

Recent progress in fungus-derived bioactive agents for targeting of signaling machinery in cancer cells

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Abstract: It is becoming increasingly understood that tumor cells may have different mutations and dependencies on diverse intracellular signaling cascades for survival or metastatic potential. Overexpression of oncogenes, inactivation of tumor suppressor genes, genetic/epigenetic mutations, genomic instability, and loss of apoptotic cell death are some of the mechanisms that have been widely investigated in molecular oncology. We partition this multicomponent review into the most recent evidence on the anticancer activity of fungal substances obtained from in vitro and xenografted models, and these fungal substances modulate expression of oncogenic and tumor suppressor miRNAs. There are some outstanding questions regarding fungus-derived chemical-induced modulation of intracellular signaling networks in different cancer cell lines and preclinical models. Certain hints have emerged, emphasizing mechanisms via which apoptosis can be restored in TRAIL-resistant cancer cells. Reconceptualization of the knowledge obtained from these emerging areas of research will enable us to potentially identify natural agents with notable anticancer activity and minimal off-target effects. Integration of experimentally verified evidence obtained from cancer cell line gene expression with large-scale functional screening results and pharmacological sensitivity data will be helpful in identification of therapeutics with substantial efficacy. New tools and technologies will further deepen our understanding of the signaling networks that underlie the development of cancer, metastasis, and resistance to different therapeutics at both a personal and systems-wide level.

Keywords: fungal products, cell signaling, cancer, apoptosis, miRNA, xenograft

Introduction

High-throughput technologies have revolutionized our current understanding of the genomically complex nature of cancer. Phenomenal breakthroughs have been made in the field of molecular oncology, as evidenced by advancements in genetics, genomics, and proteomics. With overwhelmingly expanding information about cancer, it is now more understandable that overexpression of oncogenes, inactivation of tumor suppressor genes, genetic/epigenetic mutations, genomic instability, dysregulation of spatiotemporally controlled intracellular signaling cascades, and loss of apoptotic cell death are some of the mechanisms that underlie the development and progression of cancer. Rapidly accumulating experimentally verified data are deepening our knowledge about wide-ranging mechanisms that induce resistance against different therapeutic agents. It is noteworthy that bioactive ingredients isolated from natural sources are now widely appreciated.

Anticancer activity of bioactive fungal components: in vitro and in vivo evidence

The polysaccharide fraction purified from a culture broth of *Ganoderma formosanum* has been shown to have notable anticancer activity. When administered intraperitoneally

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or orally, the polysaccharide fraction efficiently inhibited tumor growth in xenografted mice. It is noteworthy that the growth inhibitory effects exerted by the polysaccharide fraction were impaired in severe combined immunodeficiency (SCID) mice. CD4⁺ T-cells and CD8⁺ T-cells were noted to be essential for the polysaccharide fraction-mediated tumor growth-retarding effects.¹

Wnt signaling cascade

Wnt-induced intracellular signaling is an extensively studied mechanism reported to underpin the development and progression of cancer. β -catenin, a well-known downstream effector, is reported to be modulated by positive and negative regulators, and phosphorylated β -catenin is degraded via the ubiquitin pathway. Different regulators have been noted to control β -catenin, including axin, adenomatous polyposis coli, casein kinase, and glycogen synthase kinase (GSK)-3 β . Wnt-induced intracellular signaling sequesters β -catenin from GSK-3 β and promotes its accumulation in the nucleus, where β -catenin works synchronously with T-cell factor/lymphocyte enhancer binding factor to stimulate expression of target genes. Cordycepin isolated from *Cordyceps sinensis*, a parasitic fungus, has been shown to decrease protein levels as well as nuclear accumulation of β -catenin. Cordycepin exerted its biological effects by enhancing GSK-3 β -mediated

