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Potential Role of Medicinal Mushrooms in Breast Cancer Treatment: Current Knowledge and Future Perspectives

Roumyana D. Petrova,[1,2] Solomon P. Wasser,[1] Jamal A. Mahajna,[2] Cvetomir M. Denchev,[3] & Eviatar Nevo[1]

[1]Institute of Evolution, University of Haifa, Mount Carmel, Haifa, Israel; [2]Migal-Galilee Technology Center, South Industrial Zone, Kiryat Shmona, Israel; [3]Institute of Botany, Bulgarian Academy of Sciences, Sofia, Bulgaria

*Address all correspondence to Solomon P. Wasser, Institute of Evolution, University of Haifa, Mount Carmel, Haifa 31905, Israel; spwasser@research.haifa.ac.il

ABSTRACT: Breast cancer has become the most common invasive form of female cancer in the last few decades. Statistics show that the rate of newly diagnosed cases of breast cancer is rising every year depending on age, race, heredity, and ethnicity. The National Cancer Institute of US and mainly the Division of Cancer Control and Population Sciences (DCCPS) promote and conduct research that also identifies the economic, social, cultural, psychological, behavioral, and biological mechanisms that are potential reasons for breast cancer development. Advanced breast cancers do not respond well to therapy, and their gene expression arouses uncontrolled growth. Although estrogen-receptor (ER)-positive breast cancers respond to hormonal therapy, the treatment of ER-negative cancers is more complicated because of their ability for developing resistance to drugs. Lack of molecular targets in estrogen receptor-negative breast cancer is a major therapeutic hurdle. It has been known that NF-κB is significantly important in the processes of inflammation, cell survival, transformation, and oncogenesis, as well as in the etiology of breast cancer. A theory exists, according to which ER-negative breast cancer cells depend on NF-κB for aberrant cell proliferation and simultaneously avoid apoptosis, suggesting that NF-κB can be used as a potential molecular target in breast cancer treatment. Studies on new anticancer treatments and other medicinal substances from mushrooms have been significantly expanded in the last few years. This is mainly because they contain bioactive polymers such as polysaccharides and polysaccharide/protein complexes, secondary metabolites, and enzymes isolated from fruit bodies, mycelia, and culture broth. There are data showing the potential activity of medicinal mushrooms in breast cancer treatment. *Ganoderma lucidum* has shown the most significant inhibitory effect on NF-κB activity in highly invasive breast cancer cells. Other medicinal mushrooms that have also been reported to produce biologically active substances, have been tested in *in vivo* or *in vitro*, and have demonstrated breast cancer inhibitory activity are *Agaricus bisporus, A. brasiliensis, Trametes versicolor, Grifola frondosa, Inonotus obliquus, Lentinus edodes, Leucoagaricus americanus, Pleurotus ostreatus, Sparassis crispa,* etc.

KEY WORDS: medicinal mushrooms, breast cancer, nuclear factor kappa B (NF-κB), polysaccharides, polysaccharide/protein complexes, secondary metabolites

ABBREVIATIONS

AHCC: active hexose correlated compound; **Akt:** serine/therionine kinase; **AP-1:** activator protein-1; **B40:** HLA human leukocyte antigen type; **CAPE:** caffeic acid phenethyl ester; **CD40L:** CD-40 ligand; **DCCPS:** Division of Cancer Control and Population Sciences; **dnIKKβ-mut:** dominant-negative Ikkβ mutant; **EGF:** epidermal growth factor; **IAP:**

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immunosuppressive acidic protein; **IGF-1**: insulin-like growth factor-1; **ER**: estrogen receptor; **Fas-L**: Fas ligand; **HDP**: host defense potentiators; **IkB**: inhibitory proteins kappa B; **IKK**: IkB kinase comlex; **IL-1**: interleukin-1; **LM fractions**: low-molecular-weight fractions; **LPS**: lipopolysaccharide; **MEKK3**: mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 3; **NF-kB**: nuclear factor kappa B; **NK cells**: natural killer cells; **PR**: progesterone receptor; **RIP2**: receptor interacting protein 2; **SDF-1α**: stromal derived factor-1α; **TM**: tetrathiomolybdate; **TNF-α**: tumor-necrosis factor-α; **uPA**: urokinase-type plasminogen activator

INTRODUCTION

In order to treat various types of cancer, surgical operation, radiotherapy, and chemotherapy (administration of anticancer agents) are generally used. However, their side effects cause serious damage and suffering to patients. As an alternative to these treatment methods, immunotherapy is now gaining more attention than ever. Immunotherapy substantially reduces the sufferings of side effects and the inherent pain of cancer and helps to overcome the cancer growth, even in its last stage, by promoting the power of immunity with which the human body is originally equipped.

Despite the observed success of most chemotherapeutic regimes, cellular adaptations have enabled tumor cells to evade many of the chemotherapeutic drugs. One of these cellular chemoresistance factors is the transcription factor NF-κB. It is a dimeric transcription factor that belongs to the Rel/NF-κB family of transcription factors (Nakshatri et al., 1997; Pahl, 1999; Zandi and Karin, 1999; Karin and Ben-Neriah, 2000; Karin et al., 2002). NF-κB complex is maintained in the cytoplasm in an inactive form by the IkB protein. The major activator of NF-κB is known to be the IkB kinase complex (IKK) (Zandi and Karin, 1999), suggesting that the IKK complex can serve as a potential target for inhibition of NF-κB activity.

Several enzymes have already been reported as indirect activators of NF-κB. For example, MEKK3 is overexpressed in breast cancer cells and leads to activation of NF-κB, resulting in resistance to chemotherapeutic agents (Samanta et al., 2004). Another enzyme, Akt, is also known to induce the transcription function of NF-κB by stimulating its RelA/p65 subunit (Madrid et al., 2001). Because of the specificity and molecular mechanisms of these processes that must be overcome and regulated, it becomes a matter of great importance to find and apply new and more precise drugs for treatment.

Nowadays, considerable attention has been focused on the effectiveness of herbal medicines because they have enormous popularity as self-medication products. Many herbs and natural products, including mushrooms, are available on the world market and appear to have potential in the treatment of progressive cancers. Studies on the medicative activity of mushrooms have already been carried out, not only because of their traditional use in folk medicine for centuries, but also because they have shown significant activity as immunomodulators and dietary supplements (Wasser, 2002). The compounds they possess have been classified as host defense potentiators (HDPs), which can have immune system enhancement properties. That is one of the reasons they are currently used as adjuncts to cancer treatment in Far East countries.

The benefits of mushroom compounds on different clinical conditions have attracted the interest of the scientific community in the last decade in an attempt to understand the molecular mechanisms responsible for their action (Hobbs, 1995). Several classes of mushroom compounds such as proteins, polysaccharides, lipopolysaccharides, and glucoproteins have been classified as molecules that have potent effects on the immune system. They may restore and augment immunological responses of host immune effector cells, but they have no direct cytotoxic effect on tumors (Rowan et al., 2003).

Immunoceuticals isolated from more than 30 mushroom species have demonstrated antitumor activity in animal treatments. However, only a few have been tested for anticancer potential in humans (Wasser and Weis, 1999c). The few that have been tested are β-D-glucans or β-D-glucans linked to proteins. Moreover, the latter have shown greater immunopotentiation activity than the free glucans (Sakagami and Aoki, 1991; Kidd, 2000).

There are numerous clinical studies proving the cancer inhibitory effects of *Lentinus edodes* (Berk.) Singer (Furue et al., 1981; Taguchi et al., 1985; Matsuoka et al., 1997), *Grifola frondosa* (Dicks. : Fr.) Gray (Nanba, 1997a,b), *Schizophyllum commune* Fr. : Fr. (Fugimoto and Furue, 1984; Furne, 1985; Kimura et al., 1994), *Ganoderma lucidum* (W.Curtis : Fr.) Lloyd (Jong and Birmingham, 1992; Gao et al., 2002), *Trametes versicolor* (L. : Fr.) Lloyd (Liu and Zhou, 1993; Nakazato et al., 1994; Kidd, 2000), *Inonotus obliquus* (Pers. : Fr.) Pilát (Mizuno et al., 1999), and *Phellinus linteus* (Berk. et M.A. Curtis) Teng (Ikekawa et al., 1968; Kim et al., 1996; Han et al., 1999; Mizuno, 2000), etc.

