The Pharmaceutical and Chemical Journal, 2023, 10(3):86-93

Available online www.tpcj.org

Review Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Cancer Occurrence, Treatment and Antitumor Activity of Lentinan: A Review

Haiting Xu

Weifang People's Hospital, Weifang, Shandong, PR China

Abstract Cancer is a general term for a large group of malignant tumors. Cancer belongs to both chronic disease and malignant tumor. Cancer may occur in all parts of the body. Lentinan (LPS) has been proven to have various biological activities, among which the anti-tumor activity is significant. This paper provides a brief overview of tumorigenesis and treatment, lentinan structure and anti-tumor activity.

Keywords Tumor; polysaccharide; lentinan

1. Tumor development and apoptosis

Apoptosis is the process in which cells start their own "suicide" program under certain physiological or pathological conditions, ending their life, and finally the cells fall off and detached or lysis into several apoptotic bodies, which are engulfed by other cells. Apoptosis is closely related to cell proliferation and differentiation, and plays a key role in the normal development and self-stability of organisms [1]. Studies have shown that apoptosis and a variety of intracellular messengers and oncogene products, cell oncogene activation and tumor suppressor inactivation, can make the normal cell proliferation, differentiation and apoptosis regulation abnormal, namely the balance between the body of cell proliferation and death, cell proliferation, insufficient differentiation, eventually lead to tumor cell apoptosis (Apoptosis) concept, in 1972 Pathologist Kerr first pointed out that cell apoptosis, like autumn leaves or flowers fall, is a normal life phenomenon [2,3]. Kerr described the morphology of apoptotic cells. The reduction or disappearance of cell surface microvilli during early apoptosis is accompanied by an increase in cytoplasmic density, condensed nuclear chromatin, and lysis of the nuclei into fragments. After that, the cells wrinkle, the cell volume shrinks, and the cells are divided into several apoptotic bodies with smooth surfaces of intact cell membrane structures, containing a variety of different, structurally intact organelles and chromatin fragments. Finally, the apoptotic bodies are swallowed for by adjacent cells [4,5].

2. Overview of tumor treatment methods

2.1 Surgical treatment

Surgical treatment is currently one of the main treatment methods for cancer patients. In 1881, Billroth first performed oncology surgical resection with one subtotal gastrectomy. In 1890, Halsted performed radical mastectomy first clarified the principle of mass resection, that is, the resection of lymph nodes in the primary tumor area, which laid the foundation of modern tumor surgery. Surgical treatment is a local treatment, which has a good effect for small tumors detected early and without spread. For example, the 5-year survival rate for early cervical cancer surgery reached 94.69%, and the 10-year survival rate reached 94.05% [6]. Although the surgery cannot completely remove the cancer body and cancer cells, a small amount of cancer tissue and cells can be removed by

other methods due to the clearer pathological diagnosis and infiltration range after the surgery. Furthermore, surgical treatment is able to reduce the symptoms in cancer patients. For example, abdominal tumors may block the intestines and cause pain, and surgery can remove or dredge these blockages, thus alleviating these symptoms. The disadvantage of surgical treatment is that it often causes recurrence and metastasis, and postoperative recurrence and metastasis become the key factors affecting the long-term efficacy of surgery [7]. If the tumor spreads, then surgery alone cannot effectively remove the cancer cells. In particular, for tumours close to blood vessels and other more fragile organs, surgical treatment may cause severe damage to the surrounding organs and tissues. Therefore, not all cancer patients are suitable for surgical treatment. For disseminated tumors, radiotherapy, chemotherapy and immunotherapy are the more commonly used treatments at present.

