MINI-REVIEW

Progress on Understanding the Anticancer Mechanisms of Medicinal Mushroom: *Inonotus Obliquus*

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Abstract

Cancer is a leading cause of death worldwide. Recently, the demand for more effective and safer therapeutic agents for the chemoprevention of human cancer has increased. As a white rot fungus, *Inonotus obliquus* is valued as an edible and medicinal resource. Chemical investigations have shown that *I. obliquus* produces a diverse range of secondary metabolites, including phenolic compounds, melanins, and lanostane-type triterpenoids. Among these are active components for antioxidant, antitumoral, and antiviral activities and for improving human immunity against infection of pathogenic microbes. Importantly, their anticancer activities have become a hot recently, but with relatively little knowledge of their modes of action. Some compounds extracted from *I. obliquus* arrest cancer cells in the G0/G1 phase and then induce cell apoptosis or differentiation, whereas some examples directly participate in the cell apoptosis pathway. In other cases, polysaccharides from *I. obliquus* can indirectly be involved in anticancer processes mainly via stimulating the immune system. Furthermore, the antioxidative ability of *I. obliquus* extracts can prevent generation of cancer cells. In this review, we highlight recent findings regarding mechanisms underlying the anticancer influence of *I. obliquus*, to provide a comprehensive landscape view of the actions of this mushroom in preventing cancer.

Keywords: Medical mushroom - *Inonotus obliquus* - bioactive compounds - anticancer mechanism

Asian Pacific J Cancer Prev, **14 (3)**, 1571-1578

Introduction

The abilities to invade and metastasize are the defining characteristics of a cancer. After the transformation from a normal cell into a malignant cell via genetic mutation, cancerous cells proliferate rapidly, invade surrounding tissues, break off from the parent lump, migrate around the body in the blood or the lymphatic system, and set up secondary foci of cancerous growths at distant sites (Fidler, 2003). Metastasis is responsible for 90% of the deaths caused by cancer (Patel and Chen, 2012).

A report released by the World Health Organization (WHO) showed that an estimated 12.7 million people were diagnosed with cancer globally and about 7.6 million people died of it in 2008 (Zong et al., 2012). As estimated in this report, more than 21 million new cancer cases and 13 million deaths are expected by 2030. Although cancer accounts for around 13% of all deaths in the world, more than 30%of cancer deaths can be prevented by modifying or avoiding key risk factors (World Health Organization, 2012). Cancer has become currently the most intense field in life science, every year a wealthy of papers reporting the latest discoveries about cancer are published on the most top level journals such as Nature (e.g., Nawy, 2012; Victoria and Seewaldt, 2012), Science (e.g., Schwabe et al., 2012; Keller et al., 2012), Cell (e.g., Magee et al., 2012; Bernards, 2012) and some pharmaceutical journals, etc.

The current anti-cancer drugs available in market are not target specific and have been demonstrated to pose several side-effects and complications as compared with natural anticancer materials, which highlight the urgent need for novel effective and less-toxic agents such as from natural products. As such, medicinal mushrooms and their synthetic derivatives are expected to play an important role in developing innovative agents for prevention of human cancer. As a medical mushroom, *Inonotus obliquus* for containing myriad bioactive components known to exhibit potent effects of scavenging free radicals, antioxidant, hypoglycemic, antiviral, anti-inflammatory and antitumor, etc. (Chen et al., 2010; Choi et al., 2010; Shibnev et al., 2011), has become an important resource of developing nutraceutical and natural drugs for anticancer (Patel and Goyal, 2012). However, ecological harsh habitat & much slow growing speed derived limited natural resources and difficult artificial cultivation of *I. obliquus* to obtain fruiting body make it impossible to obtain large quantity of *I. obliquus*. While submerged cultures offer a promising alternative, which is fast, cost-effective, easy to control and without contamination, and which can provide abundant materials for researches on the pharmacological action of *I. obliquus* and consequently the anticancer activity of its mycelium is discovered, which

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Table 1. Bioactive Metabolites from Sclerotia of *I. obliquus*

