Chapter - 1

Introduction

Synopsis

Medicinal mushrooms have an established history of use in traditional oriental therapies. Biological properties of mushroom components with special emphasis to the mushroom polysaccharides are briefly outlined. This chapter also examines the relationship of free radicals, inflammation and apoptosis to the development of neoplasia. A brief account of the medicinal mushroom, Phellinus rimosus, used in the present study is given at the end of this chapter.

The global awareness of cancer as the second largest cause of death in people of various ages and racial background has lead to large research efforts and clinical studies in the fight against the disease (Daba and Ezeronye, 2003). Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Cancer is caused by both external factors (tobacco, chemicals, radiation, and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). These causal factors may act together or in sequence to initiate or promote carcinogenesis. Ten or more years often pass between exposure to external factors and detectable cancer (American Cancer Society, 2008).

Major modalities of cancer treatment now are surgery, radiation and chemotherapy. It has been generally recognized that in the treatment of cancer, surgery with or without radiotherapy remains the modus operandi for most cancer cures. Radiotherapy is used successfully for many forms of cancers while chemotherapy has become an integral part of a multi-disciplinary treatment of cancers and has served also as a palliative measure in cases of advanced cancer. However, cancer treatment by conventional therapies is known to have several adverse effects. The adverse effects are greatest on hematopoietic tissue, gastrointestinal mucosa, gonads and skin. Injury to hematopoietic tissue causes
severe immunosuppression and negatively affects therapy by leaving the host susceptible to infection by opportunistic and pathogenic microorganisms. It is well recognized that both radiotherapy and chemotherapy invariably damage or weaken the patient’s immunological defences which may also have been damaged by the cancer itself. Furthermore, within the holistic approach of clinical cancer therapy there is now increasing emphasis being given to patient quality of life (QOL). Survival should not be the sole criterion for assessing the treatment results.

Recent investigations have been channelled on the development of immunotherapy to target cancer cells as well as on substances such as immunopotentiators, immunoinitiators and biological response modulators (BRM) that act to prevent carcinogenesis and induce carcinostasis (Wasser and Weis, 1999a; b). Biological Response Modifiers has now evolved as the fourth method of cancer treatment in addition to surgery, radiotherapy and chemotherapy. Immunostimulating agents would possibly be useful adjuncts to conventional treatments of cancer if they do not interfere with the ability of the conventional treatment to kill tumor cells.

Medicinal mushrooms have an established history of use in traditional oriental therapies. Historically, hot water-soluble fractions (decoctions and essences) from medicinal mushrooms were used as medicine in the Far East, where knowledge and practice of mushroom use primarily originated (Hobbs, 2000; Wasser, 2002). The use of medicinal mushrooms in the fight against cancer is known for a very long time in Korea, China, Japan, Russia, USA and Canada. An old Japanese legend reports that wild monkeys rarely experience cancer, high blood pressure or diabetes. The legend suggests that perhaps it is due to some extent to their habit of wild mushroom consumption. Mushrooms belong to the group of immunocuticals by their mode of action. Nowadays, macrofungi are distinguished as important natural resources of immunomodulating and anticancer agents. The use of immunomodulating agents especially of natural origin has vital importance in diseases involving immune dysfunction, cancer and autoimmune conditions. The physiological constitution of host defence
mechanisms are improved by the intake of mushrooms and their components which restore homeostasis and enhance resistance to disease. A central premise in Oriental medicine is to regulate homeostasis of the whole body and to return the diseased individual to the normal state. Several major substances with immunomodulatory and/or anti-tumor activity have been isolated from mushrooms. These include mainly polysaccharides (in particular β-D-glucans), polysaccharoepptides (PSP), polysaccharide proteins and proteins. Furthermore, other bioactive substances, including triterpenes, lipids and phenols, have been identified and characterized in mushrooms with proven medicinal properties.

Polysaccharides are the best known and most potent mushroom derived substances with anti-tumor and immunomodulating properties. Several purified mushroom polysaccharides have been in clinical use in Japan, China, and US for several years with no reports of any significant short-term or long-term adverse effects. Five mushroom preparations have shown clinically significant efficacy against human cancers and are used as BRMs: lentinan from Lentinus edodes, D-fraction from Grifola frondosa, schizophyllan from Schizophyllum commune, PSK (krestin) and PSP (polysaccharide peptide) from Trametes versicolor. All of these preparations are chemically β-D-glucans or β-D-glucans linked to proteins. Mushroom polysaccharides offer considerable hope for cancer patients and sufferers of many devastating diseases (Chihara, 1992a; b). These compounds have been shown to be safe when consumed over long periods of treatment; and known to reduce the adverse effects of radiotherapy and chemotherapy. There are also many examples where the use of these compounds allows the reduction in dose level of the chemotherapeutic compound without reduced efficacy.

