11.1 Introduction

Tumour (neoplasm) is an abnormal mass of tissue which may be solid or fluid filled. It physically appears as a lump or swelling which may or may not pose a threat to health. Tumours vary in shape, size and kind of tissue they appear in.

Tumours are categorized as benign (non-cancerous) or pre-malignant/malignant (cancerous). A benign tumour (adenoma, fibroma, lipoma and hemangioma) cannot metastasize and is non-progressive. Although deemed unharmful to human health, benign tumours may have an impact on blood vessels or nerves and cause negative effects. Malignant tumours or cancerous tumours tend to progress and metastasize at a high rate to other parts of the body and may eventually cause death.

11.2 Current therapy for tumours

Current mainstream therapy includes surgery, chemotherapy, radiation therapy, targeted therapy, immune therapy, hyperthermia therapy, stem cell transplant, photodynamic therapy and the use of lasers. There are various side effects associated with these treatments which include, among others, fatigue, nausea, lymphoedema, effects on fertility and so on; and some treatments may increase a person’s risk of developing different types of cancer later in life. Complementary medicine such as aromatherapy, biofeedback, massage therapy, meditation, tai chi or yoga is considered to be an advantage when used along with mainstream medical care. This integrative therapy can improve the quality of life without causing problems with regular cancer treatments.

11.3 Antitumour agents from nature

Over 60% of current anticancer drugs originate from natural sources. These biologically active and diverse chemotypes from nature may serve as models for preparation of efficacious analogues using various chemical methodologies or by manipulation of biosynthetic pathways (Cragg and Newman, 2009). The US National Cancer Institute (NCI) has provided resources for preclinical screening of compounds and materials submitted by scientists worldwide for over 50 years. More than 500,000 chemicals, both synthetic and natural,
have been screened for their antitumour activity (Cragg and Newman, 2009).

Anticancer agents have been developed from plant, marine and microbial sources. Examples of these agents include vinblastine, vincristine, vinorelbine, vindesine, etoposide, Etopophos, teniposide, podophyllotoxin, paclitaxel, docetaxel, topotecan, irinotecan, camptothecin and flavopiridol (from plants); bryostatin 1, symplostatin, aplidine, kahalalide and squalamine (from marine sources); and daunorubicin, Adriamycin, ixabepilone and bleomycin (from microbial sources).

11.4 Fungi as antitumour agent

Fungi are eukaryotic organisms which include microorganisms such as yeasts, moulds and mushrooms. Genetically, fungi are more closely related to animals than to plants. According to a recent review by Cragg and Newman (2009), a number of highly effective microbial-derived chemotherapeutic agents have recently been discovered and developed and have shown potential to be a vast untapped source for drug discovery.

A familiar fungus, mushrooms, has been valued for millennia as an edible and medical source, particularly in Asian countries. It is fleshy and has an aerial umbrella shape which is the fruiting body of macrofungi. The fruiting body is usually large enough to be seen and harvested. Most of these mushrooms are grown in the wild, but of late, medicinal mushrooms are cultivated for commercial gains in traditional Chinese medicine (TCM). The main medical uses of mushrooms discovered so far are as antioxidant, anti-diabetic, hypcholesterolaemic, antitumour, anticancer, immunomodulatory, anti-allergic, nephroprotective and antimicrobial agents (Patel and Goyal, 2012).

The recognition of medicinal mushrooms as a functional food and food that promotes health has enabled its penetration into the West, and its use has been steadily increasing in complementary medical practices (Sullivan et al., 2006). Mushrooms are known to complement chemotherapy and radiation therapy by countering the side effects of cancer, such as nausea, bone marrow suppression, anaemia and lowered resistance.

