**Original Article**

**Ginsenoside-Rg5 inhibits proliferation of the breast carcinoma cells through promotion of the proteins involved in AMP kinase pathway**

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**Abstract:** The present study was aimed to investigate the effect of ginsenoside-Rg5 on the proliferation of BT-474 and T-47D breast cancer cell lines. Effect of ginsenoside-Rg5 on the proliferation of breast carcinoma cells was analyzed using MTT assay. Western blot assay was used for determination of the changes in AMP kinase and activation of p70S6K and S6. The results revealed that ginsenoside-Rg5 treatment inhibited the proliferation of BT-474 and T-47D cells in a concentration and time dependent manner. Treatment of T-47D and BT-474 cells with 10 mg/ml concentration of ginsenoside-Rg5 reduced cell viability to 23.5 and 27.7%, respectively after 48 h. Ginsenoside-Rg5 treatment promoted the activation of AMP kinase in BT-474 cells after 48 h. However, administration of siRNA for AMP kinase prevented the inhibition in cell proliferation and increase in AMP kinase activation induced by ginsenoside-Rg5 treatment. Furthermore, ginsenoside-Rg5 reduced the activation of p70S6K and S6 significantly (P<0.05) in BT-474 cells compared to the control cells. Administration of the siRNA for AMP kinase inhibited the ginsenoside-Rg5 induced reduction in p70S6K and S6 activation. Thus ginsenoside-Rg5 inhibits proliferation of breast cancer cells through promotion of protein involved in the AMPK pathway activation. Therefore, ginsenoside-Rg5 can be used for the treatment of breast cancer.

**Keywords:** Ginsenoside-Rg5, proliferation, inhibited, therapeutic, cytotoxic

**Introduction**

Breast cancer is among the leading causes responsible for high rate of morbidity and mortality of cancer patients in women throughout the globe [1]. Development of breast cancer is the result of cumulative effect arising from environmental risk factors as well genetic susceptibility of the patients [2]. It is estimated that around 20% of the patients with breast cancer have evident family history of the disease [3]. The reduced expression of the tumor suppressor gene, LKB1 is associated with the enhancement in the susceptibility to breast cancers [4, 5]. In various types of the cells LKB1 exhibits its effect through increase in the activation of AMP kinase. Despite currently available chemotherapeutic agents for the treatment of breast cancer and resection of the primary tumor the breast cancer recurrence is very high. This poor response to the available chemotherapeutic agents necessitates the discovery of novel agents for the treatment of breast cancer.

Use of herbal treatments has been demonstrated to induce positive results against various diseases either alone or in combination [6]. There are reports that traditional Chinese medicine (TCM) ginseng ginsenoