Bioactive Mycocompounds of Selected Medicinal Mushrooms for HPV

Subjects: Health Care Sciences & Services

Contributor: Tomas Rokos, Terezia Pribulova, Erik Kozubik, Kamil Biringer, Veronika Holubekova, Erik Kudela

Medicinal mushrooms have been used as a medicinal tool for many centuries and, nowadays, are used in the prevention and therapy of various diseases, including as an adjunct to cancer treatment. It is estimated that 14–16% of global cancer cases are caused by infectious events; one well-known infectious agent that leads to cancer is the human papillomavirus (HPV). HPV is responsible for more than 99.7% of cervical cancer cases and also may play a role in vaginal, vulvar, penile, anal, rectal, and oropharyngeal carcinogenesis. *Coriolus versicolor*, a basidiomycetes class mushroom, consists of glycoproteins called polysaccharide-K (PSK) and polysaccharopeptide (PSP), which are mainly responsible for its effectiveness in the fight against a variety of cancers. Its beneficial effect lies in its ability to arrest different phases of the cell cycle, immunomodulation or induction of apoptosis. *Coriolus versicolor* extractcan reduces BCL-2 expression or increases the expression of p53 tumour suppressor genes in breast tumour cell lines. Inhibition of proliferation was also demonstrated with HeLa cells, while cervical cytology abnormalities improved in patients who locally applied *Coriolus versicolor*-based vaginal gel. *Coriolus versicolor* extract itself, and also its combination with another medicinal mushroom, *Ganoderma lucidum,* leads to improved HPV clearance in HPV cervical or oral-positive patients. Medicinal mushrooms can also increase the effectiveness of vaccination.

Keywords: HPV ; cervical dysplasia ; medicinal mushrooms

1. Types of Medicinal Mushrooms and Their Biopotentials

For many years, mushrooms have been used as an effective therapeutic tool in the treatment of various diseases. For example, around 5300 years ago, Ice Man used amadou mushrooms (*Fomes fomentarius* (L.) Fr.) to survive in the inhospitable conditions of the Italian Alps. Hippocrates also described this mushroom as a potent anti-inflammatory treatment. On the other side of the world, the first inhabitants of North America used puffball mushrooms (*Calvatia* genus) to improve the wound healing process [1]. The people of Asia have also used mushrooms as a medicinal tool for many centuries. Nowadays, medicinal mushrooms have been approved in eastern countries as an adjunct to cancer treatment. Commonly used species include *Ganoderma lucidum* (Curtis) P. Karst, *Lentinus edodes* (Berk.) Singer, and *Trametes versicolor* (L.) Lloyd, which is also called *Coriolus versicolor* or turkey tail. Medical mushrooms are also distributed in other parts of the world, but in the US, for example, they are distributed as dietary supplements and regulated as food, not drugs. Manufacturing consistency is not controlled for dietary supplements, so it is not possible to guarantee that a product contains the ingredients listed on the label. The US Food and Drug Administration (FDA) has these dietary supplements as treatments for any medical condition [2].

Many countries fail to regulate the handling of medicinal mushrooms and their components, which can lead to a reduced content, a lack of effective components in the sold supplements, or even replacement of the effective components by others that can have an adverse effect on human health. Due to the fact that the fungal extract may contain a large spectrum of demonstrably or potentially bioactive compounds, it is difficult to monitor the effectiveness of sold supplements. Determining the exact dose of a substance whose beneficial effect on human health could be incorporated into a study is challenging. Therefore, it is difficult to prove the effectiveness of medicinal mushrooms; however, despite the lack of evidence, their beneficial effect on human health has been known for a long time [3].

Many bioactive compounds such as polysaccharides, proteins, fats, minerals, glycosides, alkaloids, volatile oils, terpenoids, tocopherols, phenolics, flavonoids, carotenoids, folates, lectins, enzymes, and ascorbic, and organic acids are found in medicinal mushrooms and are responsible for more than 100 medicinal functions. The most important of these functions are antioxidant, anticancer, antidiabetic, antiallergic, immunomodulating, anticholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxification, anti-inflammatory, and hepatoprotective effects [4]. Medicinal mushrooms are mainly used as dietary supplements or functional foods, but they have potential as drugs for traditional

and/or evidence-based medicine. The most important mushroom species mentioned in research are *G. lucidum*, *C. versicolor*, *Lentinula edodes* (Berk.) Pegler, *Agaricus brasiliensis* (Wasser et al.), *Cordyceps sinensis* (Berk.) Sacc., *Grifola frondosa* (Dicks.) Gray, *Hericium erinaceus* (Bull.: Fr.) Pers., and others [5].