Ying and colleagues (1987) reported that there are at least 270 species of mushroom with therapeutic properties (hence termed ‘medicinal mushrooms’) including anticancer activity. Mushrooms with potential anticancer activity include Phellinus linteus mainly found in America, Africa and Asia), Agaricus bisporus (also known as button mushroom), Pleurotus spp., Lentinula edodes and Grifola frondosa. These mushrooms have been scientifically investigated and have shown potential to possess high antitumour and immunomodulating activities (Chihara et al., 1970; Taguchi et al., 1985a, b; Nishida et al., 1988; Devita et al., 1993; Gunde-Cimmerman et al., 1993; Song et al., 1995; Kurashiga et al., 1997; Dai and Xu, 1998; Mizuno, 1999; Gunde-Cimmerman, 1999; Wasser and Weis, 1999; Jose and Janardhanan, 2000; Wasser, 2002; Kim et al., 2003; Lee et al., 2005; Chen, 2006; Gu and Gowsala, 2006; Wu et al., 2007).

Patel and Goyal (2012) recently reviewed 20 different mushrooms with anticancer potential and disclosed their pharmacologically active compounds, their antitumour potential and their underlying biological mechanism. Their findings are important as there is a lack of chemotherapeutic agents for some forms of malignant cancer, for example, oestrogen receptor-negative human breast cancer, mesothelioma, acute lymphocytic leukaemia, acute myeloid leukaemia, Hodgkin lymphoma and astrocytoma.

11.5 What component in fungi is the source of antitumour action?

Component studies done on various mushrooms have shown that mushrooms contain polysaccharides, proteins, fats, ash, glycosides, alkaloids, volatile oils, tocopherols, phenolics, carotenoids, folates, ascorbic acid enzymes and organic acids. To date, the active components discovered in mushroom responsible for conferring anticancer potential are lentinan, Krestin, hispolon, lectin, calcaelin, illudin S, psilocybin, Hericium polysaccharides A and B (HPA and HPB), ganoderic acid, schizophyllan and laccase (Patel and Goyal, 2012). Bioactive proteins with interesting biological activities (antitumour, antiviral, antimicrobial, antioxidative and immunomodulatory) have also been discovered and are being researched, such as lectins, fungal immunomodulatory proteins (FIP),
Fungi as a source of antitumour agents

The antitumour activity of mushrooms has been attributed to the attenuation of various biological activities that lead to tumour formation, which include the following:

- Immunomodulation and immunopotentiating activities (Ikewawa, 2001; Kodama et al., 2005; Han et al., 2009, Masuda et al., 2009; Masuda et al., 2010)
- Induction of apoptosis (Sliva, 2006; Chen et al., 2009; Liu et al., 2009; Tsay et al., 2009; Jang et al., 2010; Luo et al., 2010; Zhao et al., 2011)
- Inhibition of proliferation through cell cycle arrest (Pietenpol and Stewart, 2002; Chang et al., 2004; Wang et al., 2004; Ye et al., 2007)
- Antioxidation (Lee et al., 2007; Jumes et al., 2010)
- Inhibition of the NF-κB transcription factor (Kim et al., 2006; Kim et al., 2007; Liu et al., 2007; Petrova et al., 2009; Hseu et al., 2010; Jeong et al., 2010; Ruimi et al., 2010a, b; Lee and Hong, 2011)
- Inhibition of protein kinase activity (Cassinelli et al., 2000; Yassin et al., 2008; Kaneko et al., 2010; Yang et al., 2011)
- Inhibition of cyclooxygenase activity (Zhang et al., 2002; Kim et al., 2006; Narayanan et al., 2007; Jedinak et al., 2010)
- Inhibition of DNA topoisomerases (Bae et al., 2009)
- Inhibition of DNA polymerase activity (Mizushina et al., 1999)
- Anti-angiogenesis activity (Kim et al., 2004; Stanley et al., 2005; Lee et al., 2010)

Some 100 years ago, Nauts, Swift and Coley (1946) found that certain polysaccharides were able to induce complete remission of malignant tumours. Some years later, Chihara (1969) published the first report of macrofungal polysaccharide (lentinan) having antitumour activity. In the same year, there was also a report by Ikewawa et al. (1969) on the antitumour activities of essences obtained from the fruiting body of mushrooms belonging to the family Polyporaceae (Aphyllophoromycetidae). These substances (polysaccharides) are unlike antitumour drugs and are best known as the most potent mushroom-derived substance. They have been found to work by activating various immune responses (immunomodulating) and cause no harm to the body (Wasser and Weis, 1999; Wasser, 2002). The main source of these antitumour polysaccharides is from the cell walls of the fungi.

