Review Article

Possible mechanisms of action of mushroom-derived glucans on inflammatory bowel disease and associated cancer

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Abstract: Since ancient times, medicinal mushrooms have been traditionally used as a health food or supplement for the prevention and cure of a range of health-statuses or diseases, such as overt inflammation, atherosclerosis, cancer, hypertension, diabetes and others. We concentrate in this review on the effect and putative mechanism of action of glucans harvested from fungi on inflammatory bowel disease (IBD) and colitis associated cancer. Many scientists including our own group have examined the immunomodulating effect of isolated polysaccharides-glucans in general and specifically in inflammation associated with cancer. In this manuscript we reviewed the sources, the chemical composition and medicinal properties of polysaccharides extracted from edible mushrooms. In addition we brought insights into their putative mechanisms of action behind each health-promoting activity of these interesting biomolecules. The preventive and therapeutic effects of the medicinal mushrooms and their components have been well documented in mouse and rat model systems and in cancer cell lines being the most striking effects reported to their anti-inflammatory and antitumor effect. Their anticancer effects were demonstrated mainly in *in vitro* and *in vivo* experimental systems but a very limited number of studies have been conducted in human populations. We can summarize that oral consumption of several mushrooms glucans is an efficient treatment to prevent colitis-associated dysplasias through modulation of mucosal inflammation and cell proliferation. Identifying new food-derived isolates and understanding their mechanisms of action are the main challenges in using mushrooms glucans for therapeutic purposes in the field of IBD and associated cancer. Only an in-depth understanding of the mechanism of action and cross-talk between the inflammatory cell, epithelial cell and fungi derived glucans on which we have a based structural knowledge will lead to well designed intervention clinical human studies to test the efficacy of these molecules on intestinal inflammation and colitis associated cancer.

Keywords: α-glucans; β-glucans; inflammation; inflammatory bowel disease (IBD); colon cancer

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Inflammation and carcinogenesis

In the last decades, a strong connecting relationship has been demonstrated between inflammation and cancer. One of the earliest studies conducted in the 19th century by Rudolf Virchow (1) demonstrated the presence of leukocytes in malignant tissues, these observations bring this researcher to claim that tumors may arise from regions of chronic inflammation. Later, at the beginning of the 21st century, the group of Karin *et al.* (2) confirmed that cells of the innate immune system populate the microenvironment in and around tumors. He demonstrated that these cells secrete a wide range of proinflammatory cytokines and chemokines, such as TNFα, IL-1, IL-6, and IL-8, matrix-degrading enzymes, growth factors and reactive oxygen species, molecules that ultimately may support enhanced cell proliferation, cell survival, cell migration, angiogenesis, damage of DNA and thereby encourage tumor development. These studies demonstrated that

inflammation stimulates the formation of epithelial-derived tumors, the most common class of cancers, through an indirect mechanism involving activation of surrounding inflammatory cells (3). The interplay between epithelial cells and inflammatory cells is likely to be a crucial process during inflammation-associated tumor development (4). Chronic infection and consecutive inflammation may directly affect the cells that eventually become transformed as well as exert indirect effects on the tumor cell through surrounding cells.

Inflammatory bowel diseases (IBD) and colitis-associated-colorectal-cancer (CAC)

IBD, includes ulcerative colitis (UC) and Crohn's disease, is a chronic idiopathic inflammatory disease of the gastrointestinal tract affecting significant population numbers in the western world (5), the frequency of which has increased considerably over the past few decades (6). IBD is characterized by intermittent inflammation in the gastrointestinal tract (7). During the continuing inflammatory process, activated and infiltrated leukocytes produce pro-inflammatory cytokines, molecules known to play an important role in inflammation of the intestinal mucosa (8). Concomitantly, chemokines released during inflammation attract and activate additional leukocytes at the site of inflammation and up-regulate adhesion molecules that are important for leukocyte trafficking (9). Previous studies have shown that TNF-α expression is increased in blood, stools, and cultured intestinal biopsies from IBD patients (10), suggesting that TNF-α plays a central role in intestinal inflammation. Upon stimulation with TNF-α, disrupted epithelial cells in the intestinal mucosa continue the inflammatory process by inducing release of additional cytokines. Thus, increased levels of IL-1β, IL-6 and IL-8 have been reported in UC patients and their tissue levels are correlated with the degree of inflammation similarly to TNF-α (11).