2. Mechanism of Cell Proliferation and Immunomodulation Properties

The effectiveness of *C. versicolor* polysaccharides is well documented. Several studies have demonstrated the effectiveness of *C. versicolor* in the fight against a variety of cancers, mostly using polysaccharopeptide (PSP) and polysaccharide K (PSK) called krestin, extracted from this mushroom. They have proven to be helpful in ovarian [6], cervical [7], prostate [8], colon [9], lung [7], and breast [10] cancer treatment, as well as in the fight against leukemia [11] and other cancers.

The protein extract of this mushroom can cause cell cycle arrest [12]. It can also affect apoptotic pathways. Proteins BCL-2 and BCL-X_L are BCL-2 family proteins, which are regulators of the mitochondria-mediated apoptotic pathway. While BH3-only proteins, BAK, and BAX are pro-apoptotic, BCL-2 and BCL-X_L have anti-apoptotic function [13]. In breast cancer cells, 17β-estradiol stimulates overexpression of BCL-2, which decreases levels of mitochondrial apoptotic factors [14]. *C. versicolor* extract demonstrably reduces BCL-2 expression in breast cancer cells. An increased expression of genes for tumour suppressor protein p53 has also been observed in some breast tumour cell lines incubated with *C. versicolor* extract [15]. The cytotoxic effect of *C. versicolor* protein-bound polysaccharides on melanoma cells has also been confirmed via increased intracellular reactive oxygen species [16].

Caspase-3 is a death protease, one of the crucial mediators of apoptosis. Its precursor, precaspase-3, has at least 200-fold less activity than caspase-3. The overexpression of this precursor was confirmed in cancer tissue [17]. The genes of this precursor are the target of the E2F family of transcription factors. E2Fs are in an inactive form due to binding with the retinoblastoma protein (Rb) [18]. The dissociation of this bond leads to the excessive activity of the transcription factor. The dysregulation of the cell cycle based on this dissociation has been demonstrated in multiple cancers while the oncogenic potential of the E7 HPV protein also lies in this mechanism. This pRb/E2F pathway dysregulation leads to the eventual upregulation of gene transcription for precaspase-3 [17]. In promyelomonocytic leukemia cells, PSK activates caspase-3, which leads to the induction of apoptosis [19]. In the field of neurotoxicity, *C. versicolor* aqueous extract was found to have protective value in nitric oxide-induced brain diseases due to its effect on caspase-3 enzyme activity [20].

The Nuclear Factor kappaB (NF-κB) is in the transcription factor family; these affect immune response and inflammation and determine expression of p53 tumour suppressor protein genes or genes for signal transducers and activators of transcription (STAT) [21].

In interferon (IFN) signaling, after binding pathogen-associated molecular patterns (PAMP) to pathogen recognition receptors (PRR), interferon-regulatory factors drive expression of IFN genes [22]. In the next step, IFN binds to its receptors, leading to STAT activation. IFN molecules bind to cell surface receptors and initiate a signaling cascade through the Janus kinase signal transducer and activator of transcription (JAK-STAT) pathway, leading to the transcriptional regulation of hundreds of IFN-regulated genes [23]. STAT promotes expression of interferon stimulated genes (ISGs), which mediate antiviral responses [24]. STAT1-regulated genes are important targets of host gene regulation by HPV [25]. For example, HPV31 E7 can suppress STAT1 at the transcriptional level, resulting in reduced IFN-mediated gene expression [26]. HPV16 E7 inhibits IFN-induced phosphorylation, the nuclear translocation of STAT1, and the downstream expression of ISGs [27]. It has also been established that overexpressed E6 and E7 in keratinocytes repress the expression of innate immune genes [28].

Ethanolic extract of *C. versicolor* reduces prostate cancer cell growth. An in vitro study showed that this extract increased the levels of STAT1, a possible mechanism of its action [29]. On the other hand, *C. versicolor* extract showed anti-inflammatory effects in mice model inflammatory bowel disease by reducing STAT1 and STAT6 expression, leading to lower IFN-γ and interleukin-4 (IL-4) expression [30].

