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Extracts of Medicinal Mushrooms *Agaricus bisporus* and *Phellinus linteus* Induce Proapoptotic Effects in the Human Leukemia Cell Line K562

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ABSTRACT: We have studied the effects of *Agaricus bisporus* and *Phellinus linteus* ethanol extracts on transcriptional regulation of genes involved in cytokine release and apoptosis in the human leukemia cell line K562. In particular, we applied quantitative real-time PCR (Q-PCR) assays to monitor alterations of gene expression for the following genes: Bcl-2, Casp-9, NF-κB, TNF-α, IFN-γ, and IL-10. We found stronger proapoptotic activity for the *Ph. linteus* alcohol extract on K562 cells than for the *A. bisporus* extract: 4.4- and 2.2-fold increase of Bcl-2 and Casp-9 transcripts. Mushroom alcohol extracts are suggested to exert their effects on tumor cells via the induction of apoptosis. K562 leukemia cells were shown to be most responsive to the transcriptional induction of tumor necrosis factor TNF-α when stimulated with IFN-γ and then treated with *Ph. linteus* alcohol extract: up to a 4.5-fold increase. Treatment of K562 cells with *A. bisporus* extract promoted transcription of the cytokine gene IL-10.

KEY WORDS: medicinal mushrooms, *Agaricus bisporus*, *Phellinus linteus,* mushroom ethanol extracts, proapoptotic and anticancer activities

I. INTRODUCTION

The preventive and therapeutic effects of medicinal mushrooms *Agaricus bisporus* (J. Lge) Imbach (Agaricaceae, Agaricomycetideae) and *Phellinus linteus* (Berk. et M.A. Curt.) Teng (Hymenochaetaceae, Aphyllophoromycetideae) and their components have been shown to be associated with immunomodulatory effects.[1,2] This involves proinflammatory activity or adjuvant effects, as well as apoptosis, mostly with the involvement of chemokines as mediators, produced by the cells of the immune system. Mushroom extracts contain several biologically or medicinally active components. The influence of mushroom extracts on oxygen metabolism, both *in vitro* and *in vivo*, may be a

ABBREVIATIONS

Bcl-2: B-cell lymphoma 2; **BSA:** bovine serum albumin; **Casp-9:** caspase 9; **CML:** chronic myeloid leukemia; **DEAE:** diethyl diamino ethyl; **FCS:** fetal calf serum; **HMW:** high molecular weight; **IFN-γ:** interferon gamma; **IL-10:** interleukin 10; **K562:** myelogenous erythroid leukemia cell line; **NF-κB:** nuclear factor-kappa B; **Q-PCR:** quantitative real-time polymerase chain reaction; **RT-PCR:** reverse transcription polymerase chain reaction; **ROS:** reactive oxygen species; **RPMI 1640:** Roswell Park Memorial Institute cell culture medium 1640; **TNF-α:** tumor necrosis factor alpha; **TNF-R:** tumor necrosis factor receptor.

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common denominator. Redox reactions strongly influence the majority of physiological processes. Several glucans of higher Basidiomycetes were found to have a direct antioxidant effect *in vitro*.[3,4] *Agaricus bisporus*, which contains high amounts of antioxidants, has been reported to additionally harbor a pro-oxidative phenolic compound, that is, the 4-(hydroxymethyl)-phenyl radical. Phenolic compounds generally have anti- and/or pro-oxidative properties, depending on the oxidative status of their OH groups. Some are therefore able to activate the intracellular formation of reactive oxygen species (ROS). The most fundamental role of ROS might be signal transduction and regulation of diverse processes, including phagocyte activation, cell proliferation and migration, and apoptosis (or programmed cell death). Molecular damage caused by ROS in normal cells challenges the cells to repair the damage. Abnormal or affected cells are activated towards the cell death program, that is, to either apoptosis or autophagous cell death. Tumor cell lines appear particularly sensitive to ROS-induced apoptosis. In fact, antitumor agents exhibit their activity via ROS-dependent activation of apoptotic cell death.[5]

We demonstrated that partially purified polysaccharide extracts of *A. bisporus* and *Ph. linteus* are highly active in ROS generation.[6] When hot water–extracted polysaccharides of *A. bisporus* were subjected to DEAE cellulose adsorption chromatography, a nonadsorbed, colorless, high molecular weight (HMW) 1,4,α-glucan was obtained that was unable to generate ROS, suggesting the inability of some glucans to generate ROS.[7] The DEAE-adsorbed fractions that were eluted by salt gradient and consisted of a hazel-colored polysaccharide-polyphenol complex were very active in ROS generation. This suggests that the *A. bisporus* glucan needs to be associated with polyphenolic compounds to become an active ROS generator.