Based on the central pro-inflammatory role that TNF-α plays, an anti-TNF-α antibody strategy has been developed for the treatment of IBD intractable to the standard steroids treatment (12). A large number of clinical studies have demonstrated that treatment with anti-TNF-α specific antibodies induces remission in 30-40% of UC patients (13). Nonetheless, several problems have been reported as a consequence of anti-TNF-α antibody therapy, such as the high cost of this treatment and the side effects that develop several patients such as direct reactions to the drug or infectious complications (14). Therefore, there is a significant number of studies devoted to identify alternative and/or complementary medicinal molecules able to regulate specific targets associated with IBD.

Large intestinal carcinogenesis is a common malignant tumor of the digestive system and its incidence is continuously rising (15). Recent data have shown that inflammation is considered a critical risk factor for the development of many common malignancies including cancers of the colon (16). The clearest link between inflammation and colon cancer is seen therefore in patients with IBD. Chronic irritation and inflammation which is largely orchestrated by inflammatory cells affects the tumor microenvironment and provides an important event allowing the accomplishment of the neoplastic process. Inflammation plays an important role in promoting uncontrolled proliferation and acquisition of migratory characteristics of the neoplastic cell (17). The severity of inflammation directly correlates with the risk of developing colorectal cancer in patients with IBD (18). The risk of developing colorectal cancer from UC increases in direct correlation also with the duration of the disease. Therefore, colorectal cancer is one of the most serious complications of patients with IBD. The cumulative incidence of Colitis Associated Cancer (CAC) in patients with UC 25-30 years after diagnosis ranges from 8% to 43%, accounting for one sixth of all deaths in this group (18).

Medicinal glucans extracted from mushrooms

Mushrooms have been considered as an edible and medicinal resource for thousands of years specially in Asian countries. Many species of mushrooms are approved adjuvants for inflammation therapy and have been used as well as a remedy for longstanding pain (19). More than 2,000 species of mushrooms exist in nature; however less than 25 species are widely accepted as food and only a few have attained the level of an item of medicinal properties commerce. Most of the analyzed mushrooms and identified as edible macrofungi belong to the class of basidiomycetes (20). Basidiomycetes are filamentous fungi and are characterized by the presence of large structures called basidiocarps which are the sexual structures, or fruiting bodies of the fungus. Within the basidiomycestes, homopolymers of D-glucose (glucans) are very common. Glucans are polysaccharides constituents very commonly found in the fungal kingdom. Many of the basidiomycetes produce polysaccharides with very important advantageous medicinal properties. Fungal

glucans can be water soluble or insoluble. The insoluble fractions are usually structural components of the cell wall and cross linked to other polysaccharides like chitin or to proteins. The soluble fraction provides 20-50% of the total glucans whereas the unsoluble fraction provides between 50% and 80% (21).

The diversity of glucans results from the different bonds among the monomer of glucose units since there are at least eight different ways in which two glucose units can link. A condensation reaction takes place resulting in the formation of α- or β-bonds. The diversity of glucans is further increased by multiple branching of chains and substitutions on the sugar rings (22). There are many theoretically possible glucans polymers of glucose but in fact only approximately 300 are found in nature. This results in several glucans bearing different molecular weights, chemical composition and overall varied spatial configuration. All these factors affect ultimately the physical properties of mushroom glucans impinging also on the therapeutic ability of the various glucans. In summary, the glucan polysaccharides differ in their primary structure (type of basic sugar), type of linkage (α, β, etc.), degree of branching, and molecular weight, among other parameters.