The immunostimulatory effects of PSP were demonstrated in animal models, through elevation of pro-inflammatory cytokines like IL-6 and tumor necrosis factor α (TNF- α) [31]. PSP in simultaneous activation with antigens, such as lipopolysaccharide bacteria wall components, leads to activation of the PRR toll-like receptor 4 (TLR4),which increases IL-6 production. Induction of the TLR4 signalling pathway also leads to the activation of NF-κB [32]. These two inducers may also activate the signalling pathway via STAT3 [33]. On the other hand, incubation of human leukemia cells with aqueous extracts of *C. versicolor* leads to a decrease in transcription factor NF-κB and a decrease in the expression of

cyclooxygenase 2 (COX-2), whose products are responsible for higher levels of cell proliferation and angiogenesis and the reduction of apoptosis. A study of *C. versicolor* extract on human leukemia cells also shows STAT1 elevation [34].

PRR ligands such as N-acetyl glucosamine, beta glucans, and lipopolysaccharide activate innate and adaptive immunity by binding to receptors such as TLR4 or complement receptor 3. This leads to the secretion of inflammatory cytokines like IL-6 or TNF-α [35]. PSK through TLR4 plays a role in the activation of TNF-α secretion [36]. Another work describes two possible routes of *C. versicolor* extract's effect on pro-inflammatory cytokine expression. Secretion of cytokines IL-6 and TNF-α by macrophages and TLR4 expression were stimulated by the extract itself. Additionally, during treatment of cells with lower concentrations of lipopolysaccharide, the extract increased cytokine production, while higher dose of lipopolysaccharide led to their reduced synthesis. In other words, *C. versicolor* extract showed an antagonistic or additive effect according to lipopolysaccharide concentration [37].

Pleurotus ferulae [38] is another medicinal mushroom, which affects immunological response. By improving maturation and function of dendritic cells, it helps to link innate and adaptive immunity. T and B lymphocytes with antigen-specific surface receptors play an important role in adaptive immunity. Lymphocyte effector clones are formed after antigen binding to lymphocyte receptors. Cytotoxic T-lymphocytes and NK cells are the main parts of innate immunity in the immune response against viral pathogens [39]. The major histocompatibility complex (MHC) plays an important role in the process of the activation of T and B lymphocytes. By MHC, class I processes endogenous antigens as viral proteins produced by the cell. They are marked in cytoplasm by ubiquitin and are destroyed by proteasomes. Subsequently, they are moved to the endoplasmic reticulum, where α chain and β2microglobulin are synthesized, then transported to the Golgi complex, and finally transported to the cell surface, where they are recognized by CD8+ T lymphocytes. After binding CD8+ to MHC, class I CD8+ form a receptor for IL-2 and, with the help of the Th1 subpopulation of CD4+ T lymphocytes, CD8+ lymphocytes mature into mature cytotoxic Tc lymphocytes. Tc lymphocytes release perforins and granzins from cytotoxic granules—enzymes that lead to apoptosis of the target cell. Thus, after recognizing tumor cells or cells attacked by intracellular microorganisms, especially viruses, Tc lymphocytes cause their degradation.

MHC class II molecules play role in the processing and presentation of external molecules that have entered the cell by endocytosis or phagocytosis. These are antigen presenting cells—dendritic cells, monocytes, macrophages, and B-lymphocytes. After processing antigens in the endolysosome fragments, they bind to the MHC II molecules. Such a complex is transported to the cell surface and is subsequently recognized by CD4+ T lymphocytes. Subsequently, Th lymphocyte precursors are produced, which further develop into the next subpopulations. If the precursors develop in the presence of cytokine IL-12, they differentiate into the Th1 subpopulation. The Th2 subpopulation arises in the presence of IL-4 and the Th17 subpopulation is formed in the presence of IL-1 or IL-6. Subsequently, formed Th1 cells mainly produce IFN-γ and IL-2, Th2 cells produce cytokines IL-4, IL-5, IL-10, IL-13, and also influence the maturation of B lymphocytes into plasma cells and memory B cells. Th17 cells produce IL-17 and influence the production of pro-inflammatory cytokines and chemokines. Protection against intracellular microorganisms is ensured by Th1 cells [39][40].

