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Anti-tumor effects of (1 → 3)-β-D-glucan from *Saccharomyces cerevisiae* in S180 tumor-bearing mice

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ABSTRACT

(1 → 3)-β-D-Glucan from *Saccharomyces cerevisiae* is a typical polysaccharide with various biological effects and is considered a candidate for the prevention and treatment of cancer *in vitro*. Research into the function of (1 → 3)-β-D-glucan in tumor-bearing animals *in vivo*, however, is limited. Here, we investigated the effects of (1 → 3)-β-D-glucan from *S. cerevisiae* on S180 tumor-bearing mice and on the immunity of the tumor-bearing host. The molecular mechanisms underlying the observed effects were investigated. (1 → 3)-β-D-Glucan was shown to exert anti-tumor effects without toxicity in normal mouse cells. The volume and weight of S180 tumors decreased dramatically following treatment with (1 → 3)-β-D-glucan, and treatment with the polysaccharide was furthermore shown to increase the tumor inhibition rate in a dose-dependent manner. Spleen index, T lymphocyte subsets (CD_4 and CD_8), as well as interleukins (IL)-2, (IL-2, IL-6), and tumor necrosis factor-α were assayed to detect the immunoregulatory and anti-tumor effects after (1 → 3)-β-D-glucan intragastrical administration. (1 → 3)-β-D-Glucan was shown to significantly potentiate the mouse immune responses by, among other effects, decreasing the ratio of CD_4 to CD_8. The expression levels of IL-2, IL-6, and TNF-α were also significantly increased by (1 → 3)-β-D-glucan. These results suggest that (1 → 3)-β-D-glucan enhances the host's immune function during the tumor inhibition process. S180 tumor cells treated with (1 → 3)-β-D-glucan also exhibited significant apoptotic characteristics. (1 → 3)-β-D-glucan increased the ratio of Bax to Bcl-2 at the translation level by up-regulating Bax expression and down-regulating Bcl-2 expression, resulting in the initiation of cell apoptosis in S180 tumor-bearing mice. Taken together, these results indicate that the anti-tumor effects exerted by (1 → 3)-β-D-glucan may be attributed to the polysaccharide's immunostimulating properties and apoptosis-inducing features. Further investigation into these properties and their associated mechanisms will contribute to the development of potent polysaccharide-based anti-tumor agents.

1. Introduction

Cancer has become a major concern for human health owing to the high mortality associated with the disease. Many factors influence cancer initiation and growth, including various cytokines, growth factors, and tumor-induced immune system dysfunction. While all cancers induce and undergo similar changes, the response to chemotherapeutics differs significantly among different cancers [1]. Conventional chemotherapies including cell cycle inhibitors, signal transduction inhibitors, apoptosis stimulators, anti-angiogenic compounds, and anti-inflammatory compounds are cytotoxic to cancer cells; however, they are costly and cause numerous undesirable side effects, limiting their use in tumor treatment [2–4]. Accordingly, the identification of new anti-cancer drugs with higher biological activities and lower side effects has become an important research focus.

As biological response modifiers [5], the important role of biologically active polysaccharides in anti-cancer therapy was first recognized more than 60 years ago, and has gained tremendous interest in the context of the development of potential anti-cancer polysaccharide drugs [6]. Polysaccharides are biopolymers that comprise monosaccharides linked through glycosidic bonds. These

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structures are either linear or contain branched side chains. There is mounting evidence that demonstrates roles for many polysaccharides and their derivatives in signal recognition and cell-to-cell communication. Polysaccharides have a broad spectrum of biological effects and activities including antioxidant effects, anti-mutant effects, immunostimulatory effects, free radical scavenging, differentiation induction in cancer cells, and anti-tumor effects via the activation of different immune responses in the host [7,8]. Some polysaccharides have shown promising anti-cancer potential and there are several suggested mechanisms for the inhibition of tumor growth, such as via changing the activation of the immune response of the host organism or, alternatively, via anti-proliferative effects brought about by altering signal transduction within tumor cells [9]. In concrete terms, one such mechanism is direct cytotoxicity, by which polysaccharides interfere with cancer induction, proliferation, and progression by inducing cellular apoptosis and cell cycle arrest, thereby inhibiting tumor invasion, adhesion, and metastasis. The second suggested mechanism is immunoenhancement, in which the host's immune function is enhanced by polysaccharides. Immunoenhancement is considered as the main or singular mechanism by which some polysaccharides, particularly (1 → 3)-β-D-glucan from fungi, inhibit tumor progression. The third mechanism underlying the anti-cancer effects of polysaccharides is synergy with co-administered conventional anti-cancer drugs. Studies on the synergistic effects of polysaccharides administered together with other chemotherapeutic drugs have shown that co-administration of these agents distinctly improves in terms of anti-cancer potential compared with the single agents and that these synergistic effects result from enhanced tumor sensitivity as well as enhanced immune response.