Bioactive metabolites	Biological activities	References
Inotodiol (2)	Inhibiting cell proliferationa; hypoglycemic Antimutagenicb; antitumor promotingc (inhibiting 97.8% TPA-induced EBV-EA activation)	Nakata et al., 2007; Nomura et al., 2008; Ham et al., 2009; Lu et al., 2009
3β-Hydroxylanosta-8, 24-diene-21-al (4)	Antimutagenic; (IC_{50}, 232 mol ratio/32 pmol/TPA) antioxidant; hypoglycemic, Anticancer	Ham et al., 2009; Lu et al., 2009; Chung et al., 2010
Lanosta-8, 23E-diene-3β, 22R, 25-triol (6)	Antitumor promoting(IC_{50}, 231 mol ratio/32 pmol/TPA)	Taji et al., 2005
Lanosta-7, 9(11), 23E-triene-3β, 22R, 25-triol (8)	Antitumor promoting (IC_{50}, 228 mol ratio/32 pmol/TPA)	Taji et al., 2005
Lanosta-24-ene-3β, 21-diol (5)	Weak antimutagenic; antioxidant (IC_{50}, 389 mol ratio/32 pmol/TPA)	Taji et al., 2005
Ergosterol peroxide (19)	Anticancer, anti-complementary	Kim et al., 1997; Shin et al., 2001
4-Hydroxy-3, 5-dimthoxy benzoic acid-2-isopropanyl ester (22)	Antioxidant (IC_{50}, for DPPH, 345 μM, FRP activity, 0.55 μM); antitumoral (LD50 for HL-60, 166.8 μM/l, >300 μM/l for other cell lines)	Nakajima et al., 2007; 2009
Protocatechic acid (23)	Antioxidant (IC_{50}, for DPPH, 53.37 μM, FRP activity, 4.99 μM); antitumoral (LD50 for PA-1 61.3 μM/l, >300 μM/l for other cell lines)	Nakajima et al., 2007, 2009
3, 4-Dihydrobenzal aldehyde (24)	Antioxidant (IC_{50}, for DPPH, 18.06 μM, FRP activity, 5.79 μM); antitumoral (LD50 for PA-1 12.1 μM/l, U973, 105.5 μM/l, 56.0 μM/l)	Nakajima et al., 2007, 2009
Syringic acid (25)	Antioxidant (IC_{50}, for DPPH, 50.80 μM, FRP activity, 2.45 μM); weak antitumoral	Nakajima et al., 2007, 2009
2, 5-Dihydroxylterephtalic acid (26)	Antioxidant (IC_{50}, for DPPH, 24.84 μM, FRP activity, 1.64 μM), weak antitumoral	Nakajima et al., 2007, 2009
3, 4-Dihydroxyl-benzalacetone (28)	Antioxidant (IC_{50}, for DPPH, 27.75 μM, FRP activity, 4.60 μM); antitumoral (LD50 for PA-1, 12.2 μM/l, U973, 53.0 μM/l, 32.9 μM/l)	Nakajima et al., 2007, 2009
Hispolon (30)	Cell growth inhibiting (IC_{50} for human epidermoid KB cell, 4.62 μg/ml); antioxidant; antiviral	Babitskaia et al., 2000, Chen et al., 2006
Caffeic acid (27)	Antioxidant (IC_{50}, for DPPH, 41.42 μM, FRP activity, 5.46 μM); antitumoral (LD50 for PA-1 68.3 μM/l, U973, 89.1 μM/l, 27.40 μM/l)	Nakajima et al., 2007, 2009
Hispidin (31)	Antioxidant (IC_{50}, 1.31 μM for DDPH, 2.27 μM for ABTS and 47.3 for SOA); tumor cytotoxic; antiviral	Gonindard et al. 1997; Park et al. 2004; Chen et al., 2007b; Lee et al. 2009

DPPH 1,1-diphenyl-2-picrylhydrazyl, FRP ferric reduction power, SOA superoxide anion, ABTS 2.2'-azinobis(3-ethylbenzothiazoline-6-sulfonate); a, Inhibiting P388 cell proliferation with the minimum concentration at 30 mM; b, Inotodiol-containing fraction showed 73.3% inhibition to the mutagenesis induced by mutagen MNNG; c, Antitumor promoting potential was referenced by 32 ng/ml (32 pmol/ml) TPA (12-O-tetradecanoylphorbol-13-acetate)

provide abundant raw materials for *I. obliquus* study and imply a large scale market development. This paper firstly describes briefly *I. obliquus* and its bioactive chemical constituents, subsequently the anticancer mechanisms of its bioactive constituents are reviewed with highlight, which is expected to give a base for exploitation and utilization of the anticancer bioactive materials from the natural medical mushroom of *I. obliquus*.