3. Mechanism of Anti-HPV Properties and Vaccination Support

Patients affected by pre-cancerous changes of the cervix can also benefit from the use of *C. versicolor* products. A retrospective observational study evaluated the efficacy of *C. versicolor*-based vaginal gel in 183 high-risk HPV-positive women with normal or abnormal cytology. The patients applied vaginal gel for three months and were HPV DNA tested after six months. HPV negativity was confirmed in 67% of patients who applied the gel versus 37.2% of the control group. Furthermore, cytology improvement was observed in 78.5% of the treated patients versus 37.7% of controls [41]. Another study enrolled 91 HPV-positive women with low-grade Pap smear lesions. Normal Pap smears performed three months after treatment were obtained in 78% of patients in the treated group, compared to 54.8% in the control group. At their six-month visits, the high-risk HPV group showed 62.5% HPV clearance in those who applied the gel versus 40% in the control group [42]. Both studies demonstrated higher cytology improvement and HPV clearance in patients who applied *C. versicolor*-based vaginal gel. The effect of *C. versicolor* on HPV clearance was also confirmed for oral HPV infection; 61 patients underwent oral swabs for gingivitis and were positive for HPV16 or HPV18. They took capsules containing Mycelia extract from medical mushrooms *Laetiporus sulphureus* (Bull.) Murrill and a combination of extracts from *T. versicolor* and *G. lucidum* for two months. HPV was cleared in 87.8% of patients who took *T. versicolor* and *G. lucidum* extract while it was cleared in only 5% of the patients treated with *L. sulphureus* [43].

The immune system is one of the basic systems important for maintaining the homeostasis of the organism and its defense against environmental factors. Organisms, molecules or parts of molecules represent antigens that the immune system recognizes and triggers an immune response. The immune response is stimulated after the interaction between the antigen and the receptor, while the innate immune mechanisms are the first involved in defense reactions [39].

Infectious agents release PAMPs, which are recognized by receptors on the surfaces of the epithelium, that lead to the activation of the cellular and humoral mechanisms of innate immunity [44]. To recognize PAMPs, the innate immunity uses PRR, which are coded in the genome, and no further modification is required for their use. The PRRs recognize the patterns found on pathogens while such patterns are not found on the body's own cells, so innate immunity can distinguish its own structures from foreign ones. From a functional point of view, PRRs are divided into several groups while the best known are Toll-like receptors (TLRs). These are divided into 10 groups according to the ligands they can recognize [39]. Canella F. et al. [45] quantified TLR-2, 3, 4, 7, and 9 transcripts in HPV-positive and HPV-negative cervical samples from 154 women. Higher expression of TLR-9 was proved in HPV-positive samples, and extremely higher levels of this receptor were observed in patients with persistent HPV infection [45]. On the other hand, oncoproteins E6 and E7 are able to block TLR-9 induced cytokine production in keratinocytes. The mechanism of this inhibition was demonstrated by in vitro infection of keratinocyte cells with HPV16 virions. After 24 h, the expressed oncoprotein E7 caused the formation of a nuclear complex consisting of estrogen receptor 1 (ESR1 also ERα) and a dimer of two members of the NF-κB family of transcription factors (NFKB1 and RELA or p50 and p65) under the influence of IκB kinase (IKK). This complex binds to the DNA region of the TLR-9 promoter, thereby preventing the initiation of gene expression for this protein. In addition, the NF-κB family member RELA (p65) together with ERα interaction with histone deacetylase 1 (Histone Deacetylase 1–HDAC1) and lysine specific demethylase (Lysine (K)-Specific Demethylase 5B–KDM5B also JARID1B) caused histone modification of the TLR-9 promoter. These processes caused the suppression of TLR-9 transcription with a subsequent impact on weakening the function of innate immunity, mainly by reducing the production of IFN1 [46].

Macrocybe lobayensis (R. Heim) Pegler & Lodge, from the Tricholomataceae family, has been used for centuries in traditional medicine as well. A heteroglycan protein with a strong antitumor and immunomodulatory effect was isolated from this mushroom [47]. Such extract rich on polysaccharides from this mushroom is able to upregulate the expression of TLR-2 and TLR-4 [48]. Canella at al. [45] did not demonstrate a higher expression of these two receptors in low-risk and high-risk HPV positive cervical cells collected with a cytobrush from both ectocervix and endocervix samples while a study by Daud I. et al. [49] showed in endocervical specimens 80-fold greater TLR-2 the median positive change in women who cleared HPV16 infection than women who persisted this infection [49]. Although the overexpression of specific TLRs in HPV infection is disputable, the immunomodulatory effect of the *M. lobayensis,* caused by augmented macrophage activity and the TLR signalled modulated expression of immunomodulation-related genes including NF-κB, COX-2, IFN-γ, TNF-α, and Iκ-βα, stimulates the immune system in a fight against pathogens causing the infection [48].

