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ORIGINAL ARTICLE

Apoptotic properties of polysaccharide isolated from fruiting bodies of medicinal mushroom *Fomes fomentarius* in human lung carcinoma cell line

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Abstract Mushrooms are known to complement chemotherapy and radiation therapy by countering the side effects of cancer. Recently, there has been great interest in isolation of novel bioactive compounds from mushrooms due to their numerous health beneficial effects. Chemically water-extractable polysaccharide (MFKF-AP1β), with a molecular weight of 12 kDa, was isolated from fruiting bodies of mushroom *Fomes fomentarius*. In this research, we investigated the anti-tumor effects of MFKF-AP1β on human lung carcinoma A549 cells. Results showed that MFKF-AP1β markedly inhibited A549 cell growth in a dose-dependent manner based on the amount of lactate dehydrogenase (LDH) released and morphological alterations. In addition, MFKF-AP1β induced cellular apoptosis by causing single-stranded DNA breakage, as evidenced by apoptosis assay. Furthermore, MFKF-AP1β (25–100 μg/ml) significantly induced single-stranded DNA breakage in A549 cells, as shown by comet assay. Taken together, our results demonstrate that MFKF-AP1β has strong anti-tumor effects mediated through induction of apoptosis. Therefore, MFKF-AP1β could be useful in lung chemotherapy.

1. Introduction

Lung cancer has become the most common cause of cancer-related death in both men and women, accounting for 28% of all cancer-related deaths in the U.S. (Cardenal et al., 1999). Although treatment of lung cancer has improved, mortality remains high in lung cancer patients. A growing body of research suggests that naturally occurring compounds can act as antioxidants as well as cancer preventative and therapeutic agents (Parkin et al., 1999; Rahman et al., 2005; Hashibe et al., 2007). Development of chemotherapeutic agents with maximal

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anti-tumor activity and minimal toxicity has become a favorable route for cancer management. In this context, mushrooms play a pivotal role as anti-tumor agents. Edible mushrooms have been used as health nutritional supplements for several centuries, and they complement chemotherapy and radiation therapy by countering the side effects of cancer (Mizuno et al., 1995).

The bioactive compounds in mushrooms responsible for their anti-tumor potential include polysaccharides, proteins, fats, ash, glycosides, alkaloids, volatile oils, tocopherols, phenolics, flavonoids, carotenoids, folates, ascorbic acid enzymes, and organic acids (Mizuno et al., 1992, 1995; Jong and Birmingham, 1993; Ferreira et al., 2009). Among these bioactive compounds, polysaccharides are the most well-known and most potent mushroom-derived substances with anti-tumor activity (Ferreira et al., 2010). In this study, we analyzed the anti-tumor activity of a polysaccharide (MFKF-AP1β) isolated from fruiting bodies of the medicinal mushroom *Fomes fomentarius* in human lung cancer A549 cells.

F. fomentarius, a basidiomycete fungus, has been used as a traditional Chinese and Korean medicine for many centuries for the treatment of various diseases, including oral ulcers, gastroenteric disorders, hepatocirrhosis, inflammation, and various cancers. Recent studies have shown that *F. fomentarius* has antioxidant, anti-inflammatory, and anti-diabetic activities (Lee, 2005; Park et al., 2004). However, there are few reports on the apoptotic activity of *F. fomentarius* (Ito et al., 1976; Chen et al., 2008).

To discover novel agents from natural products that improve the therapeutic outcome of cancer, we tested the anti-tumor effects of a polysaccharide (MFKF-AP1β) isolated from *F. fomentarius* on A549 lung cancer cells. Specifically, we tested the apoptotic activity of MFKF-AP1β in lung carcinomas.

2. Materials and methods

2.1. Reagents

RPMI 1640, HEPES, staurosporine, Hoechst 33342, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were purchased from Sigma–Aldrich, St. Louis, USA. Fetal bovine serum was supplied by Gibco, USA. All solvents were HPLC grade and supplied by J.T. Baker (Phillipsburg, NJ, USA).

2.2. Materials

Chemically water-extractable polysaccharide (MFKF-AP1β) was isolated from hot water extracts of fruiting bodies of mushroom *F. fomentarius* by successive DEAE-Sepharose FF and concanavalin A-Sepharose 4B column chromatography. Molecular weight of MFKF-AP1β was estimated to be about 12 kDa by high performance liquid chromatography (HPLC) (Park et al., 2013). Solutions of MFKF-AP1β (25–100 μg/ml) were prepared in a water/methanol mixture (1:1, v/v).

2.3. Cell culture

Lung cancer cells A549 (KCLB) were cultivated in RPMI 1640 medium supplemented with 25 mM HEPES buffer, 25 mM sodium bicarbonate, 300 mM L-glutamate, and 10% heat-inactivated fetal bovine serum (Gibco, USA) in a CO_2 incubator (5% CO_2 in air) at 37 °C. For the experiment, cells were cultured in 12-well plates at 1×10^6 cells/well. Cells were treated with several concentrations of MFKF-AP1β (25, 50, and 100 μg/ml) for 24 h. As a positive control, 1 μM staurosporine (Sigma, St. Louis, USA) was added for 4 h to induce apoptosis. Cells were then centrifuged ($600g \times 3$ min) and harvested.

2.4. MTT assay and cell morphology

Cell viability was measured by MTT bioassay. Briefly, A549 cells (1×10^6 cells/well) were seeded in each well of a 96-well plate for 24 h. After incubation with different concentrations of MFKF-AP1β for 24 h, 10 μl of MTT solution (5 mg/ml on PBS) dissolved in PBS was added and incubated for 4 h. After color development, 100 μl of DMSO was applied. Absorbance was measured using an ELISA plate reader (Bio-Tek Instrument Co., WA, USA) at 540 nm. The viability% was measured using formula-

Sample absorbance/Control absorbance × 100.

2.5. Lactate dehydrogenase (LDH) assay

A549 cells (1×10^6 cells/well) were seeded in each well of a 96-well plate for 24 h. After incubation with different concentrations of MFKF-AP1β for 24 h, medium was collected and cleared by centrifugation. After supernatants were collected, cytotoxicity was quantified by measuring the amount of total LDH released by cells using an LDH assay kit (Sigma–Aldrich, St. Louis, USA) according to the manufacturer's protocol. Briefly, LDH, a glycolytic enzyme, is concerned with the reduction of pyruvic acid in the presence of dihydronicotinamide adenine dinucleotide (NADH). LDH assay reagent was added to supernatants and incubated for up to 30 min at room temperature in the dark, after which the reaction was stopped by adding 1 N HCl. The absorbance of samples was measured at 450 nm.

2.6. Assay for nuclear apoptosis (Hoechst staining)

To determine DNA chromatin morphological features, cells were treated with Hoechst 33342 stain according to Diaz-Ruiz et al., 2001. Briefly, cells were cultured and treated for 24 h with different concentrations of MFKF-AP1β and staurosporine (1 μM) for 4 h. After washing twice in PBS, cells were fixed with cold 4% formaldehyde. Cells were then washed with PBS again and incubated with Hoechst 33342 (1 μg/ml) at 37 °C for 10 min. After washing with PBS, cells were analyzed under a fluorescence microscope (Nikon Eclipse TS100 Epi-fluorescence microscope, Japan).

2.7. Comet assay

To evaluate DNA damage, comet assay was performed according to Singh et al. (1988), with some modifications. A549 lung cancer cells treated with MFKF-AP1β and staurosporine were harvested by washing twice with phosphate-buffered saline (PBS) and then suspended in 70 μl of 1% (w/v) low melting point agarose in PBS, pH 7.4, at