Other mushroom compounds of therapeutic interest are the secondary metabolites as lectins, lactones, terpenoids, alkaloids, antibiotics, and metal chelating agents, which are also important for the immune function of the organism (Wasser and Weis, 1999c). Mushrooms also contain a number of enzymes such as laccase, superoxide dismutase, glucose oxidase, and peroxidase. It has been shown that enzyme therapy plays an important role in cancer treatment, preventing oxidative stress and inhibiting cell growth (Ossowski and Lopez, 1996).

The rapid expansion of our knowledge concerning the processes of cell differentiation, cell cycle, apoptosis, angiogenesis, tumorogenesis, metastasis, and signal transduction control have unveiled an abundance of specific molecular targets for cancer therapy, including a variety of small-molecule compounds that inhibit or stimulate these molecular targets (Zaidman et al., 2005). Some mushroom low-molecular-weight substances have already been recorded to possess anticancer activity. For instance, the caffeic acid phenethyl ester (CAPE), which specifically inhibits DNA binding of NF-κB and has shown some promising results in human breast cancer MCF-7 cells, was found to be produced by *Agaricus bisporus* (J.E. Lange) Imbach, *Lentinus edodes*, and *Phellinus linteus* (Mattila et al., 2001; Nakamura et al., 2003). These reports proved that such substances could be used as molecular targets in malignant cells in order to treat breast cancer.

In the present article, available data on medicinal mushroom polysaccharides, polysaccharide/peptide complexes, secondary metabolites, and their antitumor activity and mechanism of action in breast cancer cells are compiled. Results of experimental tests and clinical treatments are also summarized. Moreover, some of the main therapeutic targets used in clinical trials today, as well as some novel therapeutic agents successfully applied in breast cancer treatment, are described.

BREAST CANCER

Progesterone Receptor (PR)

Progesterone is a steroid hormone that regulates a great number of processes in different tissues. Its biological action is mediated by the progesterone receptor, which is presented by two isoforms, PR-A and PR-B. They are distinguished by the additional amino acid stretch in the N terminus of PR-B. Both of them contain DNA and ligand binding domains with activation functions. PR-A is necessary for the progesterone-dependent reproductive responses required for female fertility, whereas PR-B is required for normal proliferative responses in the mammary gland. PRs regulate a great number of genes, including those involved in breast cancer development. It has been demonstrated that PR-A and PR-B express different subsets of genes involved in particular functional pathways (Richer et al., 2002; Li and O'Malley, 2003). In breast cancer cells, although some genes are regulated through both PR isoforms, most genes are uniquely regulated through PR-B.

PR can be activated not only by progesterone, but also by some kinases and growth factors (Zhang et al., 1994). Progesterone signaling is often intertwined with other hormones. For example, estrogen can induce expression of PR; thus, many of the effects attributed to progesterone are dependent on estrogens. Hormone-induced prevention of breast cancer involves combination of estrogen and progesterone (Medina et al., 2001).

Estrogen Receptor (ER)

Estrogens play a critical role in the growth, development, and maintenance of a diverse range of tissues,

including mammary glands. These hormones exert their physiological effects via the estrogen receptor, which functions as a ligand-activated transcriptional regulator. ER is a member of a large family of nuclear receptor transcription factors and consists of two isoforms, ERα and ERβ, which have equal functions in some tissues, but different in others. ER has a characteristic modular structural organization with distinct domains associated with transactivation, DNA binding, and hormone binding (White and Parker, 1998). Hormone binding to the ER ligand binding domain induces a conformational change in the receptor, which initiates a series of events that culminate in the activation or repression of responsive genes (Tsai and O'Malley, 1994). Nuclear receptors, such as ER, are also capable of regulating the transcription of genes that lack hormone responsive elements by modulating the activity of other transcription factors, such as NF-κB and AP-1, which serve as critical targets for many signaling pathways regulating cell differentiation, proliferation, and transformation. That is why ER is an important pharmaceutical target for hormone replacement in menopausal women and for chemotherapeutic drugs against certain reproductive cancers.

It is known that some growth factors, neurotransmitters, and other hormones can modulate the activity of steroid hormone receptors (Power et al., 1991). Thus, any alternation in the receptor activity and gene expression, which are involved in cell proliferation, is determined not only by hormone signals, but also by changes in other signaling pathways that take place in breast cancer progression.

Tamoxifen is a common anti-estrogenic drug that inhibits the binding of estrogen to ER. Unfortunately, breast cancer adjuvant therapy with tamoxifen is not always successful. Most patients with advanced disease develop resistance to all forms of endocrine therapy (Lykkesfeldt, 1996). Numerous studies focused on the control of the antagonistic and agonistic effects of anti-estrogens on tumor growth (Osborne et al., 2003). It has been known that tamoxifen has both antagonistic and agonistic properties—i.e., it inhibits growth in breast tissue but stimulates the proliferation of the endometrium. Endocrine therapies that lack estrogen agonistic properties, such as aromatic inhibitors, have been shown to produce higher response rates and to postpone disease progression to a greater extent compared to tamoxifen (Wolff, 2002).

Numerous natural endocrine disrupters, such as phyto-estrogens (a group of estrogenic compounds produced by plants), are also capable of binding to ER, which may affect human health. For example, Genistein, an isoflavonoid phyto-estrogen found in high quantities in soya beans and soy products, is known to bind to ER, even to its two isoforms, ERα and ERβ (Kuiper et al., 1997).

NUCLEAR FACTOR KAPPA B (NF-κB)

In inflammatory diseases and many cancers the level of active NF-κB is rather elevated. NF-κB is of significant importance in the processes of inflammation, cell survival, transformation, and oncogenesis. Moreover, there are strong suggestions that NF-κB plays an important role in the etiology of breast cancer. Elevated NF-κB DNA-binding activity is detected in both mammary carcinoma cell lines and primary human breast cancer tissues (Cao and Karin, 2003). NF-κB is also responsible for tumor metastasis in other organs. Metastasis is a nonrandom process, and each cancer type has its own preferred sites of metastasis. For example, breast cancer cells preferentially metastasize to the lymph nodes, lungs, liver, and bone (Liotta, 2001). Helbig et al. (2003) demonstrated that NF-κB directly regulates the expression of the chemokine receptor CXCR4, which appears to be critical for the motility of cancer cells in response to the SDF-1α *in vitro*.

NF-κB activators

The IKK complex has been identified as a major activator of NF-κB (Zandi and Karin, 1999). In response to a variety of stimuli, such as TNF-α, CD40L, IL-1, and LPS, the IKK complex is activated and phosphorylates the IkB inhibitory proteins, resulting in their degradation and nuclear translocation of NF-κB, where transcriptional activation of target genes begins (Karin and Ben-Neriah, 2000). Thus, IKK can serve as a potential

target for inhibition of NF-κB activity. There are a variety of target genes regulated by NF-κB such as immunoregulatory and inflammatory genes, anti-apoptotic genes, genes that positively regulate cell proliferation, and genes that encode negative regulations of NF-κB (Pahl, 1999; Karin et al., 2002). When NF-κB dimers are uncoupled from their normal mode of regulation, they can cause tumorogenesis through several mechanisms, such as promotion of cell proliferation, inhibition of apoptosis, and increasing tumor metastasis and angiogenesis (Karin et al., 2002).