2.2 Radiation therapy

Radiation therapy for tumors is to use high-energy radiation to shrink and kill tumor [8]. Its principle is the use of strong radiation source of radiation directly destroy the DNA structure of tumor cells or by tumor cells to destroy tumor cell DNA, induce tumor cell death, achieve the purpose of killing tumor cells including X-ray, γ-radiation and electron is the form of radiation therapy is divided into radiation therapy, internal radiotherapy or short distance radiotherapy and systemic radiotherapy [9-12]. External radiotherapy is to place the radioactive source in the body, while short distance radiotherapy is the radioactive source near the body of the tumor; whole-body radiotherapy is injecting radioactive material (such as radioactive iodine) into the blood to kill cancer cells, but radiotherapy not only kills the tumor cells, but also will cause normal tissue cell damage, resulting in serious side effects [13]. In China, only 27% of breast cancer patients receive radiation therapy, lower than in other countries (e. g., 40% in Korea [14], 58-68% in the Netherlands [15] and 76% in Brazil [16]. Because radiotherapy causes great pain to patients, medical scientists have been working to study how to use it more effectively and safely. Currently, the development of radio-sensitive drugs and radio-protective drugs has become a hot spot in radiotherapy.

2.3 Chemical therapy

Chemical therapy for cancer (or chemotherapy) is a way of treating cancer by using one or more chemical drugs. Chemotherapeutic drugs are generally divided into the following categories of chemical agents, diamonds, anti-metabolic drugs, anti-microtubule drugs and DNA topoisomerase [17]. Clinical commonly used chemotherapy drugs are cyclophosphamide, alin, cisplatin and so on. Chemotherapy is often used as adjuvant therapy, often in combination with other treatment modalities, such as radiotherapy, surgical treatment, and immunotherapy. Most chemotherapeutic drugs effectively target tumor cells and kill tumor cells through a variety of mechanisms, including inducing apoptosis, cell proliferation cycle arrest (inhibiting mitosis of tumor cells, while also damaging normal tissue cells. Therefore, chemotherapy has serious side effects, including digestive tract reactions, bone marrow hematopoietic suppression, cardiac damage, hepatopulmonary nephrotoxicity, and immunosuppression and neurological response [18]. Because chemotherapy affects cell mitosis, tumors growing faster (such as acute leukemia) are relatively sensitive to chemotherapy, while tumors with slower growth are relatively slow to respond to chemotherapy.

The main disadvantage of chemotherapy is the tolerance of tumors [19,20]. The drug resistance of tumor often makes its sensitivity to chemotherapy drugs reduced, leading to the recurrence and even metastasis of tumor, so the drug resistance of tumor has become one of the research hotspots today. Due to the serious side effects and drug resistance of chemotherapy, the following strategies are often adopted: (1) combination chemotherapy with multiple drugs with side effects of different mechanisms of action. Combination chemotherapy can avoid cross-resistance of tumor cells, and combination therapy often uses lower doses, greatly reducing the toxicity of chemotherapeutic drugs. (2) Chemotherapy, combined with immunotherapy, can effectively reduce the toxic and side effects of chemotherapy drugs. Chemotherapy drugs generally need to reach a certain dose, higher dose will lead to toxic side effects of drugs. Therefore, how to determine the dose of chemotherapeutic drugs is also a hot issue in current research.

3 Study on the biological activity of polysaccharides

In 1969, a polysaccharide with antitumor activity was isolated from the mushroom fruiting body and named linan, beginning a new era of biological activity of fungal polysaccharides. In the past 30 years, various polysaccharides have been extracted from mushrooms, yeast, algae, lichens and plants, which have various pharmacological activities including anti-tumor, anti-inflammation and immune regulation. Although the study of polysaccharides started later than the other two classes of biological macromolecules (proteins and nucleic acids), polysaccharides are increasingly attracting attention to because of their important physiological functions and wide applications in the life process [21]. At present, polysaccharides have become an important and effective component of natural drugs and health care products. According to incomplete statistics, there are at least 30 polysaccharides in the world that are undergoing standard clinical trials of anti-tumor, antiviral and diabetes treatment [22]. The study of polysaccharide structure and function has become the third major milestone after the study of proteins and nucleic acids in the exploration of the mystery of life. As one of the leaders, the research of its biological activity has made many progress.