Most of all medicinal mushroom glucans are derived from fruiting bodies, which have been either commercially farmed or collected from the wild. The production of medicinal mushrooms' fruiting bodies usually takes several weeks to months, and it is difficult to control the quality of the final product. A much smaller percentage of medicinal mushroom glucans are based on extracts from mycelia produced via submerged fermentation. The submerged cultivation of medicinal mushrooms has received a great deal of attention as a promising and reproducible alternative methodology for the efficient production of mushroom mycelium and metabolites. Polysaccharides with antitumor properties have been screened mostly in the fruiting bodies, less so in liquid culture medium and mycelium (23). The industrial potential of submerged cultivation of mushrooms is significant, however its success depends on up scaling and increasing product yields. The bioactive polysaccharides isolated from mushroom fruiting-bodies, submerged cultured fungal biomass, or liquid culture fermentation broths are either water-soluble α- and β-D-glucans, β-D-glucans with heterosaccharide chains of xylose, mannose, galactose, or uronic acid, or others (24).

Isolation, characterization and purification of mushrooms-derived polysaccharides at the commercial level have been made possible due to the implementation of advanced and novel biochemical technologies (23). These techniques have allowed the production of polysaccharides exhibiting advantageous medicinal properties. The medicinal properties of the isolated polysaccharides have been shown to depend not only on the producing fungus but also on their structure, molecular weight, polymeric backbone, sugar composition and their degree of branching (25).

We (26) previously compared the molecular weight and composition of the glycosyl residues and the types of glycoside bonds in polysaccharides harvested from *P. pulmonarius* under different growing conditions. Polysaccharides extracted from fruiting bodies produced by the fungus grown on straw (FBE) were compared to glucan extracted from mycelia produces in submerged culture (ME). The products of the different methodologies were shown to vary. For example we found that the glucan extracted from FBE contained 85% glucose as compared to that extracted from ME which contained only 64% glucose. Both FBE and ME glucans contained significant and equal amounts of galactose (8.3%). The ME polysaccharide also contained fucose and xylose, which were not found in the FBE glucan. The reason for the difference in carbohydrate content could be explained by the differences in carbon sources and growing conditions. ^{13}C and ^{1}H NMR analyses of the FBE preparation showed mixed α-linkages and β-anomeric carbon linkages, whereas the ME polysaccharide demonstrated mainly α-glucan linkages. (1→3)-α-glucan is found in a large number of basidiomycetes, where it is present at levels of 9% to 46% of the cell wall, which even reaches 88% in wall material of certain fruiting bodies. The chemical structure of α-glucan varies among the different fungi from polysaccharides consisting solely of (1→3)-linked α-glucose, to polysaccharides containing small percentages of (1→4)-linked residues (27).

β-glucans are a very heterogeneous class of glucose-derived polysaccharides, most of them contain β-1,3 and β-1,6 linkages with β-[1,6] side chains of varying length and distribution (9-11). The main chain is represented by β-D-glucopyranoside monomers 1,3-linked with lateral 1,6-branching. In general, β-[1,3]-D-glucans with β-[1,6] linkages have a high molecular weight (over 2,000 kDa), high viscosity and low water solubility. In addition, such β-glucans easily form gels containing high-order structures of single or triplet spirals. The biological role played by β-glucans depends on the structure of the polysaccharides and their cellular displacement. The degree of branching, the molecular weight and the secondary structure often determine the biological activity of these compounds.

β-Glucans located on the cell surface seem to have different physiological functions with respect to others secreted outside the cell wall (28).

