Anti-Proliferative Effects of Ginsenosides Extracted from Mountain Ginseng on Lung Cancer

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ABSTRACT Objective: To investigate the effect of the three major ginsenosides from mountain ginseng as anti-cancer substance and explore the underlying mechanism involved in lung cancer. Methods: The inhibitory proliferation of lung cancer by major five ginsenosides (Rb1, Rb2, Rg1, Rc, and Re) was examined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. Calculated 50% inhibition (IC50) values of five ginsenosides were determined and compared each other. Apoptosis by the treatment of single ginsenoside was performed by fluorescence-assisted cytometric spectroscopy. The alterations of apoptosis-related proteins were evaluated by Western blot analysis. Results: The abundance of ginsenosides in butanol extract of mountain ginseng (BX-MG) was revealed in the order of Rb1, Rg1, Rc, Re and Rb2. Among them, Rb1 was the most effective to lung cancer cell, followed by Rb2 and Rg1 on the basis of relative IC50 values of IMR90 versus A549 cell. The alterations of apoptotic proteins were confirmed in lung cancer A549 cells according to the administration of Rb1, Rb2 and Rg1. The expression levels of caspase-3 and caspase-8 were increased upon the treatment of three ginsenosides, however, the levels of caspase-9 and anti-apoptotic protein Bax were not changed. Conclusion: Major ginsenosides such as Rb1, Rb2 and Rg1 comprising BX-MG induced apoptosis in lung cancer cells via extrinsic apoptotic pathway rather than intrinsic mitochondrial pathway.

KEYWORDS Butanol extract of mountain ginseng, ginsenoside, lung cancer, apoptosis

Ginseng is cultivated in Eastern Asian countries such as Korea, China, and Japan. Ginseng is a species of perennial plants which belong to the Panax genus in the Araliaceae. Among eleven distinctive species of Panax genus, Panax ginseng (Asian ginseng) and Panax quinquefolius (American ginseng) are most well-characterized, in which the fresh root contains the highly valuable medicinal components.1 Panax ginseng contains many pharmacologically valuable saponins for the preventive potential against various cancers.2 In particular, the root of mountain ginseng has been used for treating chronic liver disease.3 However, mountain ginseng is rarely found in the deep mountains in Korea and is not well cultivated in laboratory. Recently, an improved culture technique of adventitious root of Panax ginseng has been developed, enabling us to provide a reliable way of commercialization of wild mountain ginseng for utilizing secondary metabolites.4,5 So far, scientific studies to elucidate the medicinal effect of mountain ginseng have not been conducted due to the lack of sample source.

In general, the ginseng species is classified by the major saponin ginsenoside profiles. The steroid saponins include two types of 20(S)-protopanaxadiol (PPD)-type and 20(S)-protopanaxatriol (PPT)-type saponins. The PPD-type saponin Rb1 and PPT-type saponin Rg1 show positive effects on glutamatergic central nervous system by enhancing glutamate release through the activation of protein kinase A in rat cerebral cortex.6 Therefore, individual ginsenoside is considered to have specific effect on the disease. Moreover, the subtypes of ginseng, i.e. Panax quinquefolius, are grouped as two chemotypes based on the relative composition of Rg1 and Re, which are determined by high-performance liquid chromatography (HPLC).7

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In the chemopreventive studies, American ginseng displayed anti-cancer therapeutic potential and enhanced killing effects with antioxidants in colorectal cancer.\(^8,9\) The structure-function relationship in ginsenosides has been extensively studied: Rh2 inhibits human ovarian and breast cancer cells\(^10,11\) and Rg3 exerts anti-proliferative effect on human colorectal cancer.\(^12\) In particular, Rg3 combined with low-dose cyclophosphamide has inhibitory effect against Lewis lung carcinoma.\(^13\) Moreover, anti-lung cancer activity of Rg3 has recently reported in human and animal model system.\(^14,15\)