HPV vaccination is an effective method of primary prevention, but its sufficient effect on already developed HPV-associated cancer has not been confirmed. On the other hand, anticancer immunotherapies have presented great development in recent years. In gynecology cancer, the two main ways of immunotherapy are promising—monoclonal antibodies in function of immune check-point blockers and T cell-based immunotherapy [50]. Dendritic cells are used in antitumor vaccines, mainly due to their ability to activate naive CD4 and CD8 T cells [51]. The positive effect of *P. ferulae* polysaccharides on the antitumor therapeutic HPV dendritic cells-based vaccine was proved in an animal model. HPV dendritic cells-based vaccine supported by *P. ferulae* polysaccharides significantly inhibited tumor growth with the increased activation of CD4+ and CD8+ T cells. Polysaccharides of this mushroom improved the antitumor efficacy of therapeutic vaccine [52]. Roopngam et al. [53] proved higher amounts of T-lymphocytes in the group of T-lymphocytes cocultured with the dendritic cells pulsed by the HPV16-E7 proteins and treated with *Pleurotus sajor-caju*-β-glucan polysaccharides in comparison with T-lymphocytes without this treatment. [53]

Another mushroom used in traditional Chinese medicine, *Flammulina velutipes* (Curtis) Singer, showed immunomodulating effect in a mice model. Fungal protein isolated from this mushrrom stimulates maturation of dendritic cells and induce antigen-specific CD8+ T-cell immune responses. [54]

References

1. Stamets, P.; Zwickey, H. Medicinal Mushrooms: Ancient Remedies Meet Modern Science. Integr. Med. 2014, 13, 46–47.
2. PDQ Integrative, Alternative, and Complementary Therapies Editorial Board. Medicinal Mushrooms (PDQ®): Health Professional Version. In PDQ Cancer Information Summaries; National Cancer Institute (US): Bethesda, MD, USA, 2022. Available online: https://www.ncbi.nlm.nih.gov/books/NBK401261/?report=classic (accessed on 7 June 2022).
3. Venturella, G.; Ferraro, V.; Cirlincione, F.; Gargano, M.L. Medicinal Mushrooms: Bioactive Compounds, Use, and Clinical Trials. Int. J. Mol. Sci. 2021, 22, 634.