The immunomodulating role of mushrooms glucan extracts

Mushrooms-derived glucans have been reported to modulate cytokine profiles and phagocyte activity, enhance protection against sepsis, infections and inflammation associated tumor and IBD development (29-31). Although the accumulating literature on the properties of glucans is extensive, the cellular and molecular mechanisms behind the reported effects remain to be solved. Contradictory findings and discrepancies in the literature, contributes to the lack of mechanistic understanding. Even though that glucans have been shown to increase resistance to infections (30), their uptake and the biological effects exerted by orally administered glucans are highly controversial (32,33). Most studies performed in animal models that have been proposed the use of glucans as pharmaceuticals for the treatment of experimental inflammation have involved their administration via intravenous, intraperitoneal, subcutaneous, or intramuscular routes (34,35). Rice *et al*. (32) showed that fungal-derived soluble glucans translocate from the gastrointestinal tract into the systemic circulation in normal animals.

The uptake of orally administered β-glucans, and biological effect thereof, has been highly controversial. Although it is now widely accepted that orally administered β-glucans may enhance host immunity, it is still not established whether β-glucans may act directly on the gastrointestinal mucosa or if entry to the blood stream is feasible and required to mediate biological effects. Gastrointestinal absorption of orally administered β-glucans has been addressed in a very limited number of publications. Chan *et al*. (36) reported uptake of particulate β-glucan from the gut mediated by intestinal macrophages that internalized the β-glucan particle, circulated throughout the body, and subsequently released bioactive soluble β-glucan into circulation. As mentioned Rice *et al*. convincingly demonstrated that three structurally distinct-1,3-glucans were internalized by a subset of epithelial cells (possibly M cells), GALT cells (i.e., macrophages and DCs) and rapidly entered circulation following a single oral dose (32).

Oral drug administration is attractive and remains the preferred delivery route despite challenges associated with low bioavailability due to poor intestinal barrier penetration and gastrointestinal degradation. Although parenteral delivery has dominated in the field of mushroom glucan research, orally administered glucans clearly mediates beneficial effects on human and animal health. Oral glucan administration had an effect on key inductive sites responsible for the appropriate functioning of the intestinal mucosal immunity. Furthermore, oral and intracolonic glucan pretreatment protected against chemically- induced mucosal injury in animal models of acute colitis (37,38), possibly by improving epithelial restoration impinging on the conservation of the barrier integrity. Most of the studies were conducted assessing the role of β-glucans. The most impressive biological properties are related to glucans. The immunomodulating ability, anti-inflammatory properties *in vitro* and *in vivo* have been reported, but in many cases the mechanistic of anti-inflammatory action extremely vary between the different publications.

Most of the investigations on the immunomodulating, anti-inflammatory and anti-tumor effects have been conducted essentially for β-D-glucans. Similar biological activities has been reported only rarely for α-(1→3)-D-glucans. Our group has been reported that α-(1→3)-D-glucan preparations from various macromycetes fungi may exhibit immunomodulatory effects (37,39).

The anti-tumor effects of mushrooms β-glucans are thought to be mediated mainly by their immunomodulatory activities such as regulators of the release of cytokines, of nitric oxide and of arachidonic acid and their metabolites (39). It has been reported that β-glucans suppress inflammatory responses in tumor animal models by decreasing the levels of pro-inflammatory cytokines, chemokines and cell adhesion molecules (37). This suggests that β-glucan is an interesting immunomodulator, causing specific effects on immune systems. An example of such activities among the polysaccharides are the β-glucan produced by *Pleurotus spp*, β-1,3 glucans (pleurans) who play an important role as modulators of the immune system exerting an antitumor effect and the inhibition of metastasis. When pleuran was locally administered, with or without concomitant parenteral pretreatment, it was effective in reducing colonic damage induced by acetic acid, but only when administered prophylactically by intraperitoneal route, but not when orally administered (38). Many other therapeutic effects have been associated with glucans isolated from *Pleurotus* species. A water β-glucan extract isolated from the mushroom *Inonotus obliquus* (40) have been demonstrated to induce anti-inflammatory effects in colitis caused by dextran sodium sulfate treatment in mice. Kim *et al*. (41) reported that the anti-inflammatory of *Inonotus obliquus* is probably due to the

inhibition of inducible NO synthase and cyclooxygenase-2 expression via the downregulation of NF-κB binding activity.