phosphorylation of β -catenin, and these effects were impaired in cells treated with GSK-3 β inhibitors. Moreover, phosphatidylinositol-4 5-bisphosphate 3-kinase (PI3K)/Akt-mediated inactivation of GSK-3 β was also inhibited, as evidenced by a decrease in phospho-Akt levels.² Protein-bound polysaccharide isolated from *Phellinus linteus* also efficiently reduced β -catenin and its target gene in SW480 colon cancer cells overexpressing β -catenin (Figure 1). Protein-bound polysaccharide inhibited T-cell factor/lymphocyte enhancer binding factor reporter activity in SW480 cells transiently transfected with a luciferase reporter construct.³ Destruxin B isolated from *Metarhizium anisopliae* has also been found to inhibit the Wnt signaling pathway. β -catenin/Tcf4 transcriptional activity was also marked inhibited in destruxin B-treated HCT116 and SW480 cells⁴ (Figure 1). *Antrodia camphorata* grows on germinated brown rice and recently it has been reported that ethanolic extract is effective against HT-29 colon cancer cells. Ethanolic extract was further characterized and ethyl acetate-mediated anticancer activity was more pronounced. The β -catenin pathway was notably inhibited in cancer cells treated with ethyl acetate.⁵

TGF signaling

A confluence of information indicates that fungal lactones, ie, (S)-curvularin, oxacyclododecindione, dehydrocurvularin,

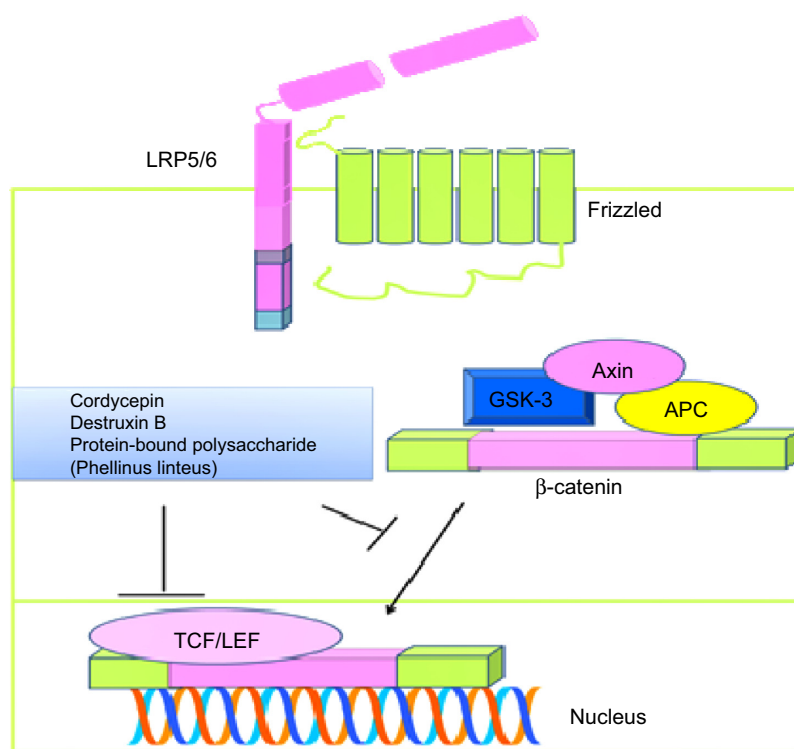


Figure 1 Wnt-induced intracellular signaling and fungal product-mediated inhibition of nuclear accumulation of β -catenin and transcriptional upregulation of target genes.

and galiellalactone, considerably inhibit transforming growth factor (TGF)-induced intracellular signaling, and SMAD2/3-mediated stimulation of the transcriptional network was also reduced in MDA-MB-231 and HepG2 cells (Figure 2).⁶ Circumstantial evidence also indicates that TGF-induced intracellular activation of SMAD2/3 is dramatically reduced in MDA-MB-231 and HepG2 cells treated with trichodimerol (a fungal metabolite).⁷ Cordycepin isolated from *Cordyceps militaris* effectively reduced production of IL-2 and TGF- β in mice xenografted with cancer cells.⁸

NF- κ B signaling

Nuclear factor kappa B (NF- κ B, p65 and p50) is cytosolic and coexists with its inhibitor, I κ B kinase. Trichothecin, a metabolite isolated from an endophytic fungus, is a potent inhibitor of trichothecin and its target genes. Detailed mechanistic insights indicated that inhibition of I κ B kinase-mediated phosphorylation of I κ B α facilitated sequestration and nuclear accumulation of p65. NF- κ B triggered expression of XIAP, cyclin D1, and Bcl-xL. However, treatment with trichothecin dramatically reduced