4. Chang, S.T.; Wasser, S.P. The role of culinary-medicinal mushrooms on human welfare with a pyramid model for huma n health. Int. J. Med. Mushrooms 2012, 14, 95–134.
5. Lindequist, U.; Kim, H.W.; Tiralongo, E.; van Griensven, L. Medicinal mushrooms. Evid. Based Complement. Altern. Me d. 2014, 2014, 806180.
6. Kobayashi, Y.; Kariya, K.; Saigenji, K.; Nakamura, K. Suppression of cancer cell growth in vitro by the protein-bound po lysaccharide of Coriolus versicolor QUEL (PS-K) with SOD mimicking activity. Cancer Biother. 1994, 9, 63–69.
7. Shnyreva, A.V.; Shnyreva, A.A.; Espinoza, C.; Padrón, J.M.; Trigos, Á. Antiproliferative Activity and Cytotoxicity of Som e Medicinal Wood-Destroying Mushrooms from Russia. Int. J. Med. Mushrooms 2018, 20, 1–11.
8. Luk, S.U.; Lee, T.K.; Liu, J.; Lee, D.T.; Chiu, Y.T.; Ma, S.; Ng, I.O.; Wong, Y.C.; Chan, F.L.; Ling, M.T.; et al. Chemoprev entive effect of PSP through targeting of prostate cancer stem cell-like population. PLoS ONE 2011, 6, e19804.
9. Roca-Lema, D.; Martinez-Iglesias, O.; Fernández de Ana Portela, C.; Rodríguez-Blanco, A.; Valladares-Ayerbes, M.; Dí az-Díaz, A.; Casas-Pais, A.; Prego, C.; Figueroa, A. In Vitro Anti-proliferative and Anti-invasive Effect of Polysaccharide -rich Extracts from Trametes Versicolor and Grifola Frondosa in Colon Cancer Cells. Int. J. Med. Sci. 2019, 16, 231–24 0.
10. Wong, J.H.; Ng, T.B.; Chan, H.H.L.; Liu, Q.; Man, G.C.W.; Zhang, C.Z.; Guan, S.; Ng, C.C.W.; Fang, E.F.; Wang, H.; et al. Mushroom extracts and compounds with suppressive action on breast cancer: Evidence from studies using cultured cancer cells, tumor-bearing animals, and clinical trials. Appl. Microbiol. Biotechnol. 2020, 104, 4675–4703.
11. Ricciardi, M.R.; Licchetta, R.; Mirabilii, S.; Scarpari, M.; Parroni, A.; Fabbri, A.A.; Cescutti, P.; Reverberi, M.; Fanelli, C.; Tafuri, A.; et al. Preclinical Antileukemia Activity of Tramesan: A Newly Identified Bioactive Fungal Metabolite. Oxidative Med. Cell. Longev. 2017, 2017, 5061639.
12. Wan, J.M.; Sit, W.H.; Yang, X.; Jiang, P.; Wong, L.L. Polysaccharopeptides derived from Coriolus versicolor potentiate t he S-phase specific cytotoxicity of Camptothecin (CPT) on human leukemia HL-60 cells. Chin. Med. 2010, 5, 16.
13. Lessene, G.; Czabotar, P.E.; Colman, P.M. BCL-2 family antagonists for cancer therapy. Nat. Rev. Drug Discov. 2008, 7, 989–1000.
14. Chauhan, P.S.; Kumarasamy, M.; Sosnik, A.; Danino, D. Enhanced Thermostability and Anticancer Activity in Breast Ca ncer Cells of Laccase Immobilized on Pluronic-Stabilized Nanoparticles. ACS Appl. Mater. Interfaces 2019, 11, 39436–39448.
15. Ho, C.Y.; Kim, C.F.; Leung, K.N.; Fung, K.P.; Tse, T.F.; Chan, H.; Lau, C.B. Differential anti-tumor activity of coriolus ver sicolor (Yunzhi) extract through p53- and/or Bcl-2-dependent apoptotic pathway in human breast cancer cells. Cancer Biol. Ther. 2005, 4, 638–644.
16. Pawlikowska, M.; Piotrowski, J.; Jędrzejewski, T.; Kozak, W.; Slominski, A.T.; Brożyna, A.A. Coriolus versicolor-derived protein-bound polysaccharides trigger the caspase-independent cell death pathway in amelanotic but not melanotic me lanoma cells. Phytother. Res. 2020, 34, 173–183.
17. Boudreau, M.W.; Peh, J.; Hergenrother, P.J. Procaspase-3 Overexpression in Cancer: A Paradoxical Observation with Therapeutic Potential. ACS Chem. Biol. 2019, 14, 2335–2348.
18. Harbour, J.W.; Dean, D.C. The Rb/E2F pathway: Expanding roles and emerging paradigms. Genes Dev. 2000, 14, 239 3–2409.
19. Hirahara, N.; Edamatsu, T.; Fujieda, A.; Fujioka, M.; Wada, T.; Tajima, Y. Protein-bound polysaccharide-K induces apopt osis via mitochondria and p38 mitogen-activated protein kinase-dependent pathways in HL-60 promyelomonocytic leuk emia cells. Oncol. Rep. 2013, 30, 99–104.
20. Kim, B.C.; Kim, Y.S.; Lee, J.W.; Seo, J.H.; Ji, E.S.; Lee, H.; Park, Y.I.; Kim, C.J. Protective Effect of Coriolus versicolor Cultivated in Citrus Extract Against Nitric Oxide-Induced Apoptosis in Human Neuroblastoma SK-N-MC Cells. Exp. Neu robiol. 2011, 20, 100–109.
21. Tilborghs, S.; Corthouts, J.; Verhoeven, Y.; Arias, D.; Rolfo, C.; Trinh, X.B.; van Dam, P.A. The role of Nuclear Factor-ka ppa B signaling in human cervical cancer. Crit. Rev. Oncol. Hematol. 2017, 120, 141–150.
22. Tamura, T.; Yanai, H.; Savitsky, D.; Taniguchi, T. The IRF family transcription factors in immunity and oncogenesis. Ann u. Rev. Immunol. 2008, 26, 535–584.
23. Stark, G.R.; Darnell, J.E. The JAK-STAT pathway at twenty. Immunity 2012, 3, 503–514.
24. Schneider, W.M.; Chevillotte, M.D.; Rice, C.M. Interferon-stimulated genes: A complex web of host defenses. Annu. Re v. Immunol. 2014, 32, 513–545.
25. Nees, M.; Geoghegan, J.M.; Hyman, T.; Frank, S.; Miller, L.; Woodworth, C.D. Papillomavirus type 16 oncogenes down regulate expression of interferon-responsive genes and upregulate proliferation-associated and NF-kappaB-responsive