Progression of the mammary carcinoma cell line RM22-F5 from an estrogen receptor ER-positive to an ER-negative was found to be accompanied by constitutive activation of NF-κB (Nakshatri et al., 1997). Increased IKK activity was shown by Romieu-Mourez et al. (2001) in transformed breast cancer cell lines, and the inhibition of IKK activity decreased NF-κB activity in tumor cell lines. Thus, the IKK complex is a potential target for controlling NF-κB activation and its functions. Samanta et al. (2004) established that another enzyme, MEKK3, also has an elevated expression in breast cancer cells. Their study indicated direct participation of MEKK3 in the activation of NF-κB, resulting in increased expression of cell survival factors and resistance to chemotherapeutic agents, thus suggesting functional cooperation between MEKK3 and other signaling molecules activated by the cytokines. Indeed, such a molecule, downstream of the IL-1 and TNF signaling pathway, is Akt, which is also reported to participate in the activation of NF-κB. Akt uses IKK to stimulate the transactivation potential of the RelA/p65 subunit of NF-κB. Akt can also use the protein kinase p38, but the latter requires coactivators to stimulate NF-κB (Madrid et al., 2001; Dugourd et al., 2003). Thus, MEKK3 and Akt may also serve as therapeutic targets to control cancer cell resistance to cytokine- or drug-induced apoptosis.

Recently, it has been reported that caspase-1, independently of its enzyme activity, can activate NF-κB, suggesting that the enzyme is involved in additional pro-inflammatory pathways. Thus, NF-κB activation is a novel function of caspase-1, in contrast to the induction of apoptosis and the maturation of pro-IL-1β. The IKK complex is the central downstream mediator of caspase-1-induced NF-κB activation, which is also RIP-2 dependent. However, the proteolytic activity of the enzyme is not important for that process (Lamkanfi et al., 2004).

Biswas et al. (2000) recorded that the epidermal growth factor (EGF) family of receptors is overproduced in ER-negative breast cancer cells and on low-level production of ER-positive cells, thus suggesting that EGF is a major growth-stimulating factor for ER-negative cells. The basal level of active NF-κB in ER-negative breast cancer cells is elevated by EGFR and inhibited by anti-EGFR antibody, thus qualifying EGFR as a NF-κB activation factor.

NF-κB inhibitors

The low-molecular-weight compound Go6976 is known to be an NF-κB inhibitor, blocking its EGF-induced activation and also causing apoptotic death, predominantly in ER-negative cells. Thus, Go6976 and similar NF-κB inhibitors were shown as potentially novel low-molecular-weight therapeutic agents for treatment of ER-negative breast cancer patients. Go6976 was shown not only to inhibit tumor growth, but also to cause the full-grown tumors in mice CSMLO cells to regress, thus confirming the previously reported inhibitory effect of Go6976 on NF-κB (Biswas et al., 2001). In a later study, Biswas et al. (2003) tested two inhibitors of NF-κB activation: Go6976 and dnIKKβ-mut. These agents modified the expression of genes related to apoptosis and specifically produced a high rate of apoptosis of the cancer cells. Results showed that treatment of animals with Go6976 caused essentially complete tumor regression. Also, dnIKKβ-mut-expressing cells did not form tumors.

In an *in vitro* study another chemical compound, TM, was demonstrated to have an anticancer effect suppressing NF-κB, leading to global inhibition of NF-κB-mediated transcription of pro-angiogenic and prometastatic genes. Moreover, the results showed that TM specifically targets NF-κB activity of cancer cells within the tumor mass (Pan et al., 2003).

Another potent inhibitor of NF-κB activity is CAPE, which specifically inhibits DNA binding of NF-κB but not other transcription factors (Natarajan et al., 1996). Watabe et al. (2004) reported results of testing CAPE in human breast cancer MCF-7 cells. This study demonstrated that CAPE not only inhibited NF-κB activity, but also activated the Fas death receptor in a Fas-L-independent manner. Moreover, CAPE induced Bax expression, caspase activation, DNA fragmentation, and apoptosis not only in breast cancer, but also in other various cancer cell lines. Results showed that cancer cells with high basal NF-κB activity are more sensitive to NF-κB inhibition by CAPE than are normal cells. This raises the possibility that cancer-cell-specific drugs could be developed if NF-κB-specific inhibitors were available for humans.

MUSHROOMS AND BREAST CANCER

Because a great number of polysaccharides and polysaccharide proteins isolated from medicinal mushrooms have shown significant anticancer activity, many studies have been conducted to prove their potential effect in the treatment of different types of cancers. Mushroom immunoceuticals act mainly by elevating the host immune system. This process includes activation of dendritic cells, NK cells, T cells, macrophages, and production of cytokines (Halpern and Miller, 2002). Several fungal products, mainly polysaccharides and especially β-glucans, were developed with clinical and commercial purposes: lentinan, isolated from *Lentinus edodes* (Chihara et al., 1970); schizopyllan, from *Schizophyllum commune* (Komatsu et al., 1969); D-fraction, from *Grifola frondosa* (Hishida et al., 1988); krestin (PSK), from *Trametes versicolor* (Sakagami and Takeda, 1993); PSP also from *T. versicolor* (Yang, 1999); AHCC; and many others.

These natural products became popular in medicinal practice mainly because of the biologically active compounds they possess, which are supposed to be related to the nature of tumor-specificity. Some of them have already been reported to possess anticancer activity in breast cancer models. While there are examples in which mushroom polysaccharides have demonstrated efficacy against specific types of cancer, as in monotherapy, the overwhelming successes have occurred when they were tested to function together with proven and accepted chemotherapeutic agents. However, some low-molecular-weight mushroom substances with fungal origin have also been reported to be active in cancer treatment.

All compounds that are not involved in the central metabolic processes of the organism, such as the generation of energy, the formation of the building blocks of proteins, nucleic acids, and cell membranes, are known as *secondary metabolites.* Secondary metabolites are found among fungi and plants and include compounds such as antibiotics (e.g., penicillin, streptomycin), dyes (e.g., indigo), flavoring and odor compounds (e.g., menthol and limonene), and even substances such as taxol (for treatment of ovarian cancer) and cyclosporin A (an immunosuppressant used to prevent transplant rejection). Some of the poisonous substances made by fungi, such as alpha-amanitin and phalloidin from the death cap mushrooms, muscarine from the fly agaric, orellanine from the false chanterelle, and lysergic acid from ergot of rye, have been purified, and scientific and medical uses have been found as a result. These substances also relate to secondary metabolites.

Several fungi were found to produce a range of antibiotics, produced under specific conditions. For instance, fungi such as *Trichoderma, Penicillium,* and *Aspergillus* produce a diverse range of antibiotics. Secondary metabolites such as penicillic acid, which is an antibacterial, aflatoxins that are mammalian toxic, and trichodermin that may have broad antifungal activity were isolated from these fungi. Some of these fungal compounds disrupt DNA and protein synthesis, others disrupt ribosomal activity or the cytoskeleton, making them extremely broad and general toxins. The ecological significance of most secondary metabolites is still uncertain, but there is no doubt that they have a function in the biochemical pathways of their producers as well as for the different properties of the latter. The recent progress made in fermentation, isolation, and structure elucidation technologies has made investigating into the secondary metabolism of Basidiomycetes seem worthwhile.

Mushrooms: Bioactive Compounds and Products Used in Breast Cancer Treatment

Lentinan is a high-molecular-weight polysaccharide with β-(1→3)-D-glucose triple helix structure and β-(1,6)-D-glucopyranoside side chains (Aoki, 1984; Hobbs, 1995). It was isolated from *Lentinus edodes*, a well-known medicinal mushroom that has antitumor, immunomodulating, antiviral, antibacterial, antiparasitic, cardiovascular, and hepatoprotective effects (Wasser and Weis, 1997). Results of some clinical applications of lentinan have proven prolongation of life span of the patients with advanced and recurrent stomach, colorectal, and breast cancer with few toxic side effects (Taguchi, 1983; Chihara, 1992).