4.1 Overview of LPS

Over the years, eating mushrooms has been considered to be extremely beneficial to human health [23]. The unique taste and flavor of mushrooms, as well as their mitigation of various degenerative diseases, have attracted the attention of scientists around the world [24]. At present, the application scope of mushrooms is expanding, not only as food, but also in drugs, nutrition and cosmetics. The nutritional and health function of mushrooms is largely attributed to the bioactive substances, such as β -glucan, which has the function of to enhance human immunity. We showed that the antitumor properties of the bioactive compounds isolated in mushrooms were mainly attributed to the polysaccharide [25-27]. Therefore, mushrooms are a powerful source of new drug products, which is of great significance for modern medicine. Lentinus edodes has always been an important food material in the daily diet of China and Japan, and it is also used as a traditional medicine and medicinal material [28,29]. The polysaccharide (lentinus edodes, Lentinan), is one of the most studied polysaccharides [30]. It is a major component in the cell wall of mushroom, and the backbone is β -1,3-D-glucan, with 2 β -1,6-glucose side groups per 5 backbone glucose residues, with certain water solubility and thermostability [31]. Lentinan shows a triple helical conformation in water and plays an important role in its biological and pharmacological activities [32]. In recent years, LPS has been used on a large scale for adjuvant drugs and dietary supplement [33]. Although the anti-tumor and anti-inflammation and antiviral biological activities of lentinan have been studied for many years, its extraction, purification, structural complexity of macromolecules and the structure-efficacy relationship of polysaccharides are still not clearly expounded, which seriously hinders the clinical use of lentinan as a medical product. Therefore, a systematic study of the chemical structure, molecular chain conformation and biological activity of lentinan are crucial for its application in disease prevention and treatment.

4.2 Structure of shiitake edodes polysaccharide

The biological activity of edodes polysaccharide is closely related to its structure. Triple helix (triple helical) lentinan showed superior antitumor activity. However, when the triple helix structure was destroyed to form a single-stranded random linear mass, the antitumor effect of lentinan significantly decreased or even disappeared [34,35]. Meanwhile, the antitumor activity of polysaccharides depends on the molecular weight size. Polysaccharides with a molecular weight of about 1×106 could strongly inhibit mouse S-180 tumor, while the activity of small molecular weight lentinpolysaccharide fragment after formic acid degradation was greatly reduced [36]. Therefore, the structure of lentinan is an important basis for its biological activity and application. Different shiitake mushrooms, different extraction methods and conditions, we have not the same polysaccharide structure [37]. The classical extraction method of lentinan is solution extraction method, that is, of lentus edodes using hot water, lye or polyethylene glycol [38]. Chihara et al., the first used hot water (80-100℃) to purify linan from mushroom, which showed significant antitumor activity of lentinan [39]. Generally, extraction methods using hot

water or organic reagents yielded lower yields, with only about 0.12% [40]. Zhang, using 1.25 M NaOH / 0.05% NaBH4 solution, obtained in 5% yield and still maintained the triple helical conformation [41,42]. The structure of natural macromers is very complex, and different structures lead to different biological activities. The chemical structure of polysaccharides is determined by the composition of the monosaccharides, the configuration of the glycosidic bond, the way of bonding, and the sequence of the monosaccharides. The earliest discovered fungus β - glucan, all have their own names derived from their sources, including mushroom polysaccharide (lentinan) extracted from mushroom (Lentinus edodes), schizonella polysaccharide (schizophyllan) in crack (Schizophyllum commune) [43], and hard glucan (scleroglucan) [44] from small nucleus (Sclerotium sp.).

4.3 Anti-tumor activity of lentinan

Since its discovery in 1969, the anti-tumor activity of LPS has been a focus of attention. "The father of shiitake edodes" Chihara used SCID mice inoculated with S-180 (Sarcoma-180) sarcoma cells to demonstrate that shiitake edodes can inhibit tumor growth, and proposed that this anti-tumor effect may come from the host immunomodulatory effect of polysaccharide rather than the direct cytotoxic effect [45,46]. Suga et al found that lentinan effectively inhibited S-180 tumors in DBA /2, SWM / M and A / J mice, but not in C3 / He and C57 / BL6 mice, and strongly inhibited fibrosarcoma (fibrosarcoma) [47] in 3-methyl cholanthene (3-methylcholanthrene, MC) -induced DBA / 2 mice. Zakany et al analyzed the effects of lentinan on arrest and regression of transplanted tumors in allogenic and homologous tumor-host systems, and showed significant inhibitory effects on both S-180 sarcoma and MC-induced sarcoma in A / Ph mice [48]. These results indicated that lentinan not only inhibited allograft tumors but also inhibited various other identical and autologous tumors, which prevented chemical and viral carcinogenesis and inhibit cancer metastasis and recurrent [49-51].