Inonotus obliquus and Bioactive Constituents

The mushroom called *Inonotus obliquus* also known as *Fuscoporia oblique* or Chaga (in Russia), belonging to the Hymenochaetaceae family of Basidiomycetes, subdivided from Aphyllophorales of Polyporaceae, is a black parasitic white-rot fungus (Sun et al., 2008) who can form sclerotia. Morphologically, *I. obliquus* is shaped like a wedge, bursts through the bark, and appears as large gall-like structures, varying in size from 5 to 40 cm in diameter, with a very irregularly cracked and deeply fissured surface. Inside the burnt charcoal surface is rusty-colored woody texture consisting of interwoven mycelia. *I. obliquus* preferably inhabits on living trunks of the mature birch such as Betula platyphylla Suk, Grebillea robusta A Cunn ex R.Br, and rarely on Ulmus pumila L. and Alnus japonica (Thunb.) (Hyun et al., 2006), approximately 20 thousands plants can be found only one that have *I. obliquus* inhabited and which results in its stiff price. Sclerotia of which can survive for as long as six years in the trunk of felled trees and its mycelium can tolerant low temperature of up to -40 °C, this fungus is restricted to cold habitats at latitudes of 45°N-50°N including North America, Finland, Poland, Russia (West Siberia, east part, the Kamchatka Peninsula), northeast of China (Lesser Khingan Range & Greater Hinggan Mountains of Heilongjiang province and Changbai Mountain of Jilin province) and Japan (Hokkaido) (Sun et al., 2008).

In nature, *I. obliquus* grows in very cold habitats and is exposed to irregular seasonal environmental stresses including freezing temperatures, UV irradiation (Hoshino et al., 1998; Zucconi et al., 2002), and the invasion of various pathogenic microbes (Bolwell et al., 2001). In response to these, *I. obliquus* has evolved a complex series of integrated defense systems. In recent years, more than 20 different kinds of bioactive components have been found in *I. obliquus* mainly including inooidiol the precursor of vitamin D2, betulinic acid, oxygenated triterpenes (Handa et al., 2010), superoxide dismutase (SOD), trametenolic acid, dextran, tannin compounds, steroids, alkaloid, lanosterol (Nakamura et al., 2009), lanoline alkane type three terpenes, lignin, melanin catechol (Zheng et al., 2009), folic acid derivatives, sheath ammonia fat analogs, mannitol, polyphenol, lanosterol (Wang et al., 2001), half the tannin compounds, aromatic substances (Zhao and Piao, 2006; Taji et al., 2008), β-glucan (Rhee et al., 2008), peptides (Hyun et al., 2006), polysaccharides (Cui et al., 2005; Zhang et al., 2007; Nakajima et al., 2007), polyphenols (Cui et al., 2005; Lee et al., 2007), triterpenoids, and steroids (Cui et al., 2005). Some components and their effects on tumor cell can be found in Table 1. These compounds are considered to be the active constituents underlying the myriad functions such as inhibiting cancer process, suggesting a high medicinal value and good prospects for market development. Since the 16th century, the sclerotia of this fungus called 'Chaga' with little toxic side effects (Chen et al., 2010) has been extensively used as a folk remedy for cancer, digestive system diseases and tuberculosis in Russia and western Siberia (Bhatnagar, 1998; Huang, 2002; Gao et al., 2006), and for heart disease and diabetes in Eastern Europe, North America and other regions. Furthermore, many studies had reported that *I. obliquus* can function in preventing and curing AIDS, anti-aging, decreasing blood lipids, lowering blood pressure, improving physical condition of allergy and strengthening the immune system of body (Kim et al., 2007; Sarikurkcu et al., 2008).