Several major substances with potent immunomodulating action such as polysaccharides, proteins, and triterpenoids have been isolated from *Ganoderma lucidum*. The pharmacological activities of this medicinal mushroom are attributed primarily to triterpenoids and polysaccharides (Wasser and Weis, 1999b). It has been known that the highly metastatic cancer cells are characterized by constitutive activation of transcription factors AP-1 and NF-κB. By using commercially available dietary supplements in the form of spores (GS) and fruiting bodies (GFB), Sliva et al. (2002, 2003) showed that *G. lucidum* inhibits constitutively active AP-1 and NF-κB in highly invasive breast and prostate cancer cells. Furthermore, both GS and GFB downregulated the expression of uPA and its receptor uPAR as well as secretion of uPA, resulting in the inhibition of cell motility. An alcohol extract of *G. lucidum* inhibited proliferation of breast cancer cells by upregulating the cell-cycle inhibitor p21/Waf-1 and by downregulating cyclin D1. The alcohol extract also induced apoptosis of breast cancer cells, which was mediated through the upregulation of expression of proapoptotic Bax protein (Hu et al., 2002).

The maitake D-fraction was tested and showed significant symptomatic improvements in a number of clinical trials in breast, prostate, lung, liver, and gastric cancers in the US and Japan, most of which were at an early clinical stage (phase I/II) (Smith et al., 2002). Nanba (1995) published some results from a study on 165 patients with various types of cancer, many of them in advanced stages and some refusing chemotherapy. D-fraction plus tablets of dried crude extract of *Grifola frondosa* were used. Dosages varied depending on the patient, D-fraction doses ranging from 35 to 100 mg per day and crude mushroom extract ranging from 4 to 6 grams. Symptomatic improvements or regression were claimed for approximately 73% of the patients with breast cancer.

PSK is a unique protein-bound polysaccharide, which has great potential as an adjuvant cancer therapy agent, with positive results seen in the adjuvant treatment of gastric, esophageal, colorectal, breast, and lung cancers (Fisher and Yang, 2002). Sugimachi et al. (1984) published results from a long-term immunotherapy with PSK in conjunction with chemotherapy to breast cancer patients and showed that the survival rate after recurrence was significantly extended by the immunochemotherapy.

Toi and Hottori (1992), in a much larger trial (914 patients) using in-depth analysis, implied that PSK significantly extended survival in ER-negative, Stage IIA patients without lymph node involvement. In 1995, Iino et al. published results from a trial on breast cancer patients with vascular invasion. Based on the knowledge that B40 antigen status had been linked to the possibility of survival with breast cancer, the researchers compared their B40-positive patients treated with PSK to the B40-negative ones. The study yielded the finding that the B40-positive patients, who were treated with PSK in addition to chemotherapy, had 100% survival after 10 years; however, the B40-negative patients had approximately 50% survival (Yokoe et al., 1997). PSP also showed high antitumor activity, but not in breast cancer patients. The Phase I trial provided PSP at doses up to 6 grams per day for 1 month to 16 healthy persons and five breast cancer patients. Appetite increased in a majority of the subjects, but no evidence was found for serious adverse effects (Xu, 1993).

The AHCC, whose active component is an oligozaharide, is obtained from several medicinal mushrooms cultured in liquid medium. Matsushita et al. (1998) carried out a study on combination therapy of AHCC plus UFT (tegafur and uracil in ratio 4:1) for *in vivo* treatment of rat mammary adenocarcinoma. Their results showed that the

combination of AHCC and UFT brought good effects, not only on primary tumor growth, but also on reducing metastasis. *In vitro* experiments showed that AHCC activated the NK cells in addition to the activation of macrophages. These effects resulted in restoring or activating the host immune system, thus suggesting that AHCC may be a good candidate for a biological response modifier.

Talorete et al. (2002) exposed the human breast cancer line MCF7 to an aqueous, hot water extract from *Agaricus brasiliensis* S. Wasser et al., to a natural estrogen (E2, 17-β-estradiol), to a proven xenoestrogen (NP, *p*-nonylphenol), and to combinations of these compounds with or without the mushroom extract to determine their proliferative effect on MCF-7 cells. Results showed no significant difference in cell proliferation between cells incubated with *Agaricus* extract and the control. Moreover, no significant difference in proliferative activity of cells incubated with NP plus E2 was noted. Weak estrogens, such as NP, can compete with 17-β-estradiol for estrogen receptors but do not elicit the same pleiotropic response. Results also showed no detectable c-*jun* mRNA signal from any of the treatments and the control. The c-*jun* mRNA was strongly expressed only in the presence of EGF and IGF-1 (Philips et al., 1993). An aqueous extract of this fungus induced c-jun protein expression and enhanced the proliferation in MCF7 cells in the presence of nonylphenol, which may be due to the involvement of the AP-1 gene regulatory complex (Talorete et al., 2002). Nevertheless, Mizuno (2002) described the case of a breast cancer patient whose tumor, 7 years after the operation, spread to the lungs. After 3 months of administration of *Agaricus brasiliensis* extract, the patient was completely recovered from the cancer.

In a clinical trial, powder of *Sparassis crispa* (Wulfen : Fr.) Fr. (300 mg/day) was given orally to several cancer patients (lung, stomach, colon, breast, ovarian, uterine, prostate, pancreas, and liver) after one course of lymphocyte transfer immunotherapy, and most of them showed significant improvement. Moreover, the results showed a complete response in breast cancer patients with advanced cancer. These facts strongly suggest that *S. crispa* is a good source for cancer immunotherapy (Ohno et al., 2003) (Table 1).

Aromatase, a cytochrome P450 enzyme complex, converts androgens to estrogens. Aromatase expression occurs in breast tumors and plays a more dominant role in tumor proliferation than circulating estradiol (Tekmal et al., 1996; Brodie et al., 1997; Yue et al., 1998). Some tests of the aromatase activity inhibition were made by Grube et al. (2001) using extracts from different mushrooms. Significant anti-aromatase effects were demonstrated by *Auricularia* spp., *Agaricus bisporus* (crimini, white button mushroom, baby button mushroom, and stuffing mushroom), *A. brasiliensis*, *Pleurotus ostreatus*, *Pleurotus* spp., *Lentinus edodes*, and *Cantharellus* spp. However, the stuffing mushroom showed the most potent but dose-dependent inhibitory effect, which was determined in MCF7aro cells cultured in the presence or absence of testosterone. With the increase of mushroom extract concentration, there was a proportional decrease in the testosterone-dependent aromatase activity. In the presence of the highest concentration of mushroom extract (10 μL), the aromatase activity was inhibited to the degree of the initial levels of cultivation (presence or absence of testosterone). This suggested that testosterone may compete with some putative components of mushroom extracts, producing a protective effect. An aromatase inhibiting compound was also isolated from *Leucoagaricus americanus* (Peck) Vellinga (Kim et al., 2000), suggesting that the species might possibly be used in the treatment of breast cancer (Table 1).

Mushrooms: Secondary Metabolites

Lorenzen and Anke (1998) precisely described the origin, structure, and activity of low-molecular-weight mushroom metabolites. As an outcome of their results, many mushroom species could be used as anticancer therapeutics. Numerous triterpenoids such as ganoderic acid, lucidenic acid, ganodermic acids, ganoderenic acids, lucidone, ganoderal, and ganoderols have been isolated from the mycelia and fruiting body of *Ganoderma lucidum* and have demonstrated antitumor and immunomodulating activity (Wasser and Weis, 1999a).

A screening of 500 strains of Basidiomycetes, Ascomycetes, and anamorphic fungi carried out

by Erkel et al. (1996), resulting in the isolation of panepoxidone from cultures of the basidiomycete *Lentinus crinitus* (L. : Fr.) Fr. Panepoxydone was shown to interfere with the NF-κB mediated signal transduction in COS-7 and HeLa S3 cells by inhibiting the phosphorylation of IkB and, therefore, sequestering the NF-κB complex in an inactive form in the cytoplasm. The same cell lines with a high affinity binding site for the transcription factor AP-1 revealed no inhibition of AP-1 DNA binding by panepoxidone, indicating preferential target within the NF-κB activating pathway. Panepoxidone, izopanepoxidone, and several related derivates were reported also as secondary metabolites from *Panus conchatus* (Bull. : Fr.) Fr. and *Penicillium utricae* (Sekiguchi and Gaucher, 1979; Shotwell et al., 2000).