Most medicinal mushroom β-D-glucans have been shown to induce most of the biological responses through binding to membrane complement receptor type three [CR3 (CD11b/CD18)] on immune effector cells, leading to ligand-receptor complex internalization (42). Two binding sites exist in type 3 complement receptors [CR3 (CD11b)]. One is for β-glucans, and is located within the C terminus, while the other for iC3b (cleaved component 3 fragment of serum complement system), is located within the N-terminus (43). Additionally, β-glucans interact following binding to an additional β-glucan receptor, Dectin-1 in neutrophils. Dectin-1 seems to be most important in macrophages while CR3 has a key role in NK cells. Dectin-1 is a lectin consisting of four components: an extracellular carbohydrate-recognition domain (CRD), a stalk, a transmembrane region, and an intracellular cytoplasmic tail. Several human dectin-1 isoforms have been cloned and characterized (44). Dectin-1 is commonly expressed in macrophages, neutrophil lineages, dendritic cells (DC), and some T-cells, but not in NK cells (45,46). Dectin-1 entails 244 amino acids containing six cysteine residues, all of which are highly conserved (47). The amino acids Trp221 and His223 located next to the fourth cysteine residue appear to be critical in its binding function (48). It was demonstrated that dectin-1 binds specifically to β-(1→3)-glucans consisting of at least 10-mer oligosaccharides (49). Binding of β-(1→3)-glucans to dectin-1 activates several signaling pathways to promote innate immune responses through activation of phagocytosis, ROS production, and induction of inflammatory cytokines (46). The intracellular cytoplasmic tail domain of dectin-1 contains an immunoreceptor tyrosine-based activation motif to activate a tyrosine kinase, which in turn stimulates ROS production but not phagocytosis (50). Activation of this tyrosine kinase also induces synthesis of TNF-α, and IL-2, IL-10, IL-12. Several pathways have been identified as being involved in dectin-1 downstream signaling. First, some evidence suggests it might act synergistically with toll-like receptor (TLR) to produce strong inflammatory responses by stimulating cytokines such as TNF-α, IL-2 and IL-12 (51). Additionally, an alternative independent of TLR pathway involves spleen tyrosine kinase (Syk) activity associated with production of several cytokines, including the macrophage inflammatory protein-2 (MIP2, CXC2) and IL-2 and IL-10 in mice DC cells (42). After binding to the ligand, dectin-1 is phosphorylated by a non-receptor tyrosine kinase Src. It was shown that β-(1→3)-glucans from *Coriolous versicolor* increase natural killer cell cytotoxic activity *in vitro*, whereas lentinan; a glucan isolated from shiitake (*Lentinula edodes*) also activates CR3 (52,53).

In a study where fluorescently-labeled β-glucans were orally administered *in vivo* it was shown that the β-glucans were taken up by macrophages by the dectin-1 receptor (54). The labeled β-glucan was then detected in the spleen, the lymph nodes, and bone marrow. It was demonstrated that within the bone marrow macrophages complex β-1,3-glucans were degraded into smaller soluble β-1,3-glucan fragments. These fragments were subsequently taken up by granulocytes via CR3. Interestingly, these granulocytes containing the CR3-bound β-glucan-fluorescein fragments were shown to kill inactivated complement 3b (iC3b)-opsonized tumor cells such as tumor cells to which monoclonal antibodies have been developed and bound (54). Additionally it was also shown that intravenous administered soluble β-glucans can be directly bound to CR3 expressed on circulating granulocytes (55). Regarding the intestinal mucosa Rice *et al*. (32) showed that soluble β-glucans are able to bind directly and undergo internalization to intestinal epithelial cells and gut associated lymphoid tissue (GALT) cells. In contrast to granulocytes and macrophages, the internalization of soluble β-glucan by intestinal epithelial cells is not dependent on dectin-1, however in GALT cells dectin-1 and TLR-2 participate in the uptake of soluble β-glucans.

