

Antitumor and Antimetastatic Activity of a Novel Water-soluble Low Molecular Weight β -1, 3-D-Glucan (branch β -1,6) Isolated from *Aureobasidium pullulans* 1A1 Strain Black Yeast

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Abstract. Though it has been reported that β -glucans or protein-binding hetero-glucans isolated from mushrooms have antitumor activity, the antitumor and antimetastatic actions of purified, structurally defined polysaccharides (such as β -glucans) have not been proven yet. A new low molecular weight (approximately 100 kDa) β -glucan was isolated from *Aureobasidium pullulans* black yeast, and was found to have low viscosity and to be water-soluble. The industrial production of this β -glucan was achieved from the culture media of *A. pullulans*. The effects of water-soluble low-molecular-weight (LMW) β -(1 \rightarrow 3) and 50-80% branched β -(1 \rightarrow 6) glucan isolated from *A. pullulans* on tumor growth and metastasis to the liver were examined in mice intrasplenically with implanted colon 26 tumor cells. In addition, to clarify the antitumor and antimetastatic actions of LMW β -(1,3-1,6) glucan, the effects on immune functions in the small intestine were also examined. The intraperitoneal (5 and 15 mg/kg) and oral (50 mg/kg) administration of LMW β -(1,3-1,6) glucan inhibited the tumor growth and liver metastasis in mice intrasplenically implanted with colon 26 cells. The numbers of natural killer (NK)- and interferon (IFN)- γ -positive cells in the small intestine of colon 26-bearing mice were lower than those in normal mice. The intraperitoneally and orally administered LMW β -(1,3-1,6) glucan prevented the reduction of the number of NK- and IFN- γ -positive cells induced by the tumor

growth after implantation of colon 26 cells. These findings suggest that the antitumor and antimetastatic actions of LMW β -(1,3-1,6) glucan may involve the enhancement of intestinal immune functions through increases in NK- and IFN- γ -positive cell numbers.

The polysaccharide (β -glucan) fractions prepared from many Basidiomycetes mushrooms, such as *Ganoderma lucidum* (1), *Phellinus linteus* (2) *Agaricus blazei* (3, 4), *Grifola frondosa* (5, 6), *Spaeassia crispa* (7, 8) and *Lentinus edodes* (9), have been the subject of several studies. For example, it was well-documented that β -glucan has anti-cancer activity through a biological response modifier (BRM) effect when administered in medicines and health foods. Lentinan from *Lentinus edodes* (10), shizophyllan (SPG) from *Schizophyllum commune* (11) and krestin (PSK) from *Corolus versicolor* (12) have been used clinically for cancer therapy in Japan. Based on the antitumor activities of polysaccharides isolated from Basidiomycetes mushrooms, it is believed that structural features, such as β -(1 \rightarrow 3) linkages in the main chain of the glucan and additional β -(1 \rightarrow 6) branch points are needed for antitumor action. On the other hand, β -glucans containing mainly β -(1 \rightarrow 6) linkages have less activity, while polysaccharides, such as β -(1 \rightarrow 6)-; β -(1 \rightarrow 3)-glucan, acidic β -(1 \rightarrow 6)-; α -(1 \rightarrow 4)-glucan and acidic β -(1 \rightarrow 6)-; α -(1 \rightarrow 3)-glucan isolated from *Agaricus blazei* have been characterized as antitumor substances (13). Thus, *A. blazei* was the first mushroom described to contain antitumor glucan with a β -(1 \rightarrow 6)-linked backbone, unlike the well-known β -(1 \rightarrow 3)-glucans. In addition, hetero- β -glucan (14), β -glucan-protein (15), α -manno- β -glucan (14) and heteroglycan-protein complex (16) were isolated from many Basidiomycetes mushrooms. It seems likely that (1 \rightarrow 3)- or (1 \rightarrow 6)- β -glucans isolated from these species have high viscosity and high-molecular-weight (over 2,000 kDa) and are insoluble. In addition, β -glucan

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