Kahlos et al. (1987) published data on some triterpenoids isolated from *Inonotus obliquus*, especially inotodiol, which had a significant anticancer effect against Walker 256 carcinosarcoma and MCF-7 human mammary adenocarcinoma. Recently, a new triterpene from this mushroom was isolated, and its anticancer properties are worth future investigations (Shin et al., 2000).

Some fermentation products from *Pleurotus ostreatus* (Jacq. : Fr.) P. Kumm. were tested in bioassays and showed their *in vitro* activity against three rodent tumor systems: sarcoma, mammary adenocarcinoma 755, and leukemia L1210 (Jong and Donovick, 1989). Yassin et al. (2003) also recorded inhibition of proliferation and differentiation of K562 human leukemia cells due to some low-molecular-weight substances from mushroom crude extracts.

Fujimiya et al. (1999) described the tumoricidal activity of LM fractions derived from *Agaricus brasiliensis* and studied their action in *in vitro* and *in vivo* systems showing a marked increase in IAP levels in the serum of mice receiving these fractions and demonstrating the possible activation of granulocytes. Results indicated that LM fractions have not only a direct tumoricidal action but also immunopotentiating activity. An acid-treated (ammonium-oxalate soluble) fraction from *A. brasiliensis* has a unique mode of action in that it has both a direct cytotoxic action on tumor cells and indirect immunopotentiating action on tumor-bearing mice (Ebina and Fujimiya, 1998). *A. brasiliensis* has progressively been propagated in the outdoors in many tropical areas, but its harvesting can be affected by climatic conditions, and thus structural analysis of low-molecular-weight molecules with biological activity is very important in the designing of possible synthetic analogues of natural products (Fujimiya et al., 1999).

Two active substances, an alphatic compound and phenol, from the mycelium of *Cordyceps ophioglossoides* (Ehrh. : Fr.) Link, were isolated. Both of them showed estrogenic activities and are supposed to be important for avoiding some side effects of carcinogenesis (Kawagishi et al., 2004).

The caffeic acid phenethyl ester (CAPE), which specifically inhibits DNA binding of NF-κB and showed some promising results in human breast cancer MCF-7 cells, was purified from culture mycelia of *Phellinus linteus*, showing a great potential anticancer activity of this medicinal mushroom (Nakamura et al., 2003). Caffeic acid has also been found in the fruit bodies of *Agaricus bisporus* and *Lentinus edodes* (Mattila et al., 2001) (Table 1).

CONCLUSIONS AND FUTURE PERSPECTIVES

All investigations on the breast cancer cell lines carried out up to date showed that NF-κB plays a major role in the processes of tumorogenesis. It is clear that NF-κB activation is associated with promotion of cell growth in mammary tumors and inhibition of NF-κB and provides an effective way for treating breast cancer. Distinct modes of NF-κB activation by IKK complex, IKKα, and IKKβ also suggest that selective inhibitors for IKK would have more specific inhibitory effects on NF-κB activity (Cao and Karin, 2003). Activated NF-κB is such a target, the removal of which (by an inhibitor) can reverse the specific anti-apoptosis of cancer cells. The low-molecular-weight compound Go6976, which selectively kills cancer cells in culture and mice, is the first potentially applicable chemotherapeutic agent that restores apoptosis by blocking the activation of NF-κB. Pro-apoptotic therapy is novel, differing from classic therapeutics directed against cell prolif-

TABLE 1. Medicinal Mushrooms Demonstrating Antibreast Cancer or NFκB Inhibition Activity in Animals and Humans

Species and Edibility	Biological Effect	References
Agaricus bisporus—edible	Suppression of aromatase activity	Grube et al., 2001
	CAPE, an inhibitor of NF-κB binding to DNA, was isolated from fruit bodies	Mattila et al., 2001
Agaricus brasiliensis—edible	Suppression of aromatase activity	Grube et al., 2001
	Completely recovering from cancer	Mizuno, 2002
*Cordyceps ophioglossoides**—inedible	Two active compounds showing estrogenic activity	Kawagishi et al., 2004
Trametes versicolor—inedible	PSK showed positive results seen in adjuvant treatment of breast cancer	Fisher and Yang, 2002
	Immunotherapy with PSK in conjunction with chemotherapy significantly extended survival rate	Sugimachi et al., 1984
	PSK significantly extended survival in ER-negative, Stage IIA patients	Toi and Hottori, 1992
	PSK in addition to chemotherapy leads to 100 percent survival	Iino et al., 1995; Yokoe et al., 1997
Ganoderma lucidum—inedible	Inhibition of AP-1 and NF-κB	Sliva et al., 2002, 2003
	G1 cell cycle arrest	Hu et al., 2002
Grifola frondosa—edible	D-fraction leads to symptomatic improvements or regression of the breast cancer	Nanba, 1995
	D-fraction showed significant symptomatic improvements in the patients at an early clinical stage of cancer (phase I/II)	Smith et al., 2002
Inonotus obliquus —inedible	Inotodiol has a significant anticancer effect on MCF-7 human mammary adenocarcinoma	Kahlos et al., 1987
Lentinus edodes—edible	Lentinan prolongs the life span of the patients with advanced and recurrent breast cancer	Taguchi, 1983; Chihara, 1992
	Suppression of aromatase activity	Grube et al., 2001
	CAPE, an inhibitor of NF-κB binding to DNA, was isolated from fruit bodies	Mattila et al., 2001
Lentinus crinitus —edible	Panepoxydone interferes with the NF-κB mediated signal by inhibiting phosphorylation of IkB	Erkel et al., 1996
Leucoagaricus americanus—edible	Suppression of aromatase activity	Kim et al., 2000; Grube et al., 2001
Panus conchatus—edible	Panepoxidone and izopanepoxidone showed potent NF-κB activity	Shotwell et al., 2000
Phellinus linteus—edible	CAPE specifically inhibits DNA binding of NF-κB	Nakamura et al., 2003
Pleurotus ostreatus—edible	*in vitro* activity against rodent mammary adenocarcinoma 755	Jong and Donovick, 1989
	Suppression of aromatase activity	Grube et al., 2001
Sparassis crispa—edible	Complete response to breast cancer patients with advanced cancer	Ohno et al., 2003

* *Cordyceps ophioglossoides* belongs to Clavicipitaceae (Ascomycetes).

eration (Biswas et al., 2003). On the other hand, the knowledge of the specific components of cytokine networks and signaling pathways and their role in the regulation of immune responses is important in designing strategies to augment these responses (Zaidman et al., 2005).

Searching for new and effective but natural compounds with NF-κB inhibitory effect is worthwhile. For many years, phytoestrogens have been the object of scientific investigations because of their beneficial effect on human health. Phytoestrogens, such as genistein, were tested *in vitro* in human breast cancer models and showed a high inhibitory activity on cell proliferation, although this was dependent on the phytoestrogen concentration (This et al., 2001). Similar natural compounds have been found in medicinal mushrooms. Unfortunately, only a small number of medicinal mushrooms have been investigated and reported to possess anti-breast-cancer activity, and further investigations should be focused on their potential antitumor effect on breast cancer development. Only 13 species of Basidiomycetes and one of Ascomycetes have been reported to possess active compounds with anticancer potential in *in vivo* and *in vitro* breast cancer models.

More attention must be paid to the isolation and testing of mushroom secondary metabolites as potential inhibitors of NF-κB, IKK, MEKK3, and Akt as main therapeutic targets in breast cancer. Thus, it will be possible to determine whether NF-κB activity in breast cancer cells can be specifically and effectively inhibited using such kinds of molecular targets.

