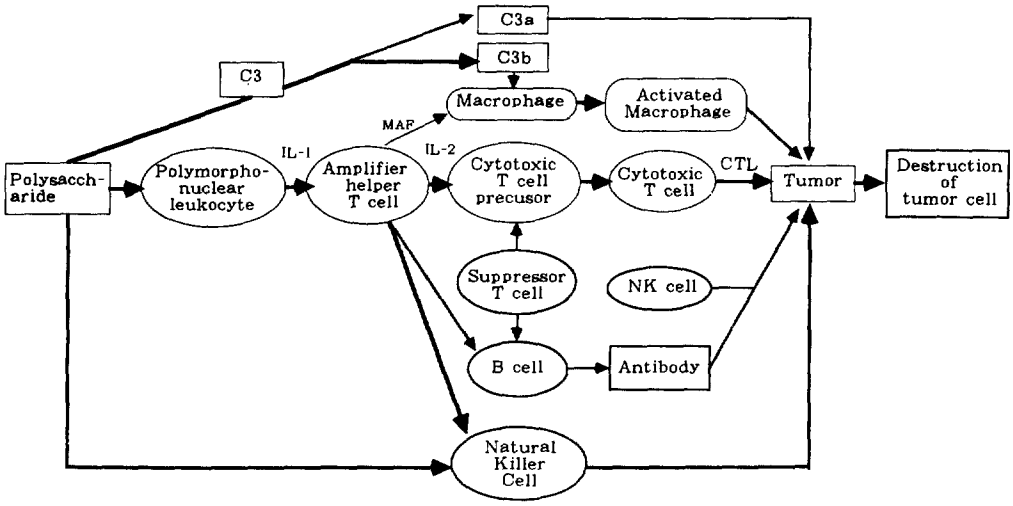

Fig. 2. Schematic mechanism of host-mediated immunitiy by immuno-modulating antitumor fungal polysaccharide (modified from Mizuno, 1994).

Immunology of Polysaccharides

To understand immuno-modulating mechanism of polysaccharide, we need the knowledge of immunology. The immune system consists of a highly complex network of cells and molecules whose special characteristics is pattern recognition. The cells (lymphocytes and macrophages) and molecules (antibody and complement) circulate through the blood stream and lymphatic system. These three kinds of cells, T, B lymphocytes, and macrophage cooperate and interact in a highly complex way to produce an immune response. Those responses are classified into humoral response due to antibody and cell-mediated response. The T lymphocytes have been shown to be responsible for cell-mediated response, whereas the B lymphocyte produce antibodies and are thus responsible for the humoral response.

Both kinds of lymphocytes require the presence of macrophage to give a functional immune response. Major role of macrophage seems to be one of presenting the antigen such as polysaccharide to the lymphocytes. The macrophage has several types of surface receptors for molecules such as antigen, antibody, and third component of complement (C3). Through these receptors, macrophage can carry out the function of presenting. It is now recognized that activation of C3 step by polysaccharide does not require specific antibody like classical pathway. Also, immune response to polysaccharide antigens has been found to be T-cell independent (Howard et al., 1971). In other words, the activation of B cell to secrete immunoglobulin does not require the cooperation of T cell. An important property of IgM produced by response to a T-cell independent antigen such as a purified polysaccharide is that memory cells do not appear to be generated. This immune response is thought to be due to T-cell independence of polysaccharides antigens. Thus the participation of T-cell is essential for the induction of IgG antibodies and memory cells (Barley-Mullens, 1980).

In a linear polysaccharide, the immunological specificity resides primarily in the terminal sugar residues and extends along the polysaccharide chain. But for branched polysaccharide antigens, immunodominant sugars are invariably those that

formthe branches.

Immuno-modulating Mechanism of Polysaccharide

The mechanisms by which fungal polysaccharides enhance immune response are poorly understood (Chihara, 1984). Yet several investigator have describe phenomena which may contribute to the immuno-stimulating activity. Phagocytosis may be stimulated to increase their rate of phagocytosis and in the process be stimulated to release cytokines such as interleukin-1 which could increase the rate of the immune response. In addition, macrophage or lymphocytes may be stimulated to release interferon-τ(IFN-τ or other cytokines which promote enhanced activity.