Clinically, the combination of mushroom polysaccharide and chemotherapy has a good anti-tumor effect, and can reduce the side effects of the latter. For example, 275 patients with advanced or recurrent gastric cancer were treated with the chemotherapy drugs filamycin C (mitomycin C) and 5-fluoruria (5-fluorouracil) or digafur (tegafur), along with partial shiitake polysaccharide injection. Based on the aspects of life extension, regression of tumor or lesion, and improvement of immune response, the results showed that the treatment effect obtained in LPS administered before chemotherapy and patients with primary lesions who had not received chemotherapy [52-54]. Similarly, in a randomized controlled study of patients with stage 3 advanced and recurrent gastric cancer, Lent PS treatment was effective in prolonging life span and no side effects [55]. LentPS was effective in prolonging overall survival of cancer patients, especially in those with gastric and colorectal cancer [56]. Similar results were also validated in patients with colon cancer and breast cancer.

Since 1985, linan has been widely used in cancer treatment in Japan. Later, other Asian countries, the United States and Europe have used it to treat cancer [57]. The addition of lentinan to the standard tumor therapy, the accepted chemoimmunotherapy [58], improves the antitumor effect of chemotherapeutic drugs and reduces its side effects. Patients with advanced gastric cancer with liver metastases treated with lentinan-assisted chemicals and trastuzumab (trastuzumab) showed prolonged overall survival (overall survival, OS) with partial response (partial response, PR) and complete response (complete response, CR). This is due to the combination of lentinan and leukocytes inducing interleukin-12 (interleukin-12, IL-12), enhancing antibody-dependent cytotoxicity and enhancing the anti-tumor effect of monoclonal antibodies [59].

Although many clinical studies have shown the excellent anti-tumor effect of lentinan involved in chemoimmunotherapy, lentinan is still currently not used as antitumor clinical agents worldwide, due to their antitumor mechanisms that are currently poorly defined. Chihara Found that lentinan can inhibit tumor growth and believed that the antitumor effect may come from the host immune regulation of polysaccharide rather than direct cytotoxicity [60]. This host-mediated antitumor activity was demonstrated in many experiments. The antitumor effects of lentinan were lost in neonatal mice (with loss of the thymus-dependent immune system), While the anti-tumor effect of polysaccharides in mice injected with serum against lymphocytes also decreased significantly [61]. Suggesting that the anti-tumor activity of polysaccharides requires an intact T lymphocyte component, While this activity is regulated by thymus-associated immune mechanisms [62]. The pathway explains the possible antitumor

mechanism of LPS, at the same time, the antitumor activity of lentinan was also inhibited after pretreatment with anti-macrophage agents, suggesting that LPS promotes the enhanced response of precursor T and giant cells to cytokines produced after certain lymphocyte-specific recognition of tumor cells. Lentinan promotes the maturation, differentiation and proliferation of immunocompetent cells in host defense mechanisms by inducing IL-1, IL-3 and interferon (interferon, IFN). LPS-induced delayed hypersensitivity at tumor sites and subsequent immune effector cells such as natural killer cells and cytotoxicity were reported.

Infiltration of T lymphocytes, is an important mechanism for the antitumor effects of lentinan. However, the exact role of lentinan-induced immune regulation is a key question to be solved.

References

[1]. Evan, G. I., Vousden, K. H. (2001). Proliferation, cell cycle and apoptosis in cancer. Nature, 411(6835):342-348.

[2]. Li, W. J., Chen, Y., Nie, S. P., et al. (2011). Ganoderma atrum polysaccharide induces anti-tumor activity via the mitochondrialapoptotic pathway related to activation of host immune response. J Cell Biochem, 112(3):860-871.

[3]. Kerr, J. F., Wyllie, A. H., Curric, A. R. (1972). Apoptosis: a basic biological phenomenonwith wide-ranging implications in tissue kinetics. Br J Cancer, 26(4):239-257.