To study whether mushroom polyphenols have a direct effect on cell growth and metabolism, we applied Q-PCR assays to monitor alterations of gene expression in the human leukemia cell line K562. The objective of the research was to analyze possible effects of *A. bisporus* and *Ph. linteus* ethanol extracts on the transcriptional regulation of genes involved in cytokine release and apoptosis.

II. MATERIALS AND METHODS

A. Chemicals

RPM1640 with L-glutamine and sodium bicarbonate was from Sigma Chemical Corp. (St. Louis, MO, USA), serum supreme and fetal bovine serum (South American origin) was from BioWhittaker (Walkersville, MD, USA), the Trizol agent was from Life Technologies (Rockville, MD, USA), and the DNase I Amp Grade and Superscript II reverse transcriptase were both from Invitrogen (Carlsbad, CA, USA).

B. Cancer Cells

The human erythroid leukemia cell line K562 was cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (fcs) and 100 U/mL^{-1} penicillin + 100 μg/mL^{-1} streptomycin and maintained in a humidified incubator with 5% CO_2 at 37°C. Cell viability was assessed by the trypan blue exclusion test.

Acridine orange/ethidium bromide staining (AO/EB) was used to visualize the induction of apoptotic bodies and was performed on live cells in cultivation medium by the addition of an equal volume of a mixture of 10 μg/mL^{-1} acridine orange and 1 μg/mL^{-1} ethidium bromide in phosphate buffered saline (PBS). The cells were then immediately observed in a Zeiss Axioscope fluorescence microscope (Carl Zeiss AG, Oberkochen, Germany) using a 40 × Neofluar objective.

C. Mushroom Extracts and Treatment

Fresh fruiting body tissue of cultivated *Agaricus bisporus* (Sylvan, strain A15) was obtained from Innerlife B.V. (Venlo, The Netherlands). Dry powdered wild-type *Phellinus linteus* was kindly provided by Amazing Grace Ltd. (Thailand). Water extracts were prepared as described previously.[6] Ethanol extracts were prepared as follows: 10 g of lyophilized or dry powdered fruiting body were homogenized to a fine powder in a mortar and then suspended in 100 mL of 96% ethanol. The

mixture was stirred at 70°C for 2 hours and then filtered and centrifuged to remove solid materials. The supernatant was concentrated to a small volume in a Buchi rotary evaporator at 45°C. The concentrate was diluted to a clear 1% (w/v) solution in absolute ethanol.

1. Cell Stimulation and Treatment

K562 cells, at a final concentration of 0.5×10^6 cells/mL^{-1}, were stimulated with 100 ng/mL^{-1} IFN-γ for 20 hours and then incubated (treated) with mushroom ethanol extracts for another 4 hours in 6- and 12-well culture plates. Various doses of mushroom ethanol extracts (1, 5, and 10 μg/mL^{-1}) and 200 μg/mL^{-1} of hot water extract were tested. Untreated and unstimulated control cells were examined at corresponding times.

D. RNA Isolation and cDNA Synthesis

Total RNA was extracted from K562 cells with Trizol according to the manufacturer's protocol. All RNA extractions were treated with 1 μL of DNase I Amp Grade for 15 min at room temperature and then heated for 10 min at 65°C to remove any traces of genomic DNA contamination. RNA concentration and purity (260/280/230 ratio) were measured by the NanoDrop (NanoDropTecnologies, Thermo Fisher Scientific Inc., Waltham, MA, USA), achieving a mean concentration of 191 ng/μL^{-1}, a 260/280 ratio of 2.03, and a 260/230 ratio of 1.89.

One microgram of total RNA was converted into cDNA using the Superscript II reverse transcriptase. For each reaction, 4 μL 5× first-strand buffer (50 mM Tris–HCl, pH 8.3, 375 mM KCl, 15 mM $MgCl_2$), 2 mL of 0.1 M dTT, 5 U RNAsin, 500 μM dNTP mix, 200 pmol Oligo-dT, 25 U Superscript II reverse transcriptase, and MQ-water were added to the RNA to a final volume of 20 μL. This reaction was then incubated at 42°C for 1 h. The reaction was carried out for 5 min at 25°C, followed by 30 s at 42°C, and was terminated at 85°C for 5 min. The finished cDNA products were stored in aliquots at –80°C until needed.