Because of this necessity, we have already started a screening of 75 strains of 67 species, kept in the Culture Collection of the Institute of Evolution, University of Haifa (HAI). The list was based mainly on the literature data on the reported active compounds in medicinal mushrooms that have already shown, or are supposed to show, activity in human breast cancer. The list was made in order to include species belonging to different taxonomical and ecological groups, such as members of Ascomycetes—e.g., *Morchella esculenta* (L. : Fr.) Pers., *M. crassipes* (Vent. : Fr.) Pers., and *Cordyceps sinensis* (Berk.) Sacc.—and many Basidiomycetes. The latter group includes members of Agaricaceae, Bolbitiaceae, Coprinaceae, Ganodermataceae, Meripiliaceae, Pleurotaceae, Pluteaceae, Polyporaceae, Strophariaceae, etc. Some of them, such as *Lentinus edodes, Lepista nuda* (Bull. : Fr.) Cooke, and *Pleurotus ostreatus,* are edible; and others, such as *Fomes fomentarius* (L. : Fr.) J.J. Kickx., *Fomitopsis pinicola* (Sw. : Fr.) P. Karst., and *Gloeophyllum abietinum* (Bull. : Fr.) P. Karst., are inedible. Moreover, there are also some poisonous species, such as *Amanita muscaria* (L. : Fr.) Hook. and *Omphalotus olearius* (DC. : Fr.) Singer. Our aim is to isolate some bioactive low-molecular-weight compounds from the mycelia and culture broth in order to investigate their potential NF-κB inhibitory effect in human breast cancer cell lines.

REFERENCES

Aoki T. 1984. Lentinan. In: Immune Modulation Agents and Their Mechanisms, Fenichel R. L. and Chirgis M. A., eds., *Immunol Stud,* **25**, pp. 62–77.

Biswas D. K., Cruz A. P., Gansberger E., and Pardee A. B. 2000. Epidermal growth factor-induced nuclear factor kB activation: a major pathway of cell-cycle progression in estrogen-receptor negative breast cancer cells. *Med Sci (PNAS),* **97**(15), 8542–8547.

Biswas D. K., Dai S. C., Cruz A., Weiser B., Graner E., and Pardee A. B. 2001. The nuclear factor kB (NF-kB): A potential therapeutic target for estrogen receptor negative breast cancers. *Med Sci (PNAS),* **98**, 10386–10391.

Biswas D. K., Martin K. J., McAlister C., Cruz A. P., Graner E., Dai S., and Pardee A. B. 2003. Apoptosis caused by chemotherapeutic inhibition of nuclear factor-kB activation. *Cancer Res,* **63**, 290–295.

Brodie A., Quing L., and Nakamura J. 1997. Aromatase in the normal breast and breast cancer. *J Steroid Biochem Mol Biol,* **61**, 281–286.

Cao Y. and Karin M. 2003. NF-kB in mammary gland development and breast cancer. *J Mamm Gland Biol Neopl,* 8(2), 215–223.

Chihara G. 1992. Recent progress in immunopharmacology and therapeutic effects of polysaccharides. *Dev Biol Stand,* **77**, 191–197.

Chihara G., Hamuro J., Maeda Y. Y., Arai Y., and

Fukuoka F. 1970. Fractionation and purification of the polysaccharides with marked antitumor activity, especially lentinan, from *Lentinus edodes* (Berk.) Singer (an edible mushroom). *Cancer Res,* **30,** 2776–2781.

Dugourd C., Gervais M., Corvol P., and Monnot C. 2003. Akt is a major downstream target of PI3-kinase involved in angiotensin II-induced proliferation. *Hypertension,* **41,** 882–890.

Ebina T. and Fujimiya Y. 1998. Antitumor effect of a peptide-glucan preparation extracted from *Agaricus blazei* in a double-grafted tumor system in mice. *Biother,* **11**(4), 259–265.

Erkel G., Anke T., and Sterner O. 1996. Inhibition of NF-kB activation by paneropoxydone. *Biochem Biophys Res Commun,* **226,** 214–221.

Fisher M. and Yang L. X. 2002. Anticancer effects and mechanisms of polysaccharide-K (PSK): implications of cancer immunotherapy. *Anticancer Res,* **22**(3), 1737–1754.

Fugimoto S. and Furue H. 1984. Clinical evaluation of Schizophyllan adjuvant immunohemotherapy for patients with resectable gastric cancer: a randomised controlled trail. *Jpn J Surg,* **14,** 286–292.

Fujimiya Y., Suzuki Y., Katahura R., and Ebina T. 1999. Tumor-specific cytocidal and immunopotentiating effects of relatively low molecular weight products derived from the basidiomycete, *Agaricus blazei* Murrill. *Anticancer Res,* **19,** 113–118.

Furne H. 1985. Clinical evaluation of Schizophyllan (SPG) in gastric cancer—randomized controlled studies. *Int J Immunol,* **7,** 333–336.

Furue H., Kitoh I., and Hattori T. 1981. Phase III study of Lentinan. *Jpn J Cancer Chemother,* **8,** 944–960.

Gao Y. H., Zhou S. F., Chen G. L., Dai X. H., and Ye J. X. 2002. A phase I/II study of a *Ganoderma lucidum* extract (Gonopoly) in patients with advanced cancer. *Int J Med Mushr,* **4,** 207–214.

Grube B. J., Eng E. T., Kao Y.-C., Kwon A., and Chen S. 2001. White button mushroom phytochemicals inhibit aromatase activity and breast cancer cell proliferation. *J Nutr,* **131,** 3288–3293.

Halpern G. M. and Miller A. H. 2002. Medicinal Mushrooms. Ancient Remedies for Moderm Ailments. M. Evans and Company, New York, 172 pp.

Han S. B., Lee C. W., Jeon Y. J., Hong N. D., Yoo I. D., Yang K-H., and Kim H. M. 1999. The inhibitory effect of polysaccharides isolated from *Phellinus linteus* on tumor growth and metastasis. *Immunopharmacol,* **41,** 157–164.

Helbig G., Christopherson K. W., Bhat-Nakshatri P., Kumar S., Kishimoto H., Miller K. D., Broxmeyer H. E., and Nakshatri H. 2003. NF-kB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine recetor CXCR4*. *J Biol Chem,* **287**(24), 21631–21638.

Hishida I., Nanba H., and Kuroda H. 1988. Antitumor activity exhibited by orally administrated extract from fruit body of *Grifola frondosa* (maitake). *Chem Pharm Bull,* **36**(5), 1819–1827.

Hobbs C. 1995. Medicinal Mushrooms: An Exploration of Tradition, Healing and Culture. Botanica Press, Santa Cruz. 251 pp.

Hu H., Ahn N. S., Yang X., Lee Y. S., and Kang K. S. 2002. *Ganoderma lucidum* extracts induce cell cycle arrest and apoptosis in MCF-7 human breast cancer cell. *Int J Cancer,* **102**(3), 250–253.

Iino Y., Yokoe T., Maemura M., Horiguchi J., Takei H., Ohwada S., and Morishita Y. 1995. Immunochemotherapies versus chemotherapy as adjuvant treatment after curative resection of operable breast cancer. *Anticancer Res,* **15**(6B), 2907–2912.

Ikekawa T., Nakanishi M., Uehara N., Chihara G., and Fukuoka F. 1968. Antitumor action of some Basidiomycetes, especially *Phellinus linteus. Jpn J Cancer Res (GANN),* **59,** 155–157.

Jong S. C. and Birmingham J. M. 1992. Medicinal benefits of the mushroom *Ganoderma. Adv Appl Microbiol,* **37,** 101–134.

Jong S. C. and Donovick R. 1989. Antitumor and antiviral substances from fungi. *Adv Appl Microbiol,* **34,** 183–261.

Kahlos K., Kangas L., and Hiltunen R. 1987. Antitumor activity of some compounds and fractions from an *n*-hexane extract of *Inonotus obliquus. Acta Pharmacol Fennica,* **96,** 33–40.

Karin M. and Ben-Neriah Y. 2000. Phosphorylation meets ubiquitination: The control of NF-[k]B activity. *Ann Rev Immunol,* **18,** 621–663.

Karin M., Cao Y., Greten F. R., and Li Z. W. 2002. NF-kB in cancer: from innocent bystander to major culprit. *Nat Rev Cancer,* **2,** 301–310.

Kawagishi H., Okamura K., Kobayashi F., and Kinjo N. 2004. Estrogenic substances from the mycelia of medicinal fungus *Cordyceps ophioglossoides* (Ehrh.) Fr. (Ascomycetes). *Int J Med Mushr,* **6,** 249–251.