Recently we reported that the boiled water extract of mountain ginseng, in particular, further butanol extract of mountain ginseng, exhibits the anti-lung cancer activity by inhibiting the nuclear translocation of nuclear factor (NF-κB).\(^16\) Lung cancer, primarily non-small cell lung carcinoma (NSCLC), is a leading cause of cancer death worldwide.\(^17\) According to cancer statistics, the 5-year survival rate of lung cancer patients was reported as only 15% in the United States. The high mortality of lung cancer was due to the delayed diagnosis at the late stage. The optimal therapeutic approaches defected for the disease. Thus, lung cancer is still considered as one of the extremely lethal diseases.\(^18\) To overcome the dilemma of lung cancer, Western medical field has recently tried to apply the alternative therapy using ginseng as herbal medicine. The clinical trial of cultivated wild ginseng pharmacopuncture to advanced lung cancer patients at late stage showed potential curative power for NSCLC.\(^19\) However, the extensive studies of other ginsenosides responsible for anti-lung cancer activity except Rg3 and Rh2 were not performed yet.

The present study was focused on the apoptotic effect of major ginsenosides comprising mountain ginseng on the lung cancer cell. Based on our preliminary study,\(^16\) we examined the anti-lung cancer activity of the prevalent ginsenoside Rb1, Rb2 and Rg1 comprising butanol-extracted Mountain Ginseng (BX-MG). The detailed apoptosis study of the ginsenosides derived from mountain ginseng would provide our understanding of anti-lung cancer therapy and application in medical field.

**METHODS**

**Preparation of Ginseng Extracts**

Mountain wild ginseng was used about 8–10 years old grown at ChonBangNongSan in Chungnam province, Korea. The preparation of BX-MG was described in detail as previously reported.\(^16\) The component ginsenosides comprising BX-MG were individually studied on the apoptotic effects on human lung cancer cell lines.

**Reagents and Chemicals**

Dulbecco’s modified eagles medium (DMEM) and RPMI-1640 were purchased from Sigma (St. Louis, MO, USA). Chemicals such as 4-(2-hydroxyethyl)-L-piperazine ethane sulfonic acid (HEPES), fetal bovine serum (FBS), phosphate buffer saline (PBS), and L-glutamate were obtained from Gibco (Paisley, Scotland). Cell culture dishes were from NUNC (Roskilde, Denmark). Whole cell lysis buffer was used with 10 mmol/L HEPES adjusted at pH 7.9. Ginsenoside Rb1 was purchased from Sigma (St. Louis, MO, USA) and ginsenosides such as Rb2, Rg1, Rc, and Re were purchased from ChromaDex (Irvine, CA, USA). The chemical structures of ginsenosides used in this work were given in Figure 1.

**Cell Culture**

Human alveolar basal epithelial carcinoma (A549), bronchiolalveolar epithelial carcinoma (NCI-H358) and adenosquamous lung carcinoma (NCI-H596) cells were purchased from the American Type Culture Collection (Rockville, MD, USA). Lung cancer cells were grown in RPMI-1640-supplemented with 10% (v/v) FBS (GIBCO, NY, USA), 1% (w/v) penicillin-streptomycin (GIBCO, NY, USA) in a 37 ℃ incubator with 5% (v/v) CO\(_2\) in a humidified atmosphere. Cells were allowed to adhere and grow for 24 h in culture medium prior to exposure to either BX-MG or individual ginsenoside such as Rb1, Rb2 and Rg1.

**Cell Viability Analysis**

The measurement of cell viability was based on the cellular reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma, St. Louis, MO, USA) by mitochondrial enzymes. Briefly, the product of MTT-formazan catalyzed by mitochondrial dehydrogenases can be measured spectrophotometrically.\(^20\) A549 cells were seeded at a density of 5 × 10\(^3\) cells/well in a 96-well plate. After 48 h, adherent cells were treated with either BX-MG or ginsenoside (Rb1, Rb2 and Rg1). To determine the appropriate dose and time that is not cytotoxic