[4]. Hale, A. J., Smith, C. A., Sutherland, L. C., et al. (1996). Apoptosis: molecular regulation of cell death. Eur J Biochem, 236(1):1-26.

[5]. Kerr, J. F., Winterford, C. M., Harmon, B. V. (1994). Apoptosis. Its significance in cancerand cancer therapy. Cancer, 73(8):2013-2026.

[6]. Surgery is the most effective and the most common treatment method. 2000. http://www.cnki.com.cn/article/cjfdtotal-klyu200009021.htm.

[7]. Xu, Z. (2001). New understanding and new model of cancer treatment. Wuhan: Hubei Science and Technology Publishing House, 171-173.

[8]. Yin, W. B., Yu, Z. H., Xu, G. Z. (2008). Radiation oncology[M]. 4th, Beijing: Chinese Peking UnionMedical College Press.

[9]. Giusti, A. M., Raimondi, M., Ravagnan, G., et al. (1998). Human cell membraneoxidative damage induced by single and factionated doses of ionizing radiation: aluorescence spectroscopy study. Int, J. Radiat. Biol, 74:595-605.

[10]. Maisin, J. R., Van-Gorp, U., De Saint-Georges L. (1982). The ultra structure of the lung at exposure to ionizing radiation as seen by transmission and scanning electron microscopy Scan Electron Microsc, (Pt 1):403-412.

[11]. Azzam, E. I., De Toledo, S. M., Little, J. B. (2003). Expression of CONNEXIN43 is highly sensitive to ionizing radiation and other environmental stresses. Cancer Res, 63:7128-7135.

[12]. Dayal, D., Martin, S. M., Owens, K. M, et al. (2009). Mitochondrial complexII dysfunction can contribute significantly to genomic instability after exposure to ionizingradiation. Radiat. Res, 172:737-745.

[13]. Marin, A., Martin, M., Linan, O., et al. (2015). Bystander effects and radiotherapy. Rep Pract Oncol Radiother, 20:12-21.

[14]. Yun, Y. H., Park, S. M., Noh, D. Y, et al. (2007). Trends in breastcancer treatment in Korea and impact of compliance with consensus recommendations on survival. Breast Cancer Res. Treat, 106:245-253.

[15]. Struikmans, H., Aarts, M. J., Jobsen, J. J., et al. (2011). Anincreased utilization rate and better compliance to guidelines for primary radiotherapy forbreast cancer from 1997 till 2008: a population-based study in the Netherlands. Radiother Oncol,100:320-325.

[16]. Lee, B. L., Liedke, P. E., Barrios, C. H., et al. (2012). Breastcancer in Brazil: present status and future goals. Lancet Oncol, 13:95-102.

[17]. Lu, X. J. (2010). Advances in research of chemical anti tumor drugs. Zhejiang Chem. Ind, 41:8-11.

