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Research article

Anti-breast cancer activity of Fine Black ginseng (*Panax ginseng* Meyer) and ginsenoside Rg5

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ABSTRACT

Background: Black ginseng (*Ginseng Radix nigra*, BG) refers to the ginseng steamed for nine times and fine roots (hairy roots) of that is called fine black ginseng (FBG). It is known that the content of saponin of FBG is higher than that of BG. Therefore, in this study, we examined antitumor effects against MCF-7 breast cancer cells to target the FBG extract and its main component, ginsenoside Rg5 (Rg5).
Methods: Action mechanism was determined by MTT assay, cell cycle assay and western blot analysis.
Results: The results from MTT assay showed that MCF-7 cell proliferation was inhibited by Rg5 treatment for 24, 48 and 72 h in a dose-dependent manner. Rg5 at different concentrations (0, 25, 50 and 100 μM), induced cell cycle arrest in G0/G1 phase through regulation of cell cycle-related proteins in MCF-7 cells. As shown in the results from western blot analysis, Rg5 increased expression of p53, $p21^{WAF1/CIP1}$ and $p15^{INK4B}$ and decreased expression of Cyclin D1, Cyclin E2 and CDK4. Expression of apoptosis–related proteins including Bax, PARP and Cytochrome c was also regulated by Rg5. These results indicate that Rg5 stimulated cell apoptosis and cell cycle arrest at G0/G1 phase via regulation of cell cycle-associated proteins in MCF-7 cells.
Conclusion: Rg5 promotes breast cancer cell apoptosis in a multi-path manner with higher potency compared to 20(S)-ginsenoside Rg3 (Rg3) in MCF-7 (HER2−/ER+) and MDA-MB-453 (HER2+/ER−) human breast cancer cell lines, and this suggests that Rg5 might be an effective natural new material in improving breast cancer.

1. Introduction

Ginseng (*Panax ginseng* Meyer) is a well characterized medicinal herb listed in the classic oriental herbal dictionary, *Shin-nong-bon-cho-kyung*. Ginseng has a sweet taste, is able to keep the body warm, and has protective effects on the five viscera (i.e., heart, lung, liver, kidney, and spleen) [1]. Ginseng can be classified by how it is processed. Red ginseng (RG; Ginseng Radix Rubra) refers to ginseng that has been steamed once. White ginseng (Ginseng Radix Alba) refers to dried ginseng. Black ginseng (BG; Ginseng Radix Nigra) is produced by repeatedly steaming fresh ginseng nine times. The fine roots (hairy roots or fibrous roots) of fresh ginseng that has been steamed nine times are called Fine Black ginseng (FBG). There are more than 30 different ginseng saponins with various physiological and pharmacological activities [2,3]. Ginsenosides are divided into two groups: protopanaxadiols and protopanaxatriols.

The root of *Panax ginseng* reportedly has various biological effects, including anticarcinogenic effects. One study showed that ginseng extracts induce apoptosis and decrease telomerase activity and cyclooxygenase-2 (COX-2) expression in human leukemia cells [4]. In addition, ginseng extracts suppress 1,2-dimethylhydrazine-induced colon carcinogenesis by inhibiting cell proliferation [5].

Until recently, research on anticancer effects of ginseng has focused on ginsenoside Rg3 (Rg3) and ginsenoside Rh2 (Rh2). Ginsenoside Rg3 is not present in raw ginseng or White ginseng, but is synthesized during heating hydrolysis; thus, only a small amount of Rg3 is present in Red ginseng. Ginsenoside Rg3 has an anticancer effect by suppressing phorbol ester-induced COX-2 expression and decreasing activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [6]. Its blood pressure lowering effects have been identified in rat aorta through enhancing endothelium-dependent relaxation [7]. Ginsenoside Rg3 in methanol extraction of heat-processed ginseng has antioxidative and antitumor effects [8].

Ginsenoside Rh2 is a major active anticancer saponin in ginseng extracts [9]. Ginsenoside Rh2 treatment modulates the protein expression level of p21 and cyclin D, and leads to a marked reduction in the proliferation of MCF-7 human breast cancer cells

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[10]. It also provokes apoptosis through activating p53 and inducing the proapoptotic regulator Bax in colorectal cancer cells [11]. In addition, Rh2 markedly reduces the viability of breast cancer cells (MCF-7 and MDA-MB-231) by arresting the G1 phase cell cycle *via* p15 ^{INK4B} and p27 ^{KIP1}-dependent inhibition of cyclin-dependent kinases [12].

Many studies on BG have been performed because interest in it has increased recently. The main component of BG is reportedly Rg5 (Fig. 1) [13]. Studies demonstrate it has diverse physiological activity such as anti-inflammatory effects on lipopolysaccharide-stimulated BV2 microglial cells [14], protective effects on scopolamine-induced memory deficits in mice [15], and inhibitory effects in a mouse model with oxazolone-induced chronic dermatitis [16]. Rg5 reportedly blocks the cell cycle of SK-HEP-1 cells at the Gl/S transition phase by downregulating cyclin E-dependent kinase activity [17].

Breast cancer is a very common cancer in women worldwide. In the United States, it is estimated that breast cancer is the leading cause of all cancers (29%) and the second leading cause of death (14%) [18]. In Korea, 16,015 new cases of breast cancer were reported in 2011 [19]. Anticancer activity of BG extract in the MCF-1 breast cancer cell line exhibited three-fold cytotoxicity, compared with Red ginseng extract [20]. However, ginseng fine roots contain a higher content of ginseng saponin than ginseng main roots [2]. In the present study, we therefore aimed to investigate anti-breast cancer activity (in the MCF-7 cell line) and the action mechanisms of FBG ethanol extract (EE), FBG butanol fraction (BF; primarily containing saponin), and Rg5 as the major saponin.