Biologically active polysaccharides have different structures and are unique to the species, strain of different species and different parts of the same species (i.e. fruiting bodies and cultured mycelia) (Reshetnikov et al., 2001). For instance, it was found that for different strains of Trametes versicolor, the polysaccharide K (PSK) and polysaccharide peptide (PSP) isolated from different strains of T. versicolor contain different peptide moieties bounded to similar polysaccharide. The potency of these polysaccharides is related to their degree of branching, solubility in water and molecular mass.

Mushroom antitumour polysaccharides are mostly glycols with a variety of glycosidic linkages, namely, (1 → 3), (1 → 6)–β–glucans and (1 → 3)–α–glucans and heteroglycans. Some bind to protein residues (PSP complexes). Glycans are polysaccharides containing glucose and other types of carbohydrate monomer as a backbone. Types of glycans include mannans, galactans, fucans and xylans – named according to the sugar components in the backbone – whereas heteroglycans side chains contain arabinose, mannose, fucose, galactose, xylose, glucuronic acid and glucose as main component or in different combinations (Wasser, 2002).

The well-known antitumour action of (1 → 3)–β–glucans has been attributed to the triple-helical conformation as an important structure for immune-stimulating activity. Maeda et al. (1988) demonstrated that when lentinan was denatured with DMSO, urea and NaOH, therefore causing it to lose its tertiary structure (while maintaining primary structure), its tumour inhibitory activity was lowered with progressive denaturation. Similar results were obtained when the triple-helical structure of schizophyllan was destroyed (Yanaki et al., 1986). The exact mechanism of how the triple-helical conformation affects the antitumour activity remains unclear to date. It has been found that the (1 → 3)–β–glucan backbone is more important than the tertiary structure of the molecule which explains the comparable antitumour action between (1 → 3)–α–mannan and (1 → 3)–β–glucan (they have similar backbone conformation). The β–glucans containing mainly 1 → 6 linkages exhibit less antitumour activity, possibly due to their inherent flexibility of having too many possible conformations (Zhang et al., 2007).

Molecular weight of glucans also plays a role in antitumour activity. Mizuno et al. (1996) reported that high-molecular-weight glucans were more effective. This is especially true for (1 → 3)–β–glucans (molecular weight ranging from 500 to 2000 kDa) with medicinal...
properties. However, Gao and colleagues (1996) reported that there was no obvious influence of molecular weight on mushroom polysaccharides such as \((1 \rightarrow 3)\)-\(\alpha\)-glucuronoxylomannans. This is an indication that the common \((1 \rightarrow 3)\)-\(\alpha\)-mannan backbone between the low and high molecular weight is an important parameter for its activity.

Chemical modifications have been described in various literatures to improve the antitumour activity of mushroom polysaccharides. It is also important to improve the water solubility and the ability to permeate stomach walls after oral digestion (Wasser, 2002). Two main procedures – Smith degradation (oxydo-reducto-hydrolysis) and activation by formolysis – have been used (Mizuno et al., 1996; Mizuno, 1999). These two schemes have successfully modified polysaccharides from *Ganoderma lucidum*, *G. frondosa* and *Leucopaxillus giganteus*. Another method to transform \(\beta\)-glucans into water-soluble form is by carboxymethylation. This has been shown to be effective in transforming insoluble, little or no antitumour effect polysaccharide to products that showed potent antitumour activity (Kiho et al., 1994; Paulik et al., 1996; Yoshida et al., 1996).