Tumor Inhibition Effects of *I. obliquus*

I. obliquus can be made into tea decoction, extracts, syrups, injections (injections), hip bath agent, aerosol for the treatment of neoplastic diseases (Zhao et al., 2004). In northwestern Russia, Siberia and Finland, the fruiting body of *I. obliquus* is decocted to treat cancer patients. At the year of 1955, the Medical Academy of Science in Moscow announced *I. obliquus* as anticancer substance,

Table 2. Biologically Active Parts and Fractions on Cancer

Bioactive parts or fractions	Biological activities on cancer	References
endo-polysaccharide	anti-cancer	Kim et al., 2006
water extract	Anticancer; 56% inhibitory against tumor cells [a]	Youn et al., 2008; Youn et al.,2009; Lee et al., 2009
Hot water extracts	antiproliferative and antioxidant (EC50, 126 μg/ml for DPPH)	Hu et al., 2009
Ethanol extract	44.2% inhibitory activity against tumor cells, 74.6% inhibitory rate for MTT; antioxidant activity and antiproliferative	Sun et al., 2011; Hu et al., 2009
Aqueous extract	Antioxidant; antitumoral; antimitotic; antitumoral; decreasing activity of LDH, HBDH, MDH and GGT and increasing CAT	Burczyk et al., 1996; Rzymowska, 1998; Chen et al., 2007a; Liang et al., 2009
Methanol extract	Anti-inflammatory[a] and anti-nociceptive[b] antimutagenic	Park et al., 2005; Ham et al. 2009
Total polysaccharides	Antitumoral and antioxidant	Song et al., 2008
Water-soluble polysaccharide	Antitumor and immunomodulatory activity	Fan et al., 2012

LDH, lactic acid dehydrogenase; HBDH, αα-hydroxylbutyrate dehydrogenase; MDH, α-malate-dehydrogenase; GGT, γ-glutamyltransferase; CAT, catalase; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; [a]Inhibiting the growth of B 16-F10 by causing cell cycle arrest at G 0 /G 1 phase and apoptosis by down-regulation of pRb, p53, and P27 expression levels; [b]Inhibiting hind paw edema in rats induced by carrageenin; [c]Induced by acetic acid-induced abdominal constriction and hot-plate test in mice

and later approved by the government to be used for development of pharmaceuticals. Then at 1960, the U.S. National Cancer Institute received a report from Australia showing the *I. obliquus* decoction could cure cancer. Up to date, a kind of brown liquid called "Befungin" is still widely used for treating cancer in Poland (Zhukovich et al., 2010).

Having an obvious inhibit effect on a variety of tumor cells, *I. obliquus* prevents the metastasis and recurrence of cancer cells favoring healthy (Wasser, 2002; Cui et al., 2005), the extracts of which can significantly inhibit the growing of cancer cells in vitro (Ham et al., 2003) as showed in Table 2. Anti-tumor experiments with n-hexane extractives of *I. obliquus* have been conducted, triterpenoids from extract of *I. obliquus*, especially Inotodiol can dramatically inhibit the walker 256 carcinosarcoma, MCF-7 human mammary adenocarcinoma and leukemia cells P388 (Kahlos et al., 1987; Handa et al., 2010). Li et al. (2003) reported that *I. obliquus* extracts with concentration of 0.5 μg•mL-1~16.0 μg•mL-1 can inhibit the proliferation and induce apoptosis of the gastric cancer cell line MGC-803, the effect of which displays a dose-response. The most abundant triterpene, inotodiol (3), was investigated by Nakata et al. (2007) for the inhibitory effect in a two-stage carcinogenesis test on mouse skin using 7,12-dimethylbenz[a]anthracene (DMBA) as an initiator and TPA as a promoter. Compound 3 was found to exhibit the potent anti-tumor promoting activity in the in vivo carcinogenesis test. In addition, administering active components of *I. obliquus* can enhance the patient's tolerance during chemotherapy or radiotherapy process and weaken the side effects caused by which (Park et al., 2005).

Anticancer Mechanisms of Bioactive Constituents Derived from *I. obliquus*

Cell proliferation and death are involved in the maintenance of homeostasis in normal cells; however, homeostasis is often disrupted in tumor cells with uncontrolled proliferation. Anti-tumor effects could be attributed to altered biochemical mechanisms, including inhibitions of proliferation, induction of cell cycle arrest at various cell cycle checkpoints, enhanced apoptosis, and regulation of signal transduction pathways, which are related to altered expressions of key enzymes (Swanton, 2004). Therefore, cell cycle arrest and the induction of cell apoptosis to prevent cancer cell proliferation becomes the major target of anti-cancer drugs.