E. Primer Design and Real-Time Q-PCR Assays

Quantitative real-time polymerase chain reaction (Q-PCR) was used for monitoring alterations of gene expression in K562 cells stimulated with mushroom ethanol extracts. Q-PCR enables a sensitive and accurate quantification of mRNA transcription levels. Selection of a housekeeping (reference) gene as an internal control is of crucial importance for data interpretation. The β-actin gene, *Actb*, was selected as a housekeeping gene from those most commonly used in the literature.[8] The expression of six genes involved in cell immune responses and apoptosis regulation (Bcl-2, Casp-9, NF-κB, TNF-α, IFN-γ, and IL-10) was evaluated. Specific cDNA sequences were retrieved from the GenBank sequence database of the National Center for Biotechnology Information (www.ncbi.nih.gov), and primers were designed by using BEACON software. Criteria for primer selection were as described in detail by Giguere and Prescott.[9] Briefly, primers were designed to span the exon-exon boundary to eliminate the possible influence of the contamination of genomic DNA. Primers were constructed to have a length of 18–25 bp, 40–60% GC content, and to produce amplicons 150–220 bp in length.

The Q-PCR was performed using SYBR Green fluorescence and a MJQ BioRad iCycler (USA). After an initial step at 95°C for 10 min, amplification was performed with 40 cycles denaturing at 95°C for 30 s, annealing at 60°C for 40 s, and extension at 72°C for 40 s. Amplification was followed by a melting curve analysis to confirm PCR product specificity. A melting curve analysis was performed after the final amplification cycle via a temperature gradient from 60°C to 95°C. A nontemplate control (NTC) was run with each assay, and all determinations were performed at least in duplicate to achieve reproducibility.

During the protocol optimization, all Q-PCR amplified products were loaded on 1.6% agarose gel using the appropriate DNA ladder to confirm appropriate fragment sizes and lack of primer dimers. No signals were detected in nontemplate controls.

Calibration curves were generated using relative concentration versus the threshold cycle (C_t). Raw data were analyzed using the $2^{-\Delta\Delta Ct}$ method,

also known as the comparative threshold cycle (C_t) or ΔΔCt method.[10] The relative gene expression was quantified based on the results obtained for β-actin as the endogenous control (the housekeeping gene).

III. RESULTS

A. Design and Quality Assessment of Q-PCR Protocol and Q-PCR Efficiency

We evaluated the expression of Bcl-2, Casp-9, NF-κB, TNF-α, IFN-γ, and IL-10 genes in K562 leukemia cells under the following conditions: (1) cells stimulated with mushroom ethanol extracts; (2) cells stimulated with ethanol extracts and preactivated (preincubated) with interferon-γ (IFN-γ); (3) cells stimulated with hot water extracts. First, we analyzed gene expression at different time points of stimulation/treatment in the time-course experiment (data not shown) in order to choose the optimal incubation time. As a result, cells stimulated at 4-h time points were subjected to comparative analysis of mRNA transcriptional level in Q-PCR experiments.

Then, we tested various doses of *A. bisporus* ethanol extract on gene expression in order to choose the optimal concentration of mushroom extracts for the induction of apoptosis and therefore applicable for the analysis (Fig. 1). In the dose-response study,