### 11.5.1 Clinical studies of antitumour activity of mushrooms

Some of the identified compounds from certain mushrooms have been tested clinically. A meta-analysis using lentinan (from the fruit body of shiitake (*L. edodes*)) was done on 650 patients with non-operable or recurrent stomach cancer (Oba et al., 2009). The group receiving chemotherapy and lentinan showed significantly longer survival time compared to the group receiving just chemotherapy only. A randomized, double-blind, placebo-controlled, multi-centre study was conducted using Ganopoly (the polysaccharide fractions extracted from *G. lucidum*) on 68 patients with advanced lung tumours (Gao et al., 2003). Forty-eight per cent of patients showed a significant increase in quality of life (as shown by Karnofsky score).

A retrospective, long-term study was conducted on post-operative colorectal carcinoma patients using PSK (Krestin), a polysaccharide preparation isolated from *Coriolus versicolor*, along with a chemotherapeutic agent (fluorouracil) (Sakai et al., 2008). Results of the study showed that there was a 31.3% increase in survival rate when treated with both Krestin and fluorouracil. There were also positive results in patient with lymph node infiltration. Clinical studies that have been done to date are limited to polysaccharides.

### 11.5.2 Lignosus rhinocerus: New discovery and new scientific data

*Lignosus rhinocerus*, the tiger milk mushroom (Figure 11.1), belongs to the Polyporaceae family and is one of the most important medicinal mushrooms in Southeast Asia and southern China and is used by natives. In Malaysia, the mushroom is also known locally as *cendawan susu rimau* – literary ‘mushroom of tiger’s milk’. It is widely used by the indigenous communities in peninsular Malaysia to treat a variety of diseases, including breast cancer, fever, cough, asthma, food poisoning and as a general tonic. The sclerotium of *L. rhinocerus* is the part with medicinal value. There have been very few studies of the biological and pharmacological activities of the mushroom due to its limited supply. The mushroom was only available by collection from the jungle. In 2009, Tan (2009)
reported successful cultivation of the mushroom in agar, solid and spawn medium with good yield, thus making it possible to obtain large quantities for investigation and therapeutic purpose.

The cold water extract of the *L. rhinocerus* cultivar contains mainly carbohydrate and a rather small amount of protein (Lee et al., 2012). The extract exhibited significant anti-proliferative activity against the breast cancer cell MCF-7 and lung cancer cell A549 and was found to be essentially not cytotoxic against the corresponding non-tumourigenic breast and lung cells (184B5 and NL 20). However, the basis of the selective anti-proliferative effect that appears to target cancer cells is yet to be elucidated. It was also reported that high-molecular-weight fraction of the cold water extract exhibited strong anti-proliferative activity against the two cancer cells tested. The low-molecular-weight fraction was devoid of anti-proliferative activity. As the high-molecular-weight fraction contains both carbohydrate and protein, the anti-proliferative agent may either be a type of protein–carbohydrate complex or proteins. DNA fragmentation studies also suggested that the cold water extract kills MCF-7 and A549 cells by inducing apoptosis.

### 11.5.3 Potential of fungi as anticancer agent

The potential for fungi to be developed as mainstream anticancer agent is vast. It has a long history of use across multiple cultures, and many new scientific evidences of its use are emerging. Conservation, cloning and various cultivation methods of therapeutic mushrooms are needed for sustainable development. To date, there have been many reports on the isolation, purification and structural studies of mushrooms having antitumour and immunostimulating/modulation effects. More studies are needed to elucidate the mechanism of action and the different roles of the isolated bioactive compounds. Owing to antitumour effects of polysaccharides found in mushrooms and the understanding that carbohydrates' basic building blocks are connected by glycosidic bonds which have great potential for structural variability, it is interesting to look into the structure–function relationship of these bioactive compounds. These studies are of great value as there are still some forms of malignant cancers for which chemotherapeutic agents have yet to be developed.

### References


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