genes in cervical keratinocytes. J. Virol. 2001, 75, 4283–4296.

26. Hong, S.; Mehta, K.P.; Laimins, L.A. Suppression of STAT-1 expression by human papillomaviruses is necessary for diff erentiation-dependent genome amplification and plasmid maintenance. J. Virol. 2011, 85, 9486–9494.
27. Zhou, F.; Chen, J.; Zhao, K.N. Human papillomavirus 16-encoded E7 protein inhibits IFN-γ-mediated MHC class I antig en presentation and CTL-induced lysis by blocking IRF-1 expression in mouse keratinocytes. J. Gen. Virol. 2013, 94, 2 504–2514.
28. James, C.D.; Fontan, C.T.; Otoa, R.; Das, D.; Prabhakar, A.T.; Wang, X.; Bristol, M.L.; Morgan, I.M. Human Papillomavi rus 16 E6 and E7 Synergistically Repress Innate Immune Gene Transcription. Msphere 2020, 5, e00828-19.
29. Hsieh, T.C.; Wu, J.M. Cell growth and gene modulatory activities of Yunzhi (Windsor Wunxi) from mushroom Trametes versicolor in androgen-dependent and androgen-insensitive human prostate cancer cells. Int. J. Oncol. 2001, 18, 81–8 8.
30. Lim, B.O. Coriolus versicolor suppresses inflammatory bowel disease by Inhibiting the expression of STAT1 and STAT6 associated with IFN-γ and IL-4 expression. Phytother. Res. 2011, 25, 1257–1261.
31. Chan, S.L.; Yeung, J.H. Polysaccharide peptides from COV-1 strain of Coriolus versicolor induce hyperalgesia via infla mmatory mediator release in the mouse. Life Sci. 2006, 78, 2463–2470.
32. Yang, S.F.; Zhuang, T.F.; Si, Y.M.; Qi, K.Y.; Zhao, J. Coriolus versicolor mushroom polysaccharides exert immunoregula tory effects on mouse B cells via membrane Ig and TLR-4 to activate the MAPK and NF-κB signaling pathways. Mol. Im munol. 2015, 64, 144–151.
33. Jedrzejewski, T.; Piotrowski, J.; Kowalczewska, M.; Wrotek, S.; Kozak, W. Polysaccharide peptide from Coriolus versic olor induces interleukin 6-related extension of endotoxin fever in rats. Int. J. Hyperth. 2015, 31, 626–634.
34. Hsieh, T.C.; Wu, P.; Park, S.; Wu, J.M. Induction of cell cycle changes and modulation of apoptogenic/anti-apoptotic an d extracellular signaling regulatory protein expression by water extracts of I'm-Yunity (PSP). BMC Complement. Altern. Med. 2006, 6, 30.
35. Ofodile, O.N. Disifin (sodium tosylchloramide) and Toll-like receptors (TLRs): Evolving importance in health and diseas es. J. Ind. Microbiol. Biotechnol. 2007, 34, 751–762.
36. Price, L.A.; Wenner, C.A.; Sloper, D.T.; Slaton, J.W.; Novack, J.P. Role for toll-like receptor 4 in TNF-alpha secretion by murine macrophages in response to polysaccharide Krestin, a Trametes versicolor mushroom extract. Fitoterapia 2010, 81, 914–919.
37. Jędrzejewski, T.; Sobocińska, J.; Pawlikowska, M.; Dzialuk, A.; Wrotek, S. Dual Effect of the Extract from the Fungus C oriolus versicolor on Lipopolysaccharide-Induced Cytokine Production in RAW 264.7 Macrophages Depending on the L ipopolysaccharide Concentration. J. Inflamm. Res. 2022, 15, 3599–3611.
38. Li, J.; Li, J.; Aipire, A.; Luo, J.; Yuan, P.; Zhang, F. The combination of Pleurotus ferulae water extract and CpG-ODN en hances the immune responses and antitumor efficacy of HPV peptides pulsed dendritic cell-based vaccine. Vaccine 20 16, 34, 3568–3575.
39. Nesuchlová, M.; Nováková, E.; Kompaníková, J. Imunológia–ako Pracuje Imunitný Systém, Martin; K-print: Bratislava, Slovakia, 2017; ISBN 978-80-8187-031-6.
40. Buc, M. Základná a Klinická Imunológia; Veda: Bratislava, Slovakia, 2012; ISBN 978-80-224-1235-3.
41. Criscuolo, A.A.; Sesti, F.; Piccione, E.; Mancino, P.; Belloni, E.; Gullo, C.; Ciotti, M. Therapeutic Efficacy of a Coriolus ve rsicolor-Based Vaginal Gel in Women with Cervical Uterine High-Risk HPV Infection: A Retrospective Observational Stu dy. Adv. Ther. 2021, 38, 1202–1211.
42. Serrano, L.; López, A.C.; González, S.P.; Palacios, S.; Dexeus, D.; Centeno-Mediavilla, C.; Coronado, P.; de la Fuente, J.; López, J.A.; Vanrell, C.; et al. Efficacy of a Coriolus versicolor-Based Vaginal Gel in Women With Human Papillomav irus-Dependent Cervical Lesions: The PALOMA Study. J. Low. Genit. Tract Dis. 2021, 25, 130–136.
43. Donatini, B. Control of oral human papillomavirus (HPV) by medicinal mushrooms, Trametes versicolor and Ganoderm a lucidum: A preliminary clinical trial. Int. J. Med. Mushrooms. 2014, 16, 497–498.
44. Tosi, M.F. Innate immune responses to infection. J. Allergy Clin. Immunol. 2005, 116, 241–249.
45. Cannella, F.; Pierangeli, A.; Scagnolari, C.; Cacciotti, G.; Tranquilli, G.; Stentella, P.; Recine, N.; Antonelli, G. TLR9 is e xpressed in human papillomavirus-positive cervical cells and is overexpressed in persistent infections. Immunobiology 2015, 220, 363–368.
46. Hasan, U. Humanpapillomavirus (HPV) deregulation of Toll-like receptor 9. Immunobiology 2014, 3, 363–368.
47. Liu, F.; Ooi, V.E.; Liu, W.K.; Chang, S.T. Immunomodulation and antitumor activity of polysaccharide-protein complex fr om the culture filtrates of a local edible mushroom, Tricholoma lobayense. Gen. Pharmacol. 1996, 27, 621–624.