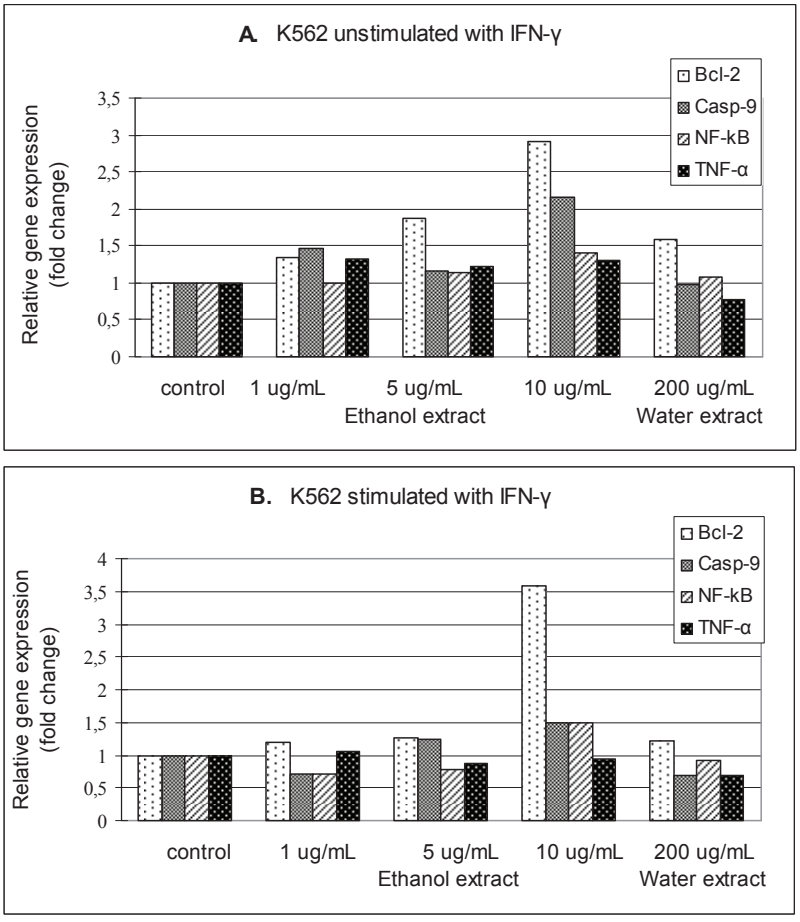

FIGURE 1. Effect of various doses of *Agaricus bisporus* extracts on gene expression in leukemia cell line K562. (**A**) Cells were treated with mushroom extracts (without IFN-γ stimulation). (**B**) Cells were stimulated with IFN-γ for 20 h and then treated with mushroom extracts. Control—untreated K562 cells without stimulation with IFN-γ. The optimal concentration of ethanol extract is 10 μg/mL^{-1}.

K562 cells were initially treated using 1, 5, and 10 μg/mL^{-1} of *A. bisporus* alcohol extract. Our results suggested that activation of gene transcription was maximal at 10 μg/mL^{-1} of *A. bisporus* ethanol extract treatment.

We also investigated the IFN-γ synergetic effect on the modulation of gene transcription in tumor cells treated with mushroom ethanol extracts. K562 cells were activated (preincubated) with IFN-γ at the concentration 100 ng/mL^{-1} for 20 h and then stimulated with mushroom extracts for another 4 h, followed by Q-PCR analysis of mRNA transcripts of genes involved in cytokine release and apoptosis (Bcl-2, Casp-9, NF-κB, TNF-α, IFN-γ, and IL-10). At time-point zero (prior to IFN-γ activation) and after the 20-h preincubation with IFN-γ and subsequent 4-h stimulation by mushroom ethanol extracts, the total RNA was isolated, reverse transcribed, and gene expression was quantitated by real-time Q-PCR. There were no apparent differences in mRNA transcription of the reference gene, β-actin, between IFN-γ-stimulated cells, mushroom extract-stimulated, and nonstimulated cells, indicating constitutive expression and the reliability of the reference housekeeping gene used in the experiment.

Therefore, the concentrations of 10 μg/mL^{-1} of ethanol extract and 200 μg/mL^{-1} of hot water extract and 20-h stimulation with 100 ng/mL^{-1} IFN-γ followed by 4-h incubation with mushroom extracts were chosen as the optimal experimental conditions for the subsequent investigation.

Agarose gel electrophoresis of PCR products during initial optimization experiments was performed to reveal single bands corresponding to amplified genes for all primer sets tested (not shown). Moreover, melting curve analysis was performed after each Q-PCR run. This demonstrated a single homogenous melting peak, confirming highly specific amplification.