Kidd P. M. 2000. The use of mushroom glucans and proteoglycans in cancer treatment. *Alt Med Rev,* **5**(1), 4–27.

Kim H. M., Han S. B., Oh G. T., Kim Y. H., Hong D.

H., Hong N. D., and Yoo I. D. 1996. Stimulation of humoral and cell mediated immunity by polysaccharide from mushroom *Phellinus linteus*. *Int J Immunopharmacol*, 18(5), 295–303.

Kim D. S., Jeong H. J., Bhat K. P., Park S. Y., Kang S. H., Yoo E. H., Lee M., Lee H. W., Krueger R. J., and Kim D. S. 2000. Aromatase and sulfatase inhibitors from *Lepiota americana*. *Planta Med*, 66, 78–79.

Kimura Y., Tojima H., and Fukase S. 1994. Clinical evaluation of sizofiran as assistant immunotherapy in treatment of head and neck cancer. *Acta Otolaryngol*, 511, 192–195.

Komatsu N., Okubo S., Kikumoto S., Kimura K., Saito S., and Sakaki S. 1969. Host mediated antitumor action of schizophyllan, a glucan produced by *Schizophyllum commune*. *Jpn J Cancer Res*, 60, 137–144.

Kuiper G. G. J. M., Carlsson B., Grandien J., Enmark E., Häggblad J., Nilsson S., and Gustafsson J-Å. 1997. Comparison of the ligand binding specificity and transcript tissue distribution of the estrogen receptors α and β. *Endocrinol*, 138, 863–870.

Lamkanfi M., Kalai M., Saelens X., Declercq W., and Vandenabeele P. 2004. Caspase-1 activates nuclear factor of the k-enhancer in B cells independently of its enzymatic activity. *J Biol Chem*, 279(23), 24785–24793.

Li X. and O'Malley B. W. 2003. Unfolding the action of progesterone receptors. Minireview. *J Biol Chem*, 278(41), 39261–39264.

Liotta L. A. 2001. In breast-cancer patients, secondary tumours often form in the lungs and bone marrow, for example, but rarely in the kidneys. The explanation for this bias involves soluble attractant molecules called chemokines. *Nature*, 410, 24–25.

Liu J. X. and Zhou J. Y. 1993. Phase II clinical trail for PSP capsules. PSP International Symposium, Fudan University Press, Shanghai.

Lorenzen K. and Anke T. 1998. Basidiomycetes as a source for new bioactive natural products. *Curr Organic Chem*, 2, 329–364.

Lykkesfeldt A. E. 1996. Mechanisms of tamoxifen resistance in the treatment of advanced breast cancer. *Acta Oncol*, 35, 9–14.

Madrid L. V., Mayo M. W., Reuther J. Y., and Baldwin Jr. A. S. 2001. Akt stimulates the transactivation potential of the RelA/p65 subunit of NF-kB through utilization of the IkB kinase and activation of the mitogen-activated protein kinase p38*. *J Biol Chem*, 276(22), 18934–18940.

Matsuoka H., Seo Y., Wakasugi H., Saito T., and Tanoda H. 1997. Lentinan potentiates immunity and prolongs the survival time of some patients. *Anticancer Res*, 17, 2751–2756.

Matsushita K., Kuramitsu Y., Ohiro Y., Obara M., Kobayashi M., Li Y.-Q., and Hosokawa, M. 1998. Combination therapy of active hexose correlated compound plus UFT significantly reduces the metastasis of rat mammary adenocarcinoma. *Anti-Cancer Drugs*, 9, 343–350.

Mattila P., Könkö K., Eurola M., Pihlava J-M., Astola J., Vahteristo L., Hietaniemi V., Kumpulainen J., Valtonen M., and Piironen V. 2001. Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *J Agric Food Chem*, 49, 2343–2348.

Medina D., Sivaraman L., Hilsenbeck S. G., Coneely O., Ginger M., Rosen J., and O'Malley B. W. 2001. Mechanisms of hormonal prevention of breast cancer. *Ann NY Acad Sci*, 925, 23–35.

Mizuno T. 2000. Development of an antitumor biological response modifier from *Phellinus linteus* (Berk. et Curt.) Teng (Aphyllophoromycetidae) (Review). *Int J Med Mushr*, 2, 19–33.

Mizuno T. 2002. Medicinal properties and clinical effects of culinary-medicinal mushroom *Agaricus blazei* Murrill. (Agaricomycetidae) (Review). *Int J Med Mushr*, 4, 299–312.

Mizuno T., Zhuang C., Abe K., Okamoto H., Kiho T., Ukai S., Leclerc S., and Meijer L. 1999. Antitumor and hypoglycemic activities of polysaccharides from the sclerotia and mycelia of *Inonotus obliquus* (Pers. : Fr.) Pil. (Aphyllophoromycetidae). *Int J Med Mushr*, 1, 301–316.

Nakamura T., Akiyama Y., Matsugo S., Uzuka Y., Shibata K., and Kawagishi H. 2003. Purification of caffeic acid as an antioxidant from submerged culture mycelia of *Phellinus linteus* (Berk. et Curt.) Teng (Aphyllophoromycetidae). *Int J Med Mushr*, 5, 163–167.

Nakazato H., Koike A., and Ito T. 1994. Efficacy of immunochemotherapy as adjuvant treatment after curative resection of gastric cancer. *Lancet*, 343, 1122–1126.

Nakshatri H., Bhat-Nakshatri P., Martin D. A., Goulet Jr. R. J., and Sledge Jr. G. W. 1997. Constitutive activation of NF-kB during progression of breast cancer to hormone-independent growth. *Mol Cell Biol*, 17, 3629–3639.

Nanba H. 1995. Results of non-controlled clinical study for various cancer patients using maitake-D-fraction. *Explore,* 6, 19–21.

Nanba H. 1997a. Maitake D-fraction: healing and preventive potential for cancer. *J Orthomol Med,* 12, 43–49.

Nanba H. 1997b. Effect of Maitake D-fraction on cancer prevention. *Ann NY Acad Sci,* 883, 204–207.

Natarajan K., Singh S., Burke Jr. T. B., Grunberger D., and Aggarwal B. B. 1996. Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kB. *Proc Natl Acad Sci USA,* 93, 9090–9095.

Ohno N., Nameda S., Harada T., Miura N. N., Adachi Y., Nakajima M., Yoshida K., Yoshida H., and Yadomae T. 2003. Immunomodulating activity of a β-glucan preparation, SCG, extracted from a culinary-medicinal mushroom, *Sparasis crispa* Wulf. : Fr. (Aphyllophoromycetidae), and application to cancer patients. *Int J Med Mushr,* 5, 359–368.

Osborne C. T., Bardou V., Hopp T. A., Chamness G. C., Hilsenbeck S. G., Fuqua S. A. W., Wong J., Allred D. C., Clark G. M., and Schiff R. 2003. Role of estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. *J Natl Cancer Inst,* 95, 353–361.

Ossowski L. and Lopez M. R. 1996. Proteolytic enzymes in cancer invasion. Introduction. *Enzyme Protein,* 49, 5–6.

Pahl H. 1999. Activators and target genes of Rel/NF-kB transcription factors. *Oncogene,* 18, 6853–6866.

Pan Q., Bao L. W., and Merajver S. D. 2003. Tetrathiomolybdate inhibits angiogenesis and metastasis through suppression of the NFkB signaling cascade. *Mol Cancer Res,* 7, 701–706.

Philips A., Chalbos D., and Rochefort H. 1993. Estradiol increases and anti-estrogens antagonize the growth factor-induced activator protein-1 activity in MCF7 breast cancer cells without affecting c-*fos* and c-*jun* synthesis. *J Biol Chem,* 268, 1403–1408.

Power R. F., Mani S. K., Codina J., Conneely O. M., and O'Malley B. W. 1991. Dopaminergic and ligand-independent activation of steroid hormone receptors. *Science,* 254, 1636–1639.

Richer J. K., Jacobsen B. M., Manning N. G., Abel M. G., Wolf D. M., and Horwitz K. B. 2002. Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. *J Biol Chem,* 277, 5209–5218.