Available novel evidence from studies utilizing high purity β-glucans indicates that these molecules are very efficient immunomodulators. Immune responses as a result of treatment with fungal β-glucans are different when compared to immune therapies based on supplementation of elements of the immune system (for example treatment with IL-2 or interferon-γ). β-glucans may have an advantage in treating diseases since they stimulate the whole immune system. Additionally, many of the β-glucans can be administered orally in a single or alternatively combinational application with other immune therapies and as a result the therapy may be significantly potentiated. For example, such a synergistic effect is believed to result from combining β-glucan application interferon-γ (56).

In human studies β-glucans have been used as adjuvant therapy in clinical trials, mainly in the Far East, with a positive effect on patients' survival and quality of life. A study describing the effect of glucan extracts from the *Trametes versicolor* mushroom improved survival and immune function in human randomized, controlled trials in cancer patients (57,58). The mechanism of action is suggested to

be through its stimulation of the immune system (59).

The role of mushrooms glucans extracts in ameliorating inflammatory bowel disease (IBD) and colitis-associated-colorectal-cancer (CAC), a mechanistic approach

CAC arises in patients with IBD, particularly UC. CACs comprise up to 5% of all colorectal cancers (60). The cumulative incidence of CAC in patients with UC 25-30 years after diagnosis ranges from 8% to 43%, accounting for one sixth of all deaths in this group (61). When colitis-associated colorectal cancer is compared to its sporadic counterpart, it is immediately clear that it follows a different histological sequence, starting in the inflamed mucosa as a hyperplastic lesion, to develop through (flat) dysplasia into adenocarcinoma. This is sometimes summarized as the "inflammation-dysplasia-carcinoma" sequence. The pathogenesis of CAC is widely believed to involve a stepwise progression from inflamed and hyperplastic epithelia through flat dysplasia to finally adenocarcinoma (62). CAC is probably promoted by chronic inflammation, but the mechanism is still unclear. Typically, the neoplastic lesions first present as aberrant crypt foci (ACF) or microadenomas and develop through a large adenoma-stage into carcinoma in situ and invasive adenocarcinoma.

We have previously demonstrated that oral administration to dextran-sulfate-induced colitic mice of different glucan preparations harvested from *P. pulmonarius* significantly attenuated the development of intestinal inflammation and other symptoms associated with chronic colitis. *P. pulmonarius* glucan preparations were fed orally to mice at daily doses of 2 or 20 mg per mouse. Both dosages delayed the occurrence of diarrhea and rectal bleeding and improved macroscopic scores (stools + haemoccult). In addition, high dosages prevented colon shortening induced by the intestinal inflammation factor dextran sulfate (DSS). Colon shortening is always found in UC patients and can serve as an indirect marker of colonic inflammation (37). Myeloperoxidase activity, which is directly related to chronic inflammation, was suppressed by *P. pulmonarius* glucans, indicating that glucans inhibit the accumulation of neutrophils in the colonic mucosa. Corroborative histological examinations clearly indicated significant attenuation of inflammation exerted by *P. pulmonarius* glucans preparations. We also demonstrated that in DSS-treated mice TNF-α release from colonic tissue samples was significantly elevated as compared with controls. The tissue levels of TNF-α protein is correlated with degree of intestinal inflammation (63). Even low daily doses of *P. pulmonarius* glucans preparations were sufficient to significantly attenuate the increase in TNF-α in colon segments. Regarding the role of mushroom-derived glucans in IBD in human studies, to the best of our knowledge only the group of Forland *et al*. (64) have reported on such studies. They have demonstrated that oral administration of AndoSan, an extract isolated from the basidiomycetes mushrooms *Agaricus blazei Murill Hericium erinaceum* and *Grifola frondosa* to IBD patients induced notable anti-inflammatory effects. They conducted a clinical pilot study (phase I trial), who included hospitalized patients with IBD (including both UC and Crohn's patients). A significant decrease in plasma levels of proinflammatory cytokines was observed after 12 days of oral intake of AndoSan. Additionally, decreased levels of the inflammatory marker calprotectin in feces of the UC patients were observed. The cytokines levels of MIP-1β, IL-6, IL-8, MCP-1, IL-1β, G-CSF, and GM-CSF were reduced in the blood of UC patients while in Crohn's patients reduced blood levels of the cytokines MIP-1β, MCP-1, IL-8, IL-1β, G-CSF, IL-17, GM-CSF, and IL-2 were measured.