B. Gene Expression Analysis in K562 Cells Treated with Ethanol Extracts

No significant differences in the level of nuclear factor-kappaB (NF-κB) and tumor necrosis factor-alpha (TNF-α) transcripts stimulated with *A. bisporus* alcohol extract were noticed between K562 cells left untreated or treated for 20 hours with IFN-γ (Fig. 1). Transcription of these genes remained at control values or was downregulated. The Bcl-2 gene transcription underwent positive regulation under the increased concentration of *A. bisporus* alcohol extract (at concentration 10 μg/mL^{-1}): more than 3.5-fold increase in IFN-γ-stimulated K562 cells and 2.8-fold increase in cells left nonstimulated. IFN-γ is shown to have immunoregulatory, antiviral, and anticancer properties. It alters transcription in up to 30 genes, producing a variety of physiological and cellular responses, including inhibition of cellular proliferation and effects on apoptosis.[11]

As another biomarker of apoptosis induction, caspase-9 activity was also slightly increased (up to 2.2.-fold) in K562 cells stimulated with *A. bisporus* alcohol extract without IFN-γ pretreatment, indicating a proapoptotic effect of the mushroom extract in K562 cells (Fig. 1A). Caspase-9 is known to be activated during programmed cell death, or apoptosis, and to be linked to the mitochondrial death pathway.[12]

Agaricus bisporus hot water extract (at concentration 200 μg/mL^{-1}) did not perform much activity either in cells stimulated with IFN-γ or in unstimulated cells: only the transcription of the Bcl-2 gene was slightly induced (1.6-fold increase), whereas the other tested genes were downregulated, including the proapoptotic gene Casp-9 (Fig. 1). This result suggested that the hot water extract of *A. bisporus* is more likely to have antiapoptotic activity and protects K562 cells against apoptosis.

In the subsequent set of experiments, K562 cells were stimulated with ethanol extracts of *A. bisporus* and *Ph. linteus* (at concentration 10 μg/mL^{-1}). The expression of six genes involved in cell immune responses and apoptosis regulation (Bcl-2, Casp-9, NF-κB, TNF-α, IFN-γ, and IL-10) was evaluated. The gene transcriptional profiles differed in K562 cells treated with *Ph. linteus* or *A. bisporus* ethanol extracts (Fig. 2). The *Ph. linteus* alcohol extract demonstrated stronger activity: an approximately 4.4-fold increase of Bcl-2 gene transcription and a 2.1-fold growth of Casp-9 in treated K562 cells. Bcl-2 family members are known to play a major role in apoptosis regulation, and they are responsive

to many cytotoxic stimuli, including limited cytokine levels, drugs, etc.[12,13]

When the K562 cells were first stimulated with IFN-γ, followed by *Ph. linteus* alcohol extract treatment, transcription of the inflammatory cytokine TNF-α was elevated up to 4.5-fold, suggesting a quite strong proinflammatory effect of the *Ph. linteus* extract on K562 cells (Fig. 2). The high expression of TNF-α transcripts stimulated by IFN-γ treatment may also indicate a proapoptotic effect of TNF-α on K562 cells. One of the possible mechanisms is that IFN-γ could induce sensitivity of the cells to the proapoptotic effects of TNF-α by promoting the surface expression of a TNF-α receptor on tumor cells.[11]

The transcription of proapoptotic genes (Bcl-2, Casp-9, and NF-κB) in K562 cells treated with *A. bisporus* ethanol extract was not much affected by IFN-γ stimulation (Fig. 2). The transcription of TNF-α and Bcl-2 genes was even higher in cells without IFN-γ preincubation: 3.2- and 2.3-fold increase, respectively, whereas the mRNA transcripts of Casp-9 and NF-κB genes were increased less than 1.5-fold (Fig. 2). Upregulation of Bcl-2 and Casp-9 gene expression indicates a proapoptotic effect of *A. bisporus* alcohol extract. Interestingly, transcription of NF-κB was higher in cells stimulated with *Ph. linteus* alcohol extract without preincubation with IFN-γ (1.7-fold increase), suggesting a positive transcriptional regulation in the K562 leukemia cells in response to treatment by the mushroom alcohol extract only.

The interleukin-10 (IL-10) gene was slightly upregulated by *A. bisporus* ethanol extract in K562 cells. The IL-10 mRNA transcription level was increased 1.7-fold in cells activated with IFN-γ for 20 h, whereas the transcription of the IFN-γ gene was always downregulated in the experiment (Fig. 2). In cells treated with the *Ph. linteus* alcohol extract, the transcription of IL-10 and IFN-γ genes was also downregulated.