Anti-tumor Mechanism of Polysaccharide

Host-mediated anti-tumor activity of fungal polysaccharides has been already reported. It has known that stimulation of acquired immunity, phagocytosis of macrophage, and growth of no nspecific T cells are involved in the anti-tumor activity of fungal polysaccharides. Also, it is widely accepted that activated macrophages, cytotoxic T cells, natural killer cells, and killer T cells usually play important roles in tumor immunity. Fig. 2 showed the proposed mechanism of host-mediated immunity by fugal polysaccharides (Mizuno, 1994). But the exact anti-tumor mechanism waits for further study. The following is the known roles of each cells in anti-tumor activity of polysaccharides.

Macrophage Involvement in Anti-tumor Activity

Some kinds of fungi found to be very strong inducers for the activation of macrophages. Macrophage could be activated to become cytotoxic by two different mechanisms i) direct activation by polysaccharides and ii) activation of polymorphonuclear leukocytes (PMN) which produce IL-1. Holmberg et al (1987) have shown that macrophage-mediated anti-tumor activity may be limited to certain tissues. And the potential consequences of cellular interactions between polysaccharide and phagocytic cells are numerous. For example, zymogen treatment of macrophage induces thrombosane A synthesis (Bienkowski et al., 1989), reactive oxygen intermediates, arachidonic acid, and prostaglandin synthesis. But the role of these compounds as mediators of macrophage-anti-tumor cytotoxicity is controversial (Domer and Garner, 1991). It may be noted that Czop and Austin (1985) recently reported the presence of a receptor for β-D-glucan on human monocytes, in relation to stimulation of macrophage activities by zymosan.

It has also been reported that 3H-SPG (Schizophyllan) is mainly accumulated in the reticuloendothelial cells such as macrophages and in the tumor capsular belt area of tumor-inoculated ICR mice (Tabata et al., 1990). When 3H-labeled glucan-polyol was intraperitoneally administered to tumor-bearing mice, the radioactivities appeared in the serum 12~23 hr after the injection, and the serum contained a tumor-inhibiting factor. Chemical identification of the inhibiting factor must await a sufficient amount of serum. But it was suggested that it seems to be glycoprotein or protein in nature, although it might not be purified yet (Kishida et al., 1989).

Natural Killer (NK) Cell Involvement in Anti-tumor Activity

Natural killer cell activity has been noticeable in mice and in human peripheral blood mononuclear cells (PBMC) in response to exposure to fungal polysaccharide (Ausiello et al., 1989). In mice, heat- or merthiolate-killed intact *C. albicans* have been shown to induce NK activity, as well as treatment with mannoprotein from the fungus (Scaringi et al., 1988).

Direct Interaction Between Fungal Polysaccharide and Tumor Cells

While it is clear that host effector mechanisms such as activated macrophages or enhanced NK cell function are important in reducing the tumor burden, it is also clear that tumor cell shave different sugar receptors, i.e., lectins or lectin-like molecules which provide for binding of carbohy-

Table 3. Structural factors affecting anti-tumor activity of fungal polysaccharides.

1. Triple helix conformation
2. Degree of branching and branching components
3. Polyhydroxyl groups
4. Molecular weight
5. Anomeric configuration type and monosaccharide components
6. Glycosidic linkage type
7. Other component except sugar

drates by tumor cells. Gabius et al (1985) have proposed that such lectins may play a role in the establishment of metastasis or other cell-cell interactions critical to tumor sustenance. By binding to the surface of the tumor, fungal polysaccharide might prevent cell-cell interactions or metastasis. The interaction of the sereceptors with fungal polysaccharide has been explicitly demonstrated.

Ohmori et al (1989) showed that a protein-bound galactosaminoglycan (CO-N) from polysaccharide fraction (SN-C) from *Cordyceps ophioglossoides* showed a direct antitumor activity. The direct effect results from binding of CO-N to cell membrane of tumor cell. In other words, CO-N binds to the tumor cell surface and inhibits the membrane permeability to glucose and consequently exhibited an inhibiting effect on tumor cells.

Conclusions

Various kinds of structural factor of anti-tumor polysaccharides were considered with respect to their anti-tumor activity. But the correlation of polysaccharide structures to their anti-tumor effect is not yet fully understood, except for dependence on the molecular weight. Table 3 summarizes above-discussed factors which have an effect on anti-tumor activity of polysaccharides. Fungal polysaccharides contribute to the overall health of the tumor-bearing host by prolonging chanisms that are suppressed or by reversing survival through restoration of resistance metumor-induced suppression.

A better knowledge for relationships between the chemical structure and its anti-tumor activity can allow us to synthesize anti-tumor polysaccharide in future. And the usefulness of therapy with immunomodulator such as fugal polysaccharides will not be clear until the specific mechanisms of immunomodulation are investigated and understood.

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