Regarding the effect of glucans in CAC impressive data provided by Okamoto *et al*. (65), demonstrated that lentinan prevented carcinogenesis in chronic UC animal models by inhibiting expression of P450 1A2, which catalyses pre-carcinogenic compounds. This inhibition was mediated by an increase in TNF-α levels and DNA-binding activity of the NF-κB. We have recently demonstrated a chemopreventive ability of *P. pulmonarius* glucans preparations assessed at the same dose levels previously tested for IBD: 2 and 20 mg/mouse/day in diet (39). The *P. pulmonarius* glucans preparations delayed the occurrence of colonic inflammation, ulceration and histological evaluation and additionally the formation of ACFs. *P. pulmonarius* glucans reduced the number of colonic ACF formation in a dose-dependent manner. Cell proliferation plays an important role in multi-step carcinogenesis. In the colon, the number of cryptal cells is strictly regulated by a balance between cell proliferation and cell death that maintains homeostasis (66). Changes in cell proliferation and apoptosis are regarded as a common denominator in the pathogenesis of tumor formation (67). Reduced tumor incidence is generally associated with decreases in cellular proliferation and/or increases in apoptosis. Feeding mice with *P. pulmonarius* glucans significantly lowered the expression of the proliferating-associated marker proliferating cell nuclear antigen (PCNA) in adenocarcinomas, thus suggesting that *P. pulmonarius*

glucans suppresses the abnormal proliferative activity of preneoplastic and neoplastic cells, thereby inhibiting carcinogenesis (39).

Mushrooms α or β-glucans are able to inhibit the cancer growth directly. In this regard, recently Chen *et al*. showed that exopolysaccharide from the mushroom *Fomes fomentarius* has a direct antiproliferative effect *in vitro* on human gastric cancer cells in a dose- and time-dependent manner (68). Xie *et al*. showed that *Ganoderma lucidum* glucan extract inhibits proliferation of SW480 human colorectal cancer cells (69). We isolated a soluble α-glucan from *P. ostreatus* and demonstrated to control colon cancer cell proliferation via direct interaction of the glucan with the colon cancer cells and their apoptosis induction (70). A crude β-(1→6)-glucan extracted from *Agaricus blazei* caused apoptosis or programmed cell death in human ovarian cancer HRA cells. As the apoptotic effect of this β-glucan could be abolished by applying the p38 MAPK-specific inhibitor, SB203580 (71), this would seem to involve activation of the p38 MAPK pathway by translocation of an apoptosis activator Bax from the cytosol to the mitochondria, cytochrome c release and caspase 9 activation.