IL-10 is an anti-inflammatory cytokine that has pleiotropic effect in immune regulation. Interestingly, the lack of IL-10 transcription in K562 cells was always correlated with downregulated IFN-γ transcription. This is not surprising because

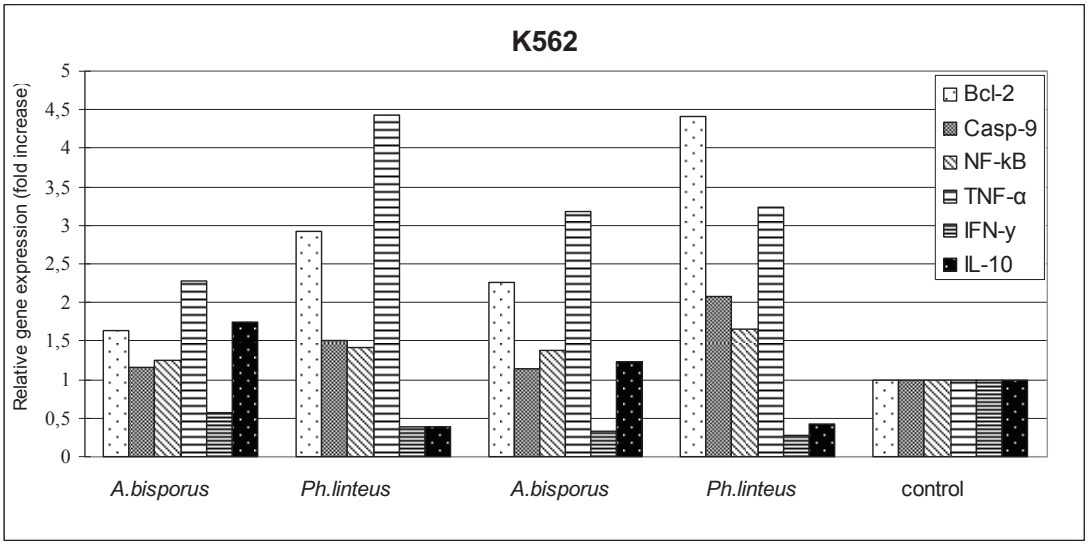

FIGURE 2. Induction of gene expression in leukemia cells K562 stimulated *in vitro* with ethanol extracts of *Agaricus bisporus* and *Phellinus linteus*. mRNA transcripts were quantified without stimulation and after 20 h of IFN-γ stimulation followed by 4-h treatment with 10 μg/mL^{-1} ethanol extracts. For each sample, one cDNA was prepared (Q-PCR assay for each gene was performed in duplicate).

IL-10 is able to inhibit proinflammatory cytokines (including IFN-γ),[10] and, vice versa, IFN-γ itself can specifically downregulate the cytokine IL-10 transcription.[14]

IV. DISCUSSION

The K562 cell line, derived from a chronic myeloid leukemia (CML) patient, is known to be particularly resistant to apoptotic death.[15] However, analysis of gene expression in Q-PCR experiments has demonstrated that K562 cells that were preactivated with IFN-γ for 20 h can be triggered to apoptosis (or at least become more responsive to apoptosis) by a 4-h mushroom alcohol extract treatment.

Apoptosis, or programmed cell death, is a form of cell deletion/elimination aimed at the control of cell differentiation and proliferation that can be triggered by either intracellular or extracellular signals, followed by activation of a group of enzymes called caspases.[16] Caspases work like a cascade pathway by targeting and cleaving key cellular structures and thus promoting cell decomposition.[17]

Apoptosis can be considered as a promising mechanism to fight tumor cells. That is why it was of interest to evaluate the variety of genes involved in the programmed cell death. For example, TNF-α, when it binds to the TNF-R1 receptor, can act as an extracellular trigger for apoptosis.[18] TNF is shown to be a major mediator of apoptosis as well as of inflammation and immunity. TNF-α is a multifunctional cytokine that stimulates the acute-phase reaction in the inflammatory process[19] and can cause apoptotic cell death and activation of the NF-κB transcriptional factor.[20] The central role of TNF in inflammation has been demonstrated by the ability of agents that block the activity of TNF to treat a range of inflammatory conditions, including rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis.[21] TNF-α can deliver both proapoptotic and antiapoptotic signals to the cells. For example, interaction of TNF-α with its membrane receptor TNF-R1 can activate the NF-κB pathway that, in turn, leads to death receptor-mediated apoptosis.[18] However, some other factors, such as the amount of reactive oxygen species (ROS), can shift the intercellular balance in favor of the proinflammatory or proapoptotic pathway.