I κ B α phosphorylation and nuclear accumulation of NF- κ B. Enforced expression of constitutively active I κ B kinase- β considerably reduced trichothecin-induced apoptosis.⁹ Western blot analysis confirmed a decrease in I κ B kinase, p50, and p65 protein levels in fumigaclavine-treated MCF-7 cells.¹⁰ Destruxin B isolated from *M. anisopliae* inhibited I κ B kinase- α and NF- κ B, and interestingly the I κ B α level was enhanced.⁴

PI3K/Akt signaling pathway

Phosphoinositide-dependent protein kinase 1 (PDK1) is a serine/threonine kinase reported to modulate wide-ranging downstream effectors, particularly PI3K and Akt. It is now known that ligand-binding sites present in the PDK1 kinase domain can be targeted using different natural agents. Neobalbacanol, a novel molecule isolated from the *Albatrellus confluens* fungus, has been shown to dock into the PDK1 adenosine triphosphate-binding pocket, thus forming hydrogen bonds with the PDK1 backbone. This inhibition abrogated PDK1-mediated levels of phospho-Akt at the 308th and 473rd serine residues in C666-1 nasopharyngeal carcinoma cells.¹¹

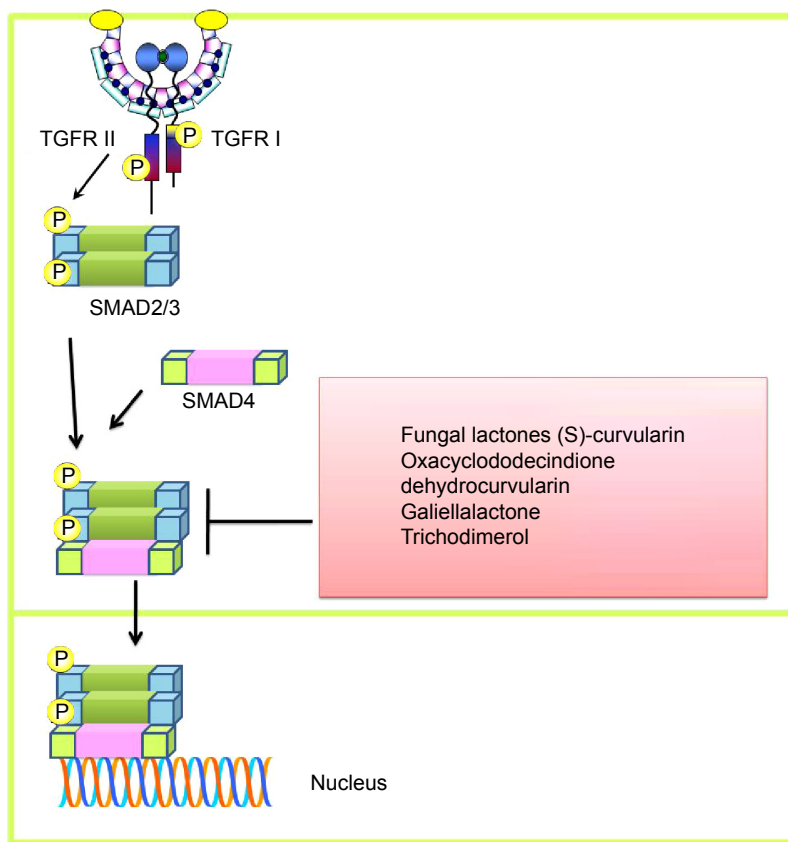


Figure 2 Transforming growth factor-induced intracellular signaling and fungal product-mediated inhibition of nuclear accumulation of SMAD and transcriptional upregulation of target genes.

Abbreviation: TGFR, transforming growth factor receptor.

