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EVALUATION OF THE EXPRESSION LEVEL OF TOLL-LIKE RECEPTORS TYPE 4 IN TUMOR CELLS EXPOSED TO THE CYTOKININ EXTRACTS FROM THE MEDICINAL FUNGI

Background/Aim. Medicinal fungi demonstrate significant cytotoxic, antiproliferative, and immunomodulatory effects against various types of cancer due to the numerous bioactive components, including cytokinin plant growth regulators. Biological mechanism of these effects seems to be associated with activation of the immune system, the crucial proteins of which are toll-like receptors (TLR). The objective of our study was to elucidate the influence of cytokinin extracts from medicinal fungi on the TLR expression in vitro. **Methods.** Total RNA was isolated by phenol-chloroform extraction and measured by ThermoScientific NanoDrop-1000. cDNA was obtained from total RNA by RT-PCR using the "High Capacity cDNA Reverse Transcription Kit". The expression level of TLR-4 was evaluated by 7500 Real-Time PCR Systems using specific primers and SYBR Green fluorochrome. Cytokinin extracts were purified by ion-exchange and thin layer chromatography and analyzed by the HPLC-MS system Agilent 1200. The expression level of TLR-4 was determined in HepG2 (human hepatocarcinoma) and MT-4 (human T-cell leukemia) cells. **Results.** Mycelium of medicinal fungi from the IBK Mushroom Culture Collection was obtained by cultivation in liquid nutrient medium. The total cytokinin content in mycelial biomass was detected in the range $14.49 \pm 0.49 - 70.22 \pm 2.32$ nmol/g DW. Purified cytokinin fractions were added to the incubation medium of Hep-G2 and MT-4. Cytokinin fractions isolated from the mycelium of *Pleurotus nebrodensis*, *Pleurotus osteratus* and *Sparassis crispa* stimulated the expression of TLR-4 in cells of both lymphocytic and hepatocytic origin. Simultaneously, cytokinin fractions from the mycelium of *Morchella esculenta* increased the level of TLR-4 expression in MT-4 cells by 1.5 times, whereas in HepG2 cells their effect was almost similar to the control. Both crude extracts and cytokinin fractions isolated from *Flammulina velutipes*, *Cyclocybe aegerita* and *Herichium erinaceus* induced a change in the expression of pro-inflammatory cytokines in MT-4 cells. The effect of purified fractions on the level of TGF β (transforming growth factor β) expression compared to crude extracts was significantly greater. **Conclusions.** The effect of purified cytokinin extracts isolated from mycelium was divergent in various cell lines and strongly depended on the fungi species. Their influence on the level of TLR-4 expression suggests the immunomodulatory properties of these biologically active substances in certain cell lines.

Keywords: medicinal fungi, cytokinins, Toll-like receptors.

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