Induction of cell apoptosis has been the target mechanism for cancer treatments (72). Certain mushroom polysaccharides possess proapoptotic properties in a variety of tumor cell lines *in vitro*. For example, Hu *et al*. reported that the mushroom *Inonotus obliquus* induces apoptosis with differing sensitivity in human colon cancer DLD-1 cells (73). Zhang *et al*. (74) found growth-inhibitory effects of a β-glucan from the mycelium of *Poria cocos* on human breast carcinoma MCF-7 cells. This treatment resulted in decrease expression of the antiapoptotic protein Bcl-2 and also in elevation of Bax/Bcl-2 ratio. Caspase cascade is a well-known key pathway in the apoptotic signal transduction. Since caspase-3 is the main executioner of apoptosis, immunohistochemistry to active form of caspase-3 has been widely used to assess apoptosis (75,76). Furthermore, increased caspase-3 processing has been previously associated with DSS-induced colonic tissue damage and colitis (77,78). We demonstrated that dietary feeding with *P. pulmonarius* glucans increased, in a dose dependent manner, immunohistochemical expression of active-caspase-3 (39). The Bcl-2 family members regulate cytochrome-c release from mitochondria into the cytosol. While Bax were shown to induce cell death, Bcl-2 can protect it apparently by preventing mitochondrial disruption. We demonstrated that dietary feeding with *P. pulmonarius* glucans increased also, the expression of Bax. We confirmed these *in vivo* results with *in vitro* effects of *P. pulmonarius* glucans preparations on colon carcinoma cell lines (HCT-116 and HT29) (39). The release of cytochrome c, which is usually present in the mitochondrial intermembrane space, into the cytosol is a key early step in the apoptotic process. In the *P. pulmonarius* glucans treated colon carcinoma cell lines, we demonstrated that there is an increase in the level of cytosolic cytochrome c. Additionally, the decrease in the expression level of the anti-apoptotic protein Bcl-2 was evident during the induction of apoptosis by *P. pulmonarius* glucans as compared to increase in the expression level of the pro-apoptotic protein Bax.

Regarding the effect of β-glucans on CAC in humans we did not found any publication on this specific neoplastic disease. Nonetheless, recent evidence is just now beginning to emerge in the peer-reviewed literature regarding additional types of neoplasia (79). There are few human studies that have examined the ability of fungi-derived-glucans to affect tumor progression since most human data have concentrated mainly in the immune response. A recent study reported that β-glucan harvested from lentinan was safe and effective for use in patients suffering from advanced pancreatic cancer (80). Similarly, it was reported that lentinan can improve quality of life in advanced colorectal cancer (81) and survival in hepatocellular patients (82). Although several clinical trials have shown possible treatment benefits of β-glucans the data are still preliminary and controversial. Evidenced-based firm conclusions of the clinical significance of glucans treatment cannot be concluded hitherto. In summary, the most promising evidence to date in human trials has come from recent studies on a benefit of β-glucan on quality of life and survival when given in combination with cancer treatment. This encouraging data inquires to perform additional studies that will compare the specific effects of highly purified and characterized mushroom-glucans from different sources to further support our understanding of the mechanisms of action and aid in the development of clinical studies in cancer patients.

Concluding remarks

In summary, fungal glucans appear to be beneficial to prevent or cure IBD and CAC and as the gastrointestinal tract could be the target for the glucans, oral administration seems to be the preferred delivery route despite challenges associated with low bioavailability due to poor intestinal barrier penetration and gastrointestinal degradation.

However, most data, to date, were produced using model organisms or cell cultures.

The mechanisms of action of these fungal glucans preparations appear to depend on their capabilities to bind to cell receptors, which are known to include dectin-1, CR3, and others or alternatively to attack directly cancer epithelial cells. This event then leads to multiple signal pathways which in turn promote immune responses or apoptosis in the affected cells. Data suggest that different fungal glucans preparations have different effectiveness probably through their different binding affinity to each receptor or apoptosis induction, although the chemical purity of the glucans used in these studies is not always known. Correspondingly, the choice of which glucan to use may be important. Unfortunately, we still do not understand what structural features are best for inducing activities. Future studies need to characterize mechanistically how individual glucans of known structure activate each pathway. Only then can we rationally improve the use of mushroom glucans preparations and start more wisely broad human clinical studies.

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