PI3K-mediated phosphorylation of Akt was also inhibited in fumigaclavine-treated MCF-7 cells.¹⁰ Destruxin B isolated from *M. anisopliae* inhibited the PI3K/Akt pathway by reducing Akt expression.⁴ Extract of *Pleurotus pulmonarius* also inhibited phospho-Akt levels, and enforced expression of constitutively active Akt impaired the extract-mediated inhibitory effects on Huh7 and Hep3B liver cancer cells. Moreover, sensitivity to cisplatin was enhanced in cancer cells treated with the extract.¹² Ochratoxin, a secondary metabolite of *Penicillium*, has recently been noted to potentiate carcinogenesis. It was shown mechanistically that ochratoxin-treated cells displayed active PI3K/Akt and MEK/ERK1–2 intracellular signaling cascades. It was further shown that c-Met, a cell surface receptor, was also functionally active upon treatment of cells with ochratoxin.¹³ There is a recent report suggesting that sterigmatocystin in combination with ochratoxin or citrinin worked with effective synergy against Hep3B hepatocellular cancer cells.¹⁴

MMPs: novel targets of fungal products

Contemporary studies indicated that 3-O-methylfunicone dramatically reduced α V β 5 integrin and matrix metalloproteinase (MMP)-2 expression by exerting inhibitory effects on ERK-mediated signaling in human mesothelioma cells.¹⁵ Fumigaclavine C, a chemical isolated from *Aspergillus fumigatus*, also efficiently targeted MMPs. MMP-2 and MMP-9 protein expression was remarkably reduced in fumigaclavine-treated MCF-7 cells. The decrease in MMPs reduced the migratory and invasive potential of MCF-7 cells. ERK, JNK, and p38 mitogen-activated protein kinase (MAPK) are also targeted by fumigaclavine, as evidenced by reduced phospho-ERK, JNK, and p38 MAPK levels in fumigaclavine-treated MCF-7 cells.¹⁰ Protein-bound polysaccharide isolated from *P. linteus* and destruxin B isolated from *M. anisopliae* exerted inhibitory effects on MMP-2 and MMP-9 activity in HT1080 and SW480 colon cancer cells.^{3,4} Cordycepin and zhankuic acid, isolated from *Antrodia cinnamomea*, considerably enhanced tissue inhibitors of MMP (TIMP-1 and TIMP-2) in CL1-0 lung adenocarcinoma cells.¹⁶

Cyclins and cyclin-dependent kinases

Mitogenic signals promote heterodimerization of cyclin D1 and cyclin E1 with cyclin-dependent kinase (CDK)-4 and CDK-2, respectively. These phosphorylate the Rb proteins, causing release of E2Fs. Cyclin E is also a target gene of E2F that later complexes with CDK-2 to phosphorylate Rb. Fumigaclavine-treated MCF-7 breast cancer cells displayed

a marked decrease in CDK-2, CDK-4, and cyclin E protein expression levels.¹⁰ Moreover, the fungus-derived compound cephalochromin downregulated cyclin D1, cyclin E, CDK-2, and CDK-4 in A549 lung cancer cells, thus abrogating the association of cyclins with their respective CDKs.¹⁷

Targeting of negative regulators of death signaling

Paxilline, isolated from *Penicillium paxilli*, has been shown to effectively induce apoptosis in TRAIL-resistant glioma cell lines. Paxilline-treated glioma cells displayed a marked increase in the TRAIL receptor, DR5. Interestingly, paxilline markedly reduced cellular levels of FLICE-inhibitory protein and survivin by potentiating proteasome-triggered degradation.¹⁸ Fungus-derived cephalochromin effectively reduced Bcl-xL and survivin expression and activated caspase-3 via caspase-8 and caspase-9 in A549 lung cancer cells.¹⁷ Caspase-8-mediated proteolytically cleaved truncated Bid and Bax were also elevated in DU145 prostate cancer cells treated with glionitrin A, isolated from *A. fumigatus*.¹⁹