Mushroom polysaccharides display a wide array of biopharmacological activities. While much attention has been drawn to various immunological and anti-cancer properties of the mushroom compounds, they also offer other potentially important therapeutic properties including antioxidants, anti-hypertensive, cholesterol-lowering, liver protection, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-viral and anti-microbial. Unlike proteins and nucleic acids, polysaccharides contain repetitive structural features which are
polymers of monosaccharide residues jointed to each other by glycosidic linkages. Among these macromolecules, polysaccharides offer the highest capacity for carrying biological information because they have the greatest potential for structural variability. For example, the number of possible permutations for four different sugar monomers can be up to 35,560 unique tetrasaccharides, whereas four aminoacids can form only 24 different permutations (Ooi and Liu, 2000). Therefore, this enormous potential variability in polysaccharide structure allows the necessary flexibility for the precise regulatory mechanisms of various cell-cell interactions in higher organisms.

Polysaccharides, which are the major constituents of mushrooms, have been demonstrated to play an important role as dietary free-radical scavenger for the prevention of oxidative damage. Free radicals and active oxygen can induce oxidant damage. There are increasing evidence indicating that reactive oxygen species (ROS) produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes show a wide variety of pathological effects, such as causing DNA damage, carcinogenesis and cellular degeneration related to aging (Blander et al., 2003; Harman, 1993; Liu et al., 1997). The oxyradicals attack DNA causing change in genomic sequences leading to mutation, deletion, gene-amplification or rearrangement. In this manner, oxidative DNA damage was found important in the etiology of many human cancers (Irshad and Chaudhuri, 2002).

Free radicals have long been implicated in damage of connective tissues in inflammation. It is becoming more evident that many aspects of tumor promotion arise from persistent and unresolving inflammation. Chronic inflammation may be one of the driving forces of transformation that together with other determinants, including the intrinsic properties of pre-malignant cells supports the initiation of cancer. Obviously, if inflammatory conditions prevail at the tumor site, they may further support the progression of tumor into more advanced stages and also promote metastasis. Acceleration of neoplastic processes by chronic inflammation was clearly observed in many malignant diseases (Coussens and Werb, 2002; Schwartzburd, 2003).
In normal tissues, a homeostasis is maintained by a balance between cell proliferation and apoptosis. Apoptosis or programmed cell death is a regulated process involving activation of a series of molecular events leading to cell death. Recent scientific advances indicate that reduced apoptosis and/or increased cell proliferation play a central role in carcinogenesis. Therefore, treatments of human neoplasia have been directed to promote apoptosis of tumor cells. Agents that specifically induce apoptosis in tumor cells without affecting the corresponding normal cells could serve as key factors in successful treatment of human cancers. Mushroom polysaccharides are widely being used as nonspecific immunostimulants for cancer patients. Now it has been suggested that the anticancer effects of mushroom polysaccharides are not only immunomodulatory, but may result from cell cycle arrest and/or induction of apoptosis. It is generally believed that polysaccharides do not induce direct toxicity in cancer cells. Recently, many mushroom polysaccharides have been shown to exert a direct cytotoxic effect on cancer cells in vitro.

*Phellinus* is a large and widely distributed genus belonging to the *Hymenochaetaceae* of basidiomycetes. There are approximately 220 known species of *Phellinus* mushrooms in the world, and they were found mainly in tropical areas of America and Africa (Kim et al., 2003d). Many kinds of *Phellinus* species including *P. igniarius*, *P. hartigii*, *P. gilvus*, *P. pini*, etc. are known to have several medicinal effects such as anti-tumor and immunostimulating activities (Ayer et al., 1996; Jung et al., 1997; Rew et al., 2000; Shibata et al., 1968; Shon and Nam, 2001). Among them, *P. linteus* is well known as one of the most popular medicinal mushrooms in traditional Chinese medicine due to its high anti-tumor (Han et al., 1999) and immunostimulating (Lee et al., 1996b; Kim et al., 1996) activities. About 18 species of *Phellinus* are found to occur in Kerala, most of them are wood inhabiting (Leelavathy and Ganesh, 2000). *Phellinus rimosus* (Berk) Pilat, commonly called ‘Bracket fungus’ or ‘Cracked cap polypore’, referred to as ‘Plamanjal’ or ‘Plachanam’ in Malayalam, is a polyporous macrofungus, parasitic mostly on jack fruit tree trunks in Kerala (Figure 1.1). It has reddish-brown cork-like texture and resembles a shelf,
growing on the trunk of living trees. The dorsal side has woody appearance, whereas the ventral side has velvet like appearance (Figure 1.2). It lacks stalk and grows up to 25 cm. It is a kind of wood rotting species, which causes so-called “whitening”, that is, decaying a host tree to the point where it shows a white color due to its strong lignin decomposition ability. It causes white pocket rot initially but later the heartwood is transformed into a white spongy mass. The present study was focused on the isolation and characterization of the total and neutral polysaccharides from the fruiting bodies of *P. rimosus* and on the anti-tumor, antioxidant, anti-inflammatory, cytotoxic, anti-proliferative and apoptotic activities of the isolated polysaccharides. The findings are presented in this thesis.
Figure 1.1. Photograph of fruiting body of *Phellinus rimosus* growing on natural habitat

Kingdom: **Fungi**  
Phylum: **Basidiomycota**  
Class: **Basidiomycetes**  
Order: **Hymenochaetales**  
Family: **Hymenochaetaceae**  
Genus: **Phellinus**  
Species: *Phellinus rimosus* (Berk.) Pilat
Figure 1.2. Fruiting body of *Phellinus rimosus* (a) dorsal and (b) ventral view