Involving in the arrest of cell cycle in cancer cells

In eukaryotic cells, cell cycle checkpoints help to lead the orderly progression and completion of critical events, such as DNA replication, chromosome segregation and induction of differentiation (Elledge, 1996). Thus, the arrest of cell cycle is the first event that occurs at the moment when the fate of the cells is decided to either differentiation or proliferation. Usually, the decision of cells to differentiate is made in the G0/G1 phase of the cell cycle, and the induction of differentiation is believed to follow G0/G1 cell cycle arrest (Zhu and Skoultchi, 2001). The regulation of cells entering from the G1 phase into S phase is particularly important, as the cells normally must pass through a restriction point in late G1 to progress to the S phase (Pardee, 1989). The activities of two types of cyclins (cyclin D and cyclin E), Cdks (cyclin-dependent kinases 2, 4 and 6), and Cdks inhibitors are necessary for entering from the G1 phase into S phase of the cell cycle (Weinberg, 1989). Thus, most of bioactive substances from natural products have been reported to exert their anticancer activity by blocking cell cycle progression and triggering tumor cell apoptosis (Chang et al., 2004; Ye et al., 2005; Hsieh et al., 2006).

Youn et al. (2008) confirmed that *I. obliquus* water extract (IWE) previously used for treating cancers and digestive system diseases could selectively and significantly inhibited the viability and proliferation, and induced apoptotic cell death in human hepatoma HepG2 cells, while this effect was not found in the human immortalized non-tumor cell line. HepG2 cells were dose-dependently arrested by IWE at the G0 /G1 phase of the cell cycle, and cyclin D1 and Cdk2, and Cdk6, which were responsible for most of the cell cycle arrest, were effectively deceased at the lowest dose of IWE. Meanwhile, HepG2 cells that had a functional p53 were more sensitively damaged by IWE than Hep3B cells who did not have a functional p53, i.e., delete p53. G1-phase arrest of cell cycle progression provides an opportunity for cells to either undergo repair mechanisms or proceed by the apoptotic pathway (Mantena et al., 2006). p53 is a tumor suppressor gene encoding a transcription factor, the tumor-suppressive activity of which involves inhibition of cell proliferation through cell cycle arrest and/or apoptosis or subject to NDA repairing. The apoptosis-inducing effect

of *I. obliquus* extract on cancer cells may be closely related to p53. However, which exactly part of its components and how they induce the up-/down-regulation of these genes are still under the dark. And what other components in the cancer cells that participate in this process needs further investigation.

Besides, the anti-proliferation effect of IWE on melanoma B16-F10 cells by Youn et al. (2009) suggested that it not only inhibited the growth of cancer cells by causing cell cycle arrest at G0 /G1 phase and apoptosis, but also induced cell differentiation. While, the association of these effects with the down-regulated expression of pRb, p53 and p27, further show that *I. obliquus* extracts result in a G0/G1 cell cycle arrest with reduction of cyclin E/D1 and Cdk2/4 expression levels (Youn et al., 2009). Intraperitoneal administration of *I. obliquus* extract significantly inhibited the growth of tumor mass in B16-F10 cells implanted mice, resulting in a 3-fold inhibition at dose of 20 mg/kg/day for 10 days. The ethanolic extract of sclerotium and fruiting body of *I. obliquus* elicited significant anti-tumor activity, 74.6 and 44.2%, respectively (Sun et al., 2011), which could be attributed to the promoting effect in cell differentiation.

Direct participatiuon in the cell apoptosis pathway

Beside through arresting tumor cells in G0/G1 phase following induce cell apoptosis or differentiation, water extract of *I. obliquus* (WEI) can directly and specifically act on the apoptosis pathway. When human colon cancer cells (HT-29) were treated by 1.0 mg/mL WEI for 48 h, the maximum inhibitory effect (56%) was observed, accompanying with down-regulation of Bcl-2 and up-regulation of Bax and caspase-3 (Lee et al., 2009). Among them, Bcl-2 can form ion channels in biological membranes, which then influence the permeability of intracellular membranes, and thus the mitochondrial contents released into the cytoplasm (Kim et al., 2003), potentially resulting in the activation of caspase-3 and thereby leading to the induction of apoptosis. However, the overexpression of Bcl-2 protein can rescue cells from apoptosis by maintaining membrane integrity and preventing the release of mitochondrial contents (Frémont, 2000). Bax is a pro-apoptotic factor that translocates from the cytosol to the outer mitochondrial membrane, where it forms heterodimers with Bcl-2 protein to create pores and mediate the release of cytochrome c (Park et al., 2007). The ultimate goal of down-regulating the Bcl-2 expression and up-regulating the expression of Bax and caspase-3 induced by WEI is to induce the cancer cells undergo apoptosis, achieving the anti-cancer effect.