48. Ghosh, S.; Khatua, S.; Acharya, K. Crude polysaccharide from a wild mushroom enhances immune response in murine macrophage cells by TLR/NF-κB pathway. J. Pharm. Pharmacol. 2019, 71, 1311–1323.
49. Daud, I.I.; Scott, M.E.; Ma, Y.; Shiboski, S.; Farhat, S.; Moscicki, A.B. Association between toll-like receptor expression and human papillomavirus type 16 persistence. Int. J. Cancer 2011, 128, 879–886.
50. Di Tucci, C.; Schiavi, M.C.; Faiano, P.; D'Oria, O.; Prata, G.; Sciuga, V.; Giannini, A.; Palaia, I.; Muzii, L.; Benedetti Panici, P.; et al. Therapeutic vaccines and immune checkpoints inhibition options for gynecological cancers. Crit. Rev. Oncol. Hematol. 2018, 128, 30–42.
51. Fu, C.; Zhou, L.; Mi, Q.S.; Jiang, A. DC-Based Vaccines for Cancer Immunotherapy. Vaccines 2020, 8, 706.
52. Li, J.; Aipire, A.; Zhao, H.; Yuan, P.; Li, J. Pleurotus ferulae polysaccharides improve the antitumor efficacy of therapeutic human papillomavirus dendritic cell-based vaccine. Hum. Vaccines Immunother. 2019, 15, 611–619.
53. Roopngam, P.E. Increased Response of Human T-Lymphocytes by Dendritic Cells Pulsed with HPV16E7 and Pleurotus sajor-caju-β-glucan (PBG). Iran. J. Immunol. 2018, 15, 246–255.