As one of the most important classes of polysaccharides, fungal polysaccharides have attracted a great deal of attention. Some fungal polysaccharides (e.g., *Lentinus edodes* polysaccharide) have been developed into immune-enhancing and anti-tumor drugs for clinical use [10,11]. As a representative polysaccharide, (1 → 3)-β-D-glucan from *Saccharomyces cerevisiae* is the focus of many polysaccharide-based anti-tumor researches. The co-administration of yeast (1 → 3)-β-D-glucan and bevacizumab in SKOV-3 human ovarian carcinoma cells, for example, was shown to yield augmented efficacy in terms of tumor progression and long-term survival compared with treatment with bevacizumab alone [12]. The polysaccharide sulfoethyl glucan has also been shown to exhibit DNA protective, anti-mutagenic and anti-clastogenic effects [13]. Furthermore, Tumors have been shown to induce the generation of immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs) in a tumor microenvironment, thereby contributing to evasion of immunological attack. (1 → 3)-β-D-glucan was shown to directly abrogate the immune suppression of tumor-educated dendritic cells (TEDCs) involved in tolerance induction and to promote Th1 cell differentiation to improve antitumor responses [14]. (1 → 3)-β-D-Glucan was also shown to enhance CD_4 T cell proliferation and to phagocytose tumor cells by inducing phenotypic changes in macrophages to negatively modulate the immunosuppressive tumor microenvironment [15].

To the best of our knowledge, although there have been researches about the anti-tumor effects of yeast (1 → 3)-β-D-glucan [16,17], comprehensive and systematic studies on the anti-tumor effects and immune regulation exerted by *in vivo* (1 → 3)-β-D-glucan treatment in tumor-bearing animals still remain unknown. More in-depth investigation is also required, and it is essential that *in vivo* studies are performed to confirm the *in vitro* results. Accordingly, the aim of the present study was to investigate both the anti-tumor and immunoregulatory activities of (1 → 3)-β-D-glucan in S180 tumor-bearing mice.

2. Materials and methods

2.1. Chemicals and reagents

S. cerevisiae (1 → 3)-β-D-glucan was provided by the Institute of Modern Physics, Chinese Academy of Sciences (Lanzhou, China), and it is linked by β-D-glucose one another by 1 → 3 glycosidic chain with 1 → 6 glycosidic branches. The nomenclature of (1 → 3)-β-D-glucan by the way of IUPAC is [3-β-D-glucopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-1 →]$_n$. In addition, the (1 → 3)-β-D-glucan is water soluble, and purity ≥95%. The average molecular weight is about 1.7×10^4 Da, degree of branching is 0.03–0.2, and degree of polymerization is approximately 106. RPMI-1640 medium, fetal bovine serum (FBS), pancreatin, penicillin, streptomycin, DMSO, AnnexinV-PI kit, and cytokine detection kits were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Animals

Six- to eight-week-old Kunming SPF male mice (18 ±2 g) were purchased from Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences. The mice were housed under normal laboratory conditions and were allowed to acclimate in the facility for 1 week prior to experiments. All animal experimental procedures were approved by the Animal Care and Use Committee of Lanzhou University.

2.3. Cell culture

Mouse sarcoma S180 cells were obtained from the Cell Bank of the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences and were cultured in RPMI 1640 medium containing 10% FBS and were incubated at 37 °C in an incubator containing 5% CO_2.

2.4. Ascites tumor-bearing mouse model

To establish an S180-bearing mouse model, ascite tumor cells (4×10^5 cells in 0.2 mL PBS) were intraperitoneally injected into the right axillary region of the mice. After 7 days, the tumor cells of the S180 mice were collected and diluted 3-fold with 0.9% NaCl solution before being assessed for cell viability using the trypan blue dye exclusion method. The mice were subsequently subcutaneously inoculated with 0.2 mL diluted S180 tumor cell solution for the establishment of a S180-bearing model.

2.5. Drug administration

The S180 tumor-bearing mice were divided randomly into four groups: a control group (normal saline) and three groups for oral gavage of (1 → 3)-β-D-glucan at dosages of 50, 100, and 200 mg/kg, respectively. The mice were administered the treatment (0.2 mL) intragastrically once a day for 16 days.

2.6. Body weight, tumor growth rate, and tumor inhibition rate analysis

To monitor body weight, the mice were weighed at 2, 4, 6, 8, 10, 12, 14, and 16 days after the initiation of (1 → 3)-β-D-glucan treatment. After 16 days of drug administration, the resulting tumors