Romieu-Mourez R., Landesman-Bollag E., Seldin D. C., Traish A. M., Mercurio F., and Sonenshein E. 2001. Roles of IKK kinases and protein kinase CK2 in activation of nuclear factor-kB in breast cancer. *Cancer Res,* 61, 3810–3818.

Rowan N. J., Smith J. E., and Sullivan R. 2003. Immunomodulatory activities of mushroom glucans and polysaccharide-protein complexes in animals and humans (A review). *Int J Med Mushr,* 5, 95–110.

Sakagami H. and Aoki T. 1991. Induction of immunopotentation activity by a protein bound polysaccharide PSK (Review). *Anticancer Res,* 11, 993–1000.

Sakagami H. and Takeda M. 1993. Diverse biological activity of PSK (Krestin), a protein-bound polysaccharide from *Coriolus versicolor.* In: Mushroom Biology and Mushroom Products, Chang S. T., Buswell J. A., and Chiu S. W., eds. Chinese University Press, Hong Kong, pp. 237–245.

Samanta A. K., Huang H. J., Bast Jr. R. C., and Liao W. S.-L. 2004. Overexpression of MEKK3 confers resistance to apoptosis through activation of NF-kB*. *J Biol Chem,* 279(9), 7576–7583.

Sekiguchi J. and Gaucher G. M. 1979. Isoepoxydon, a new metabolite of the patulin pathway in *Penicillium urticae. Biochem J,* 182(2), 445–453.

Shin Y., Tamai Y., and Terazawa M. 2000. Chemical constituents of *Inonotus obliquus* (Pers. : Fr.) Pil. (Aphyllophoromycetidae) III: A new triterpene, 3β,22,25-trihydroxy-lanosta-8-ene from sclerotia. *Int J Med Mushr,* 2, 201–207.

Shotwell J. B., Hu S., Medina E., Abe M., Cole R., Crews C. M., and Wood J. L. 2000. Efficient stereoselective synthesis of isopanepoxidone and panepoxidone: a re-assignment of relative configuration. *Tetrahedron Lett,* 41, 9639–9643.

Sliva D., Labarrere C., Slivova V., Sedlak M., Lloyd F. P. Jr., and Ho N. W. Y. 2002. *Ganoderma lucidum* suppresses motility of highly invasive breast and prostate cancer cells. *Biochem Biophys Res Commun,* 298(4), 603–612.

Sliva D., Sedlak M., Slivova V., Valachovicova T., Lloyd F. P. Jr., and Ho N. W. Y. 2003. Biologic activity of *Ganoderma lucidum* for the inhibition of highly invasive breast and prostate cancer cells. *J Altern Complement Med,* 9(4), 491–497.

Smith J. E., Rowan N. J., and Sullivan R. 2002. Medicinal mushrooms: their therapeuthic properties and current medical usage with special emphasis on cancer treatments. Cancer Research UK. University of Strathclyde, Glasgow. 256 pp.

Sugimachi K., Inokuchi K., and Matsuura H. 1984. Hormone conditional cancer chemotherapy for recurrent breast cancer prolongs survival. *Jpn J Surg,* 14, 217–221.

Taguchi T. 1983. Effects of Lentinan in advanced or recurrent cases of gastric, colorectal, and breast cancer. *Jpn J Cancer Res (GANN),* 10(2), 387–393.

Taguchi T., Furure H., Kimura T., Kondo T., Hattori T., Ito F., and Ogawa N. 1985. End-point result of a randomised controlled study on the treatment of gastrointestinal cancer with a combination of Lentinan and chemotherapeutic agents. *Excerp Medic,* 1985, 151–165.

Talorete T. P. N., Isoda H., and Maekawa T. 2002. *Agaricus blazei* (Class Basidiomycotina) aqueous extract enhances the expression of c-Jun protein in MCF7 cells. *J Agricult Food Chem,* 50, 5162–5166.

Tekmal R., Ramachandra N., Gubba S., Durgam V. R., Mantione J., Toda K., Shizuta Y., and Dillehay D. 1996. Overexpression of int-5/aromatase in mammary glands of transgenic mice results in the induction of hyperplasia and nuclear abnormalities. *Cancer Res.,* 56, 3180–3185.

This P., De la Rochefordiere A., Clough K., Fourquet A., Magdelenat H., and The Breast Cancer Group of the Institute Curie. 2001. Phytoestrogens after breast cancer. *Endocr Relat Cancer,* 8, 129–134.

Toi M. and Hottori T. 1992. Randomised adjuvant trial to evaluate the addition of tamoxifen and PSK to chemotherapy in patients with primary breast cancer. *Cancer,* 70, 2475–2483.

Tsai M.-J. and O'Malley B. W. 1994. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Ann Rev Biochem,* 63, 451–486.

Watabe M., Hishikawa K., Takayanagi A., Shimizu N., and Nakaki T. 2004. Caffeic acid phenetyl ester induces apoptosis by inhibition of NF-kB and activation of Fas in human breast cancer MCF-7 cells. *J Biol Chem,* 279(7), 6017–6026.

Wasser S. P. 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Appl Microbiol Biotechnol,* 60, 258–274.

Wasser S. P. and Weis A. L. 1997. Shiitake mushroom —*Lentinus edodes* (Berk.) Singer. In: Medicinal Mushrooms, Nevo, E., ed. Pedelfus, Haifa, p. 45.

Wasser S. P. and Weis A. L. 1999a. Medicinal properties of substances occurring in Higher Basidiomycetes mushrooms: current perspectives (Review). *Int J Med Mushr,* 1, 31–62.

Wasser S. P. and Weis A. L. 1999b. General description of the most important medicinal Higher Basidiomycetes mushrooms. 1. *Int J Med Mushr,* 1, 351–370.

Wasser S. P. and Weis A. L. 1999c. Therapeutic effects of substances occurring in Higher Basidiomycetes mushrooms: a modern perespective. *Crit Rev Immunol,* 19, 65–96.

White R. and Parker M. G. 1998. Molecular mechanisms of steroid hormone action. *Endocr Rel Cancer,* 5, 1–14.

Wolff A. C. 2002. Systemic therapy. *Curr Opin Oncol,* 14, 600–608.

Xu G. 1993. Phase I clinical test report of PSP capsules. In: PSP International Symposium 1993, Yang Q. & Kwok C., eds., Fudan University Press, Hong Kong, pp. 179–182.

Yang Q. 1999. History, present status and perspectives of the study of Yun Zhi polysaccharopeptide. In: Advanced Research in PSP, Yang Q., ed. Hong Kong Assotiation for Health Care Ltd., pp. 5–15.

Yassin M., Mahajna J. A., and Wasser S. P. 2003. Submerged cultured mycelium extracts of Higher Basidiomycetes mushrooms selectively inhibit proliferation and induce differentiation of K562 human chronic myelogenous leukemia cells. *Int J Med Mushr,* 5, 261–276.

Yokoe T., Ino Y., Takei H., Horiguchi J., Koibuchi Y., Maemura M., Ohwada S., and Morishita Y. 1997. HLA antigen as predictive index for the outcome for breast cancer patients with adjuvant immunochemotherapy with PSK. *Anticancer Res,* 17, 2815–2818.

Yue W., Wang J. P., Hamilton C., Demers L., and Santen R. J. 1998. In situ aromatization enhances breast tumor estradiol levels and cellular proliferation. *Cancer Res,* 58, 927–932.

Zaidman B., Yassin M., Mahajna J., and Wasser S. P. 2005. Medicinal mushrooms modulators of molecular targets as cancer therapeutics. *Appl Microbiol Biotechnol* (in press).

Zandi E. and Karin M. 1999. Bridging the gap: composition, regulation, and physiological function of the IkB kinase complex. *Mol Cell Biol,* 19, 4547–4551.

Zhang Y., Bai W., Allgood V. E, and Weigel N. L. 1994. Multiple signaling pathways activate the chicken progesterone receptor. *Mol Endocrinol,* 8, 577–584.