Hereafter, Zhong et al. (2010) also found the decreased expression of Bcl-2 and increased expression of caspase-3 and Bax after co-culture of 80 mg /L *I. obliquus* extract with human gastric BGC2823 cell line for 48 h. However, beside the up-regulated expression of p53 and Bax proteins and the down-regulated Bcl-2 protein, the expression of Ki-67 whose synthesis and expression are closely related to cell proliferation (Nariculam et al., 2009) found to decreases with the increase of Inotodiol concentration and exposure time when A549 cells are arrested in S phase by Inotodiol (Zhong et al., 2011).

Indirect anticancer effects via immunostimulating

Recently, accumulated evidence has demonstrated that polysaccharides have a broad spectrum of biological effects, such as antibiotic, antioxidant, anti-mutant, anticoagulant, and immunostimulation activies (Ali et al., 2009; Wijesekara et al., 2011). The mechanisms polysaccharides from natural resources exert their tumor inhibition effects are reviewed by Zong et al. (2012) and can be assigned into the following four aspects: (1) the prevention of tumorigenesis by oral consumption of active preparations; (2) direct anticancer; (3) immunopotentiation activity in combination with chemotherapy; and (4) the inhibition of tumor metastasis.

The endo-polysaccharide from *I. obliquus* suppressed the in vivo growth of B16F10 murine melanoma cells, highly metastatic-malignant neoplasm of melanocytes, in mice after both oral and intraperitoneal administration with intraperitoneal being more effective (Kim et al., 2006). In most cases, intraperitoneal administration is more rapid and effective than oral (Bae et al., 2005). Intraperitoneal administration of the endo-polysaccharide significantly prolonged the survival rate of B16F10-implanted mice, approximately 67% of the initial number of mice survived with no tumor incidence after 60 days of feeding (Kim et al., 2006). Before this, documents have demonstrated that this mycelium endo-polysaccharide did show no any direct cytotoxic effect on most of melanoma cells and no cytotoxicity for normal cells, instead significantly activated the macrophage function of mouse immunocytes (Kim et al., 2005). Afterwards, the signaling pathway of macrophage activation by *I. obliquus* polysaccharide was demonstrated to induce the phosphorylation of three MAPKs as well as the nuclear translocation of NF- ϰB (Won et al., 2011). Thus, the anti-cancer effect of endo-polysaccharide from *I. obliquus* is not directly tumorcidal but rather is humoral immune related immuno-stimulating, which different from that of directly inhibition of tumor cell growth and protein synthesis by sclerotia polysaccharides.

I. obliquus polysaccharide was capable of promoting NO/ROS production, TNF-α secretion and phagocytic uptake in macrophages, as well as cell proliferation, comitogenic effect and IFN-γ/IL-4 secretion in mouse splenocytes (Won et al., 2011). Fan et al. (2012) purified a water-soluble polysaccharide (ISP2a) from *I. obliquus* by DEAE-Sepharose CL-6B and Sepharose CL-6B column chromatography and then the anti-tumor activity of ISP2a was tested. The ISP2a exhibited no significant anti-tumor activities and the growth of SGC-7901 cells was not affected by ISP2a treatment in vitro, but significantly inhibited the growth of transplantable SGC-7901 in mice in vivo, and this inhibition effect increase with dose. Meanwhile, the proliferation activity of splenocyte and macrophage was significantly enhanced in vitro by ISP2a, which may directly resulted in the obviously elevation of relative weights in spleen and thymus, beside, the concentration of TNF-α in serum of mice was increased significantly as well. This demonstrates that the anti-tumor activity of polysaccharide from *I. obliquus* may through indirectly pathway of immunomodulatory.