[18]. Chen, Y. Z., He, X. B. (1999). Chemotherapy side effects common syndrome differentiation, China Pharmaceuticals, 8:21-22.
[19]. Holohan, C., Schaeybroeck, S., Longley, D., et al. (2013). Cancer drug resistance: An evolving paradigm. Nat. Rev.Cancer, 13:714-726.
[20]. Dean, M., Fojo, T., Bates, S. (2005). Tumor stem cells and drug resistance. Nat, Rev. Cancer, 5:275-284.
[21]. Werz, D. B., Seeberger, P. H. (2005). Carbohydrates as the next frontier in pharmaceutical research. Chem. Eur. J, 11:3194-3206.
[22]. Lin, J., Li, P., Chen, K. S. (2013). Advance in studies on anti-tumor activity of polysaccharides inlatest five years. China J. Chin, Materia Medica, 38:1116-1125.
[23]. Rathore, H., Prasad, S., Sharma, S. (2017). Mushroom nutraceuticals for improved nutrition andbetter humman health: A review. Pharma Mutrition, 5:35-46.
[24]. Roupas, P., Keogh, J., Noakes, M., et al. (2012). The role of edible mushroomsin health: Evaluation of the evidence. J. Funct. Foods, 4:687-709.
[25]. Wasser, S. P. (2014). Medicinal mushroom science: Current perspectives, advances, evidences, andchallenges. Biomed. J, 37:345-356.
[26]. Wasser, S. (2002). Medicinal mushrooms as a source of antitumor and imnunomodulatingpolysaccharides. Appl. Microbiol. Biotechnol, 60:258-274.
[27]. Lemieszek, M., Rzeski, W. (2012). Anticancer properties of polysaccharides isolated from fungiofthe Basidiomycetes class. Contemporary oncology (Ponan, Poland), 16:285-289
[28]. Mohamed Yahaya, N. F., Rahman, M. A., Abdullah, N. (2014). Therapeutic potential ofmushroomns in preventing and ameliorating hypertension. Trends Food Sci. Technol, 39:104-115.
[29]. Hobbs, C. (2000). Medicinal Value of Lentinus edodes (Berk) Sing. (Agaricomycetideae). A Literature Review. Int. J. Med. Mshrooms, 2:287-302.
[30]. Rop, O., Mlcek, J., Jurikova, T. (2009). Beta-glucans in higher fungi and their health effects. Nutr Rev, 67:624-631.
[31]. Bisen, P. S., Baghel, R. K., Sanodiya, B. S., et al. (2010). Lentinus edodes:A macrofungus with pharmacological activities. Curr. Med. Chem, 17:2419-2430.
[32]. Mizuno, T. (1999). The extraction and development of antitumor-active polysaccharides frommedicinal mushrooms in Japan (review). Int. J. Med. Mushrooms, 1:9-29.
[33]. Zhang, Y., Zhang, M., Jiang, Y., et al. (2018). Lentinan as an immunotherapeutic for treating lungcancer: A review of 12 years clinical studies in China. J. Cancer Res. Clin. Oncol, 144:2177-2186.
[34]. Unursaikhan S., Xu X, Zeng F. et al. (2006). Antitumor activities of O-sulfonated derivatives of (13)-a-D-glucan from different Lentinus edodes. Biosci, Biotechnol. Biochem, 70:38-46.
[35]. Zhang, L., Li, X., Xu, X., et al. (2005). Correlation between antitumor activity, molecular weight, and conformmation of lentinan. Carbohydr: Res, 340:1515-1521
[36]. Sasaki, T., Takasuka, N., Chihara, G., et al. (1976). Antitumor activity of degraded products of lentinan: Its correlation with molecular weight. JPNJ. Cancer Res, 67:191-195.
[37]. Ahmad, A., Anjum, F. M., Zahoor, T., et al. (2012). Beta glucan: A valuable functional ingredient in foods. Crit. Rev. Food Sci. Mtr, 52:201-212.
[38]. Rasmy, G. E., Botros, W. A, Kabeil S. S., et al. (2010). Preparation ofglucan from Lentinulaedodes edible mushroom and elucidation ofits edicinal value. Australian Journal of Basicand Applied Sciences, 4:5717-5726.
[39]. Chihara, G., Hauro, J., Maeda, Y.Y., et al. (1970). Fractionation and purificationof the polysaccharides with marked antitumor activity, especially Lentinan, from Lentinusedodes (Berk.) Sing.(an Edible Mushroom). Cancer Res, 30:2776-2781.
[40]. Yap, A. T., Ng, M.L. (2001). An improved method for the isolation oflentinan from the edibleand medicinal Shiitake mushroom, Lentinus edodes (Berk.) Sing.(Agaricomycetideae). Int.J.Med.Mushrooms, 3:9-19.