Verrucarin A, isolated from *Myrothecium verrucaria*, is a protein synthesis inhibitor reported to activate caspase-3 and upregulate Bax in MDA-MB-231 and T47D breast cancer cells. Moreover, Bcl-2 expression was notably reduced in verrucarin-treated breast cancer cells.²⁰ The ratio of Bax to Bcl-2 was also enhanced in human laryngeal carcinoma cells treated with polysaccharides of *Lepista sordida*. Release of cytochrome c was noted in Hep-2 cells along with a marked increase in caspase-9 activity.²¹

Fungal products awake “guardian angels”

Polysaccharides isolated from *Ganoderma atrum* considerably enhanced cell surface expression of Toll-like receptor-4 in macrophages and induced intracellular signaling. Moreover, there was evident nuclear accumulation of the p65 subunit of NF- κ B and degradation of I κ B α . Further, phosphorylated levels of p38-MAPK, ERK-1/2, and c-JNK were also increased in macrophages.²² In another study, a similar mode of action was demonstrated, as polysaccharides did not kill CT26 colorectal carcinoma cells directly but exerted inhibitory effects on proliferation of CT26 cells by activating peritoneal macrophages. Tumor growth was also remarkably inhibited in mice with CT26 xenografts.²³

mTOR signaling

Extract of *Ganoderma lucidum* efficiently decreased levels of phospho-mTOR (mammalian target of rapamycin) and

its downstream effectors, including p70S6K, S6, p-S6, and p-4E-BP1, in SUM-149 inflammatory breast cancer cells.²⁴ Monascuspiloin efficiently induced apoptosis in LNCaP prostate cancer cells by inhibiting PI3K/Akt/mTOR-induced downstream signaling.²⁵ Antrocin isolated from *A. camphorata* decreased phospho-Akt and phospho-mTOR levels in MDA-MB-231 cells.²⁶

Targeting of Rho GDP dissociation inhibitor

RHO-specific guanine nucleotide dissociation inhibitors (RHoGDIs) have emerged as versatile regulators of RHO proteins and are reported to protect RHO proteins from degradation. It has previously been convincingly demonstrated that secalonic acid isolated from *Penicillium* spp. considerably enhanced caspase-3-mediated cleavage of RhoGDI 2. Secalonic acid-treated HL60 cells displayed a marked increase in the cleaved product of RhoGDI 2.²⁷ Alcohol extracts of *A. camphorata* mycelia also downregulated RhoGDI- α expression in A549 lung carcinoma cells.²⁸

VEGF-induced signaling

Vascular endothelial growth factor (VEGF) is a proangiogenic molecule for vascular endothelial cells and a potent vascular permeability enhancer. Mounting evidence indicates that VEGF has a key role during angiogenesis, proliferation, and migration of endothelial cells. Toluquinol exerted inhibitory effects on VEGF-induced and fibroblast growth factor-induced intracellular signaling. Interestingly, it has been suggested that capillary tube formation of endothelial cells on Matrigel is substantially reduced upon treatment with toluquinol, a methylhydroquinone isolated from a marine fungus. Additionally, the invasive, migratory and proteolytic activities of endothelial cells were also suppressed.²⁹ Cytochalasin isolated from *Diplodia macrospora* efficiently inhibited positioning of hypoxia-inducible factor-1 α at the VEGF promoter in ovarian cancer cells.³⁰ Arthrinins isolated from *Arthrinium* spp. markedly inhibited VEGF receptor-2 in cancer cells.³¹

Preliminary results for fungal products

Citriquinone is a benzoquinone isolated from *Penicillium citrinum* and has notable activity against cancer cells.³² Chaetoglobosin, a secondary metabolite isolated from *Penicillium aquamarinum*, effectively induced cell cycle arrest and apoptosis in leukemia cells. It was also noted to exert inhibitory effects on cell migration and membrane ruffling.³³