The chemical components, modifications, structure and

other physical properties of fungal polysaccharide all can influence the anticancer activity of *I. obliquus*. When an alien substance enters into the body, the immune system will see it as an antigen firstly and attack it to eliminate this potential dangerous invader. *I. obliquus* polysaccharides may contain some antigen fragments of or the structure of which may alike some component of cancer cells, bridging the cancer cells with immunocytes and facilitating the immunocytes to discriminate cancer cells with more precision. Until now, however, how exactly the *I. obliquus* polysaccharide stimulate human immune system is unclear, and whether this stimulate effect on the cancer, at the same time indirectly impact other disease process (e.g. cardiovascular and cerebrovascular diseases). Furthermore, the signaling pathway toward the activation of immunocytes such as macrophages and T cells by polysaccharides isolated from *I. obliquus* is still under the dark.

Other anticancer mechanisms of I. obliquus

I. obliquus polysaccharide isolated via water extraction following alcohol precipitation could decrease sialic acid concentration and the level of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) in serum, suppressing the promoting effects of NO on blood vessel generation (Jin et al., 2004). Additionally, *I. obliquus* extracts can via decreasing the number of proteins and mitosis indexes, while increasing the cell number of metaphase in cell division to interfere with cell mitosis so as to inhibit cancer cell proliferation. At the same time, the metabolism of cancer cells can also be affected by *I. obliquus* extracts, such as induce the decrease in lactate dehydrogenase (LDH) hydroxybutyrate dehydrogenase (HBDH), malate dehydrogenase (MDH), γ-gamma glutamyl transpeptidase (GGT) and the increase in catalase(CAT) activity (Zeng, 2007).

Reactive oxygen species (ROSs) play important roles in degenerative or pathological processes in conditions such as aging (Burns et al., 2001), cancer, coronary heart disease, Alzheimer's disease (Ames et al., 1983; Gey, 1990; Diaz et al., 1997), neurodegenerative disorders, atherosclerosis, cataract formation, and inflammation (Aruoma, 1998). Excessive production of ROSs may lead to oxidative damage to proteins, DNA, genomic instability and other macromolecules, accumulation of which with time favors the acquisition of mutations and ultimately results in the cellular transformation to cancer cells (Vera-Ramirez et al., 2011). However, a rush of studies have reported that substances from *I. obliquus* harbor the antioxidative properties (Huang et al., 2012; Mu et al., 2012), which can prevents the generation of cancer cells. Hu et al. (2009) found that hot water and ethanol extract of *I. obliquus* could induce the apoptosis in colon cancer cells (DLD-1) byway of hindering reactive oxygen species caused tissue damage.

Current Problems and Future Perspectives

Although a large number of pharmaceutical studies have focused on the treatment of cancer currently, further studies are needed on the complex molecular mechanisms respect to cancer formation, proliferation, and metastasis due to cancer generation is controlled by multiple genes and influenced by a variety of factors in vitro and in vivo. As such, the anti-cancer process is still very arduous and cancer is still a leading cause of death worldwide. The significant inhibition effects on cancer cells by *I. obliquus* extracts have been demonstrated by both in vivo and in vitro cell toxicological tests on tumor cells. Nevertheless, the various *I. obliquus* extraction methods as well as the diversity and complexity of the components extracted lead to a baffle that whether anticancer effects of *I. obliquus* is attributed to a kind of single component, or to the synergistic effect of the many individual components, and whether antagonism exists between each components in term of anticancer is unclear. Although some studies have shown the anticancer effects of a single component from *I. obliquus*, such as polysaccharide, but the anti-cancer mechanisms of which needs further explored, whether they act effectively solely on a single anticancer pathway or not.

The limited provision of natural fruiting body of *I. obliquus* leads to the utilization of submerged fermentation technology, by which a great quantity of *I. obliquus* mycelium can be obtained. However, the types and yields of anticancer active components are influenced by fermentation conditions. Therefore, the future research should be starting from the metabolic pathways of *I. obliquus* active components to explore their synthesis pathway, fermentation and purification, and the metabolic pathways based construction of engineering bacteria can be conducted for the production of its active constituents, which is expected to provide a theoretical basis base for scientifically developing of anticancer health care products, as well as microcapsules.

Acknowledgments

This study was supported by National Forestry Public Welfare industrial special study (201004079), Youth Science Foundation of Heilongjiang University (QL2011), Innovation Laboratory Project of Heilongjiang University (CX11083) and Laboratory Opening Foundation of Heilongjiang University.

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