[41]. Zhang, L., Zhang, X., Zhou, Q., et al. (2001). Triple helix of p-D-glucanfromm Lentinus Edodes in 0.5 M NaCl aqueous solution characterized by light scattering. Polym. J, 33:317-321.
[42]. Wang, X., Xu, X., Zhang, L. (2008). Thermally induced conformation transition of triple-helicallentinan in NaCl aqueous solution. J. Phys. Chem. B, 12:10343-10351
[43]. Zhang, M., Cui, S. W, Cheung, PC. K. et al. (2007). Antitumor polysaccharides frommushrooms: A review on their isolation process, structural characteristics and antitumoractivity. Trends Food Sci. Technol, 18:4-19.
[44]. Tabata, K., Ito, W., Kojima, T., et al. (1981). Ultrasonic degradation ofschizophyllan, an anttumor polysaccharide produced by Schizophyllum commune fries.Carbohydr: Res, 89:121-135.
[45]. Coviello, T., Palleschi, A., Grassi, M., et al. (2005). Scleroglucan: A versatile polysaccharide for modified drug delivery. Molecules, 10:6-33.
[46]. Chihara, G., Maeda, Y., Hamuro, J., et al. (1969). Inhibition of mouse Sarcoma180 by polysaccharides from Lentinus edodes (Berk) Sing. Nature.
[47]. Suga, T., Shiio, T., Maeda, Y.Y. (1984). Antitumor activity of lentinan in murine syngeneic and autochthonous hosts and its suppressive effect on 3-methylcholanthrene- induced carcinogenesis. Cancer Res, 44:5132-5137.
[48]. Zákány, J., Chihara, G., Fachet, J. (1980). Effect of lentinan on tumor growth in murine allogeneic and syngeneic hosts. Int. J. Cancer, 25:371-376.
[49]. Hamuro, J., Lentinan, G. C. (1985). A T-cell oriented immunopotentiator: its experimental and clinical applications and possible mechanism of immune modulation., in Immunomodulation agents and their mechanisms, Fenichel R.L. and M.A., C., Editors, New York: Dekker. P: 409-436.
[50]. Hobbs, C. (2002). Medicinal mushrooms: an exploration of tradition, healing, and culture. Santa Cruz, California: Botanica Press.
[51]. Wasser, S.P., AL, W. (1997). Shiitake mushrooms Lentinus edodes (Berk.)Sing.], in medicinal mushrooms, E., N., Editor. Haifa, Israel: Peledfus.
[52]. Wasser, S. P., Weis, A. L. (1999). Medicinal properties of substances occurring in higher basidiomycetes mushrooms: Current perspectives (review). Int. J. Med. Mushrooms, 1:31-62.
[53]. Ikekawa, T. (2001). Beneficial effects of edible and medicinal mushrooms on health care. Int. J. Med. Mushrooms, 3:291-298.
[54]. Furue, H., Kitoh, I. (1981). Phase 111-study on Lentinan. Japanese Journal of Cancer and Chemotherapy, 8:944-960.
[55]. Taguchi, T., Furue, H., Kimura, T., et al. (1985). End point results of phase III study of lentinan. Japanese Journal of Cancer and Chemotherapy, 12:366-378.
[56]. Taguchi, T., Furue, H., Kimura, T., et al. (1985). End-point results of a randomized controlled study on the treatment of gastrointestinal cancer with a combination of lentinan and chemotherapeutic agents, in Rationale of biological response modifiers in cancer treatment, Tsubura, E., Urushizaki, I., and Aoki, T., Editors. Amsterdam Excerpta Medica. p. 151-165.
[57]. Higashi, D., Seki, K., Ishibashi, Y., et al. (2012). The effect of lentinan combination therapy for unresectable advanced gastric cancer. Anticancer Res,
[58]. Ina, K., Kataoka, T., Ando, T. (2013). The use of lentinan for treating gastric cancer. Anticancer Agents Med. Chem, 13, 681-688.
[59]. Ina, K., Kabeya, M., Furuta, R., et al. (2016). P1- 035Chemo-immunotherapy using lentinan for the treatment of inopeable gastric cancer with multiple liver metastases. Ann. Oncol, 27:vii94–vii104.
[60]. Maeda, Y.Y., Chihara G. (1971). Lentinan, a new immuno-accelerator of cell-mediated responses. Nature, 229:634-634.
[61]. Chihara, G. (1992). Immunopharmacology of lentinan, a polysaccharide isolated from Lentinus edodes: its application as a host defense potentiator. Int. J. Orient. Med, 17:57-77.

[62]. Suzuki, M., Iwashiro, M., Takatsuki, F., et al. (1994). Reconstitution of anti-tumor effects of lentinan in nude mice: Roles of delayed-type hypersensitivity reaction triggered by CD4-positive T cell clone in the infiltration of effector cells into tumor. Jap. J. Cancer Res, 85:409-417.