Diterpene alkaloids known as penitremes are also bioactive ingredients isolated from *Penicillium* with considerable anticancer activity against MCF-7 breast cancer cells.³⁴ Fermented ginseng extracts isolated from *Penicillium simplicissimum* have activity against ES2 human clear-cell ovarian carcinoma cells.³⁵ Eremophilane-type sesquiterpenes isolated from *Penicillium* spp. have activity against A-549 cancer cells.³⁶ Dihydrothiophene-condensed chromone isolated from *Penicillium oxalicum* is effective against SW-620, A375, and HeLa cancer cells.³⁷ There is a direct piece of evidence highlighting the autophagic cell death-inducing role of peneciraistin, a spiroketal molecule isolated from *Penicillium raistrickii*, in A549 lung cancer cells. Moreover, use of autophagy inhibitors induced apoptotic cell death in A549 cells.³⁸ It is noteworthy that the mammosphere-forming ability of MCF-7 cells decreased significantly upon treatment with the 3-O-methylfunicone metabolite isolated from *Penicillium pinophilum*.³⁹ Patulin isolated from *Penicillium* spp. has been shown to induce activation of caspase-3 and to considerably enhance levels of phospho-EGR-1; indepth analysis revealed an EGR-1-mediated increase in expression of ATF3 in colorectal cancer cells.⁴⁰

miRNA regulation

miRNA is a non-coding RNA consisting of 21–22 nucleotides. The RNA polymerase binds to the promoter region of a specific DNA sequence and forms a hairpin structure of the pri-miRNA. The pri-miRNA is recognized by nuclear protein DGCR8. DGCR8 works synchronously with Drosha by cleaving nucleotides from hair pin of pri-miRNA to form pre-miRNA. This “pre-miRNA” transcript has the capacity to form a stem-loop structure, and is then processed into 21–22 nucleotide long miRNA by an enzyme called Dicer. Increasingly, it is being realized that miRNAs regulate other genes by binding to complementary sequences in the target gene. Perfect complementarity of binding leads to mRNA degradation of the target gene. Imperfect pairing inhibits translation of mRNA to protein. miRNAs have emerged as master regulators of the gene network.

Diaporine A (D261), a natural product of endophytic fungus, has been shown to effectively inhibit proliferation of non-small cell lung cancer cells by upregulating expression of miR-99a. It was shown mechanistically that miR-99a quantitatively controlled expression of mTOR. Diaporine A also induced regression of tumor growth in mice xenografted with NCI-H460 non-small cell lung cancer cells.⁴¹ Antrocin, a sesquiterpene lactone isolated from *A. camphorata*, a medicinal mushroom, considerably inhibited interleukin

6-induced activation of STAT3, constitutive activation of STAT-3, and its nuclear accumulation. Let-7c was markedly increased in antrocin-treated cancer cells. More importantly, intraperitoneally administered antrocin induced regression of tumor growth in lung cancer tumor xenografts.⁴²

Bostrycin, isolated from marine fungi, considerably upregulated miR-923 and miR-638 in A549 lung carcinoma cells.⁴³ miR-638 is known to suppress progression of cancer, as evidenced by cell culture studies. Future studies should focus on identifying the potential protein network involved in cancer progression.

A. cinnamonomea, a medicinal fungus, has considerable efficacy against SK-Hep-1 liver cancer cells. The MAPK and PI3K/Akt signaling cascades were extensively reduced in SK-Hep-1 cells treated with extracts of *A. cinnamonomea*. Surprisingly, the oncomirs miR-21 and miR-191, together with oncogenic clusters miR-106b-25 and miR-17-92, were effectively downregulated in SK-Hep-1 cells.⁴⁴

Trametes robiniophila murriss (Huaier) is a fungus with notable anticancer activity. Recently, it has been shown that Huaier stimulates expression of miR-26b-5p in A549 pulmonary adenocarcinoma cells. A549 cells transfected with miR-26b-5p displayed a higher apoptotic rate, and targeted inhibition of miR-26b-5p in A549 cells dramatically abrogated the Huaier-mediated anticancer effects. It was shown mechanistically that miR-26b-5p negatively regulated EZH2 in A549 cells.⁴⁵