The considerably high transcription of TNF-α and Bcl-2 genes stimulated by alcohol extracts of *A. bisporus* and *Ph. linteus* in our experiments indicates their proapoptotic effect on K562 leukemia cells (Fig. 2). The highest level of Bcl-2 transcripts (4.4-fold increase) was observed in cells treated with the *Ph. linteus* extract, which indicates its stronger effect on apoptosis induction as compared to the *A. bisporus* ethanol extract. Morphologically, no obvious signs of increased apoptotic activity were observed after 4 h of incubation, when live stimulated K562 cells were stained with ethidium bromide and immediately visualized microscopically. After overnight incubation with ethanol extracts, the situation was quite different: most IFN-γ-stimulated and ethanol extract–treated K562 cells had died, mostly through apoptosis, leaving apoptotic cells and granular nuclear remnants in the culture. The apoptotic cells and the nuclear remnants incorporated ethidium bromide and showed bright orange fluorescence under the fluorescence microscope.

The increased level of TNF-α transcripts observed in cells preincubated with IFN-γ also proves the proapoptotic effect of IFN-γ stimulation. This trend was clearly observed in cells treated with the *Ph. linteus* alcohol extract: in cells preincubated with IFN-γ, the transcription of Bcl-2 was decreased, whereas the transcription of TNF-α was elevated up to 4.5-fold as compared to cells without IFN-γ stimulation. Therefore, it is suggested that IFN-γ stimulation induces the sensitivity of K562 leukemia cells to the proapoptotic action of TNF-α. Many reports indicate that IFN-γ can arrest the cell cycle and provide a proapoptotic signal.[11,22]

In our experiment, NF-κB transcription was slightly upregulated in K562 cells treated with *A. bisporus* and *Ph. linteus* alcohol extracts (from 1.2- to 1.7-fold increase), the gene transcription being not much affected by IFN-γ activation (Fig. 2). NF-κB is a universal transcriptional factor that regulates the expression of a large number of genes involved in immune-inflammatory responses, cell cycle progression, and inhibition of apoptosis.[23] The NF-κB transcriptional factor can be activated in response to various stimuli, including cytokines,

viral infection, oncoproteins, membrane receptor's overexpression, and other stressful conditions (e.g., ROS). All these processes require rapid reprogramming of gene expression.

As shown in a number of research studies, NF-κB is constitutively expressed in some tumor cells.[24] In our experiment, the level of NF-κB transcripts was slightly decreased in K562 cells preactivated with IFN-γ, as compared to the cells stimulated with mushroom ethanol extracts only: from 1.6- to 1.4-fold for *Ph. linteus* and from 1.4- to 1.2-fold for the *A. bisporus* extract (Fig. 2). This might suggest the potent antitumor activity of mushroom ethanol extracts applied in combination with IFN-γ stimulation because the repression of NF-κB gene transcription is believed to stop the proliferation of tumor cells. Therefore, it might be suggested that the mushroom ethanol extracts are able to inhibit/repress NF-κB transcription and to block activation of the NF-κB signaling pathway. For example, many natural products, including antioxidants that were suggested to have anticancer and anti-inflammatory activity, have also been shown to inhibit the transcriptional factor NF-κB.[25] Several recent studies on medicinal mushrooms (*Ganoderma lucidum, Pleurotus* spp., *Schizophyllum commune, Marasmius oreades, Trametes versicolor*, etc.) also indicate that fungal low-molecular-weight metabolites are able to modulate the activity of NF-κB.[26]

In conclusion, we demonstrated a suppressive effect/activity of *Ph. linteus* and *A. bisporus* alcohol extracts on erythroid leukemia cell line K562 (preactivated with IFN-γ). Mushroom alcohol extracts are suggested to exert their anticancer effect via the induction of apoptosis. The *Ph. linteus* alcohol extract demonstrated stronger proapoptotic activity on K562 leukemia cells than the alcohol extract of *A. bisporus*. Therefore, even tumor cells generally resistant to programmed cell death, such as K562, can be triggered to apoptosis by treatment with *Ph. linteus* alcohol extract. The K562 cells were more responsive to transcriptional induction of proapoptotic genes (particularly, Bcl-2 and TNF-α) when stimulated with IFN-γ. Treatment of K562 cells with *A. bisporus* extract selectively promoted transcription of the cytokine gene IL-10.

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