2. Materials and methods

2.1. Materials

Fine Black ginseng (*Panax ginseng* Meyer) for experiments was purchased from Kumsan Town, Chungcheongnam Province, the Republic of Korea in August 2009. All other chemicals were of an analytical reagent grade. Distilled water for high-performance liquid chromatography (HPLC) and acetonitrile were purchased from J.T. Baker SOLUSORB (Philipsburg, NJ, USA). The standards were purchased from Chromadex (Santa Ana, CA, USA) and Ambo Institute (Seoul, South Korea). Proton magnetic resonance, carbon magnetic resonance, heteronuclear multiple quantum coherence and heteronuclear multiple bond coherence spectra were measured with INOVA-500 (500 MHz) (Varian, palo alto, CA, USA).

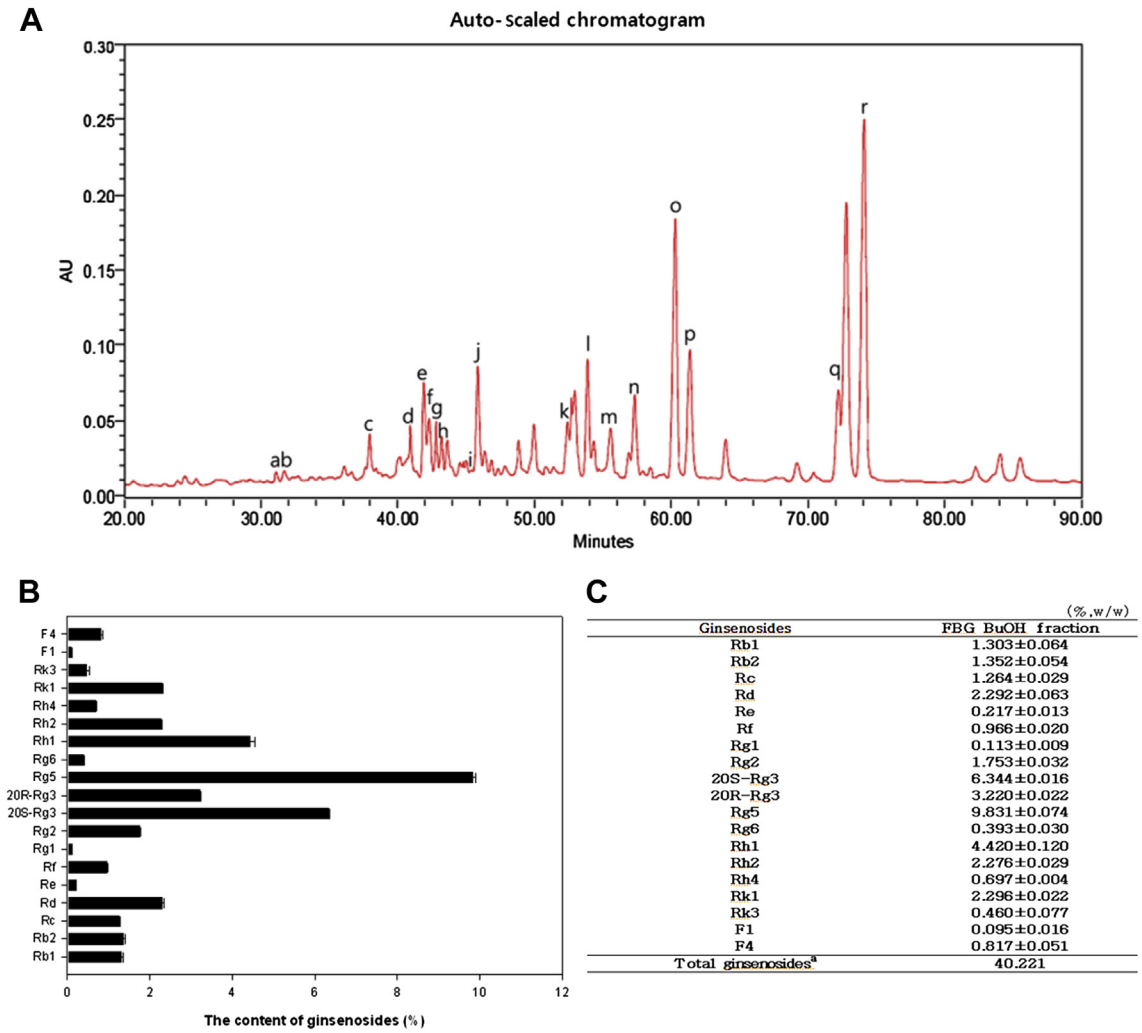

Fig. 1. (A) High-performance liquid chromatography chromatogram of ginsenosides in the butanol (BuOH) fraction of Fine Black ginseng (FBG), compared with the chromatogram of the ginsenoside standards: a, Rg1; b, Re; c, Rf; d, Rb1; e, Rg2; f, Rh1; g, Rc; h, Rb2; i, F1; j, Rd; k, Rg6; l, F4; m, Rk3; n, Rh4; o, (20S) Rg3; p, (20R) Rg3; q, Rk1; r, Rg5. (B) The content of ginsenosides and (C) the composition of ginsenosides in BuOH fraction of Fine Black ginseng.