Pharmacokinetic studies

Aspergiolide (ASP)-A, an anthracycline isolated from a marine fungus, significantly inhibited tumor growth in H22 hepatoma-bearing mice. The distribution and pharmacokinetic parameters of ASP-A were studied by intraperitoneal injection of 15 mg/kg and 30 mg/kg of ASP-A at varying times. ASP-A achieved an effective concentration of drug and was noted to be broadly redistributed in vivo with a shorter half-life.⁴⁶ Xyloketal B, isolated from *Xylaria* spp. (mangrove fungus), exerted inhibitory effects on cytochrome P450 (CYP)3A-mediated metabolism of midazolam in rats by markedly reducing hepatic CYP3A activity and expression. Xyloketal B may behave as a CYP3A4/Cyp3A2 substrate and/or inhibitor, which dramatically alters the pharmacokinetics of CYP3A4/Cyp3a2-mediated metabolism of coadministered drugs.⁴⁷

Clinical trials

Schizophyllan (SPG) has previously been used in combination with conventional chemotherapy in a randomized

controlled trial including 367 patients with inoperable recurrent gastric cancer, resulting in a considerable increase in median survival. SPG in combination with radiotherapy notably improved the time to recurrence and survival time in patients with stage II cervical cancer in this randomized controlled trial but not in those with stage III disease.^{48,49} In a prospective, randomized controlled trial involving 312 patients treated with surgery, chemotherapy (5-fluorouracil), radiotherapy, and SPG in various combinations, survival rates were notably longer in SPG-treated patients than in those who did not receive SPG. It is noteworthy that a markedly increased proportion of CD8⁺ cells (HLA-DR⁺) activation from total CD8⁺ subset was evident with progressing disease. SPG-induced acceleration was observed in terms of recovery of activated CD8⁺ T-cells in the CD8⁺ T-cell subsets when compared with patients not treated with sizofiran (SPG) after radiotherapy.⁵⁰ SPG was also tested for efficacy in 15 patients with head and neck cancer, and the SPG-treated group had an 86.7% cumulative 5-year survival rate.⁵¹

Basidiomycetes are a rich source of natural agents and a widely studied product is the protein-bound polysaccharide, PSK (Krestin[®]), with notable biological activity. There is a surprising piece of evidence suggesting that PSK-mediated anticancer effects were not significant either in early-stage cancers or in more advanced cases (>S3 or >N3) in patients treated combinatorially with carbazilquinone + PSK. However, survival of patients whose disease was classified as S1 + S2(N1-2) was better on treatment with carbazilquinone + PSK.⁵²

In a randomized double-blind trial, PSK was given to colorectal cancer patients, and the results revealed improved disease-free survival ($P < 0.05$) and overall survival ($P < 0.05$) rates in the PSK group. Importantly, analysis of patients with polymorphonuclear leukocytes and undergoing treatment with PSK indicated notable biological activity, such as phagocytosis and chemotactic locomotion.⁵³ There is also a direct piece of evidence suggesting that the 5-year disease-free survival rate was considerably high (73.0%) in a group treated with chemotherapy + PSK when compared to that in patients treated with chemotherapy alone. Disease recurrence was reduced by 43.6% (95% confidence interval [CI] 4.5–66) and mortality by 40.2% (95% CI –12.5, 68.3) in the PSK-treated group. Disease-free survival and overall survival in PSK-treated patients with stage III disease increased significantly, with a notable decrease in recurrence, particularly of lung metastases.⁵⁴

Meta-analysis of three randomized controlled trials conducted in patients who had undergone curative resection also highlighted the effective role of PSK. A comparative analysis

of chemotherapy alone and chemotherapy + PSK concluded that the disease-free survival risk ratio was 0.72 (95% CI 0.58–0.90, $P=0.003$) and the overall survival risk ratio was 0.71 (95% CI 0.55–0.90, $P=0.006$), thus signifying notable survival rates because of the effective synergy induced by the combinatorial approach.⁵⁵

Conclusion

Rapidly developing resistance against a wide range of therapeutic interventions has added another layer of complexity and future studies must focus on identification of natural agents with clear anticancer activity. Fungal products have been shown to have considerable potential in delaying the progression of cancer and induction of apoptosis, as evidenced by in vitro and in vivo studies. It is becoming increasingly evident that fungal products can modulate the intracellular protein network, and a better understanding of the different cancers and their subtypes will be helpful in identification of most efficient bioactive ingredients will minimal off-target effects.

Disclosure

The authors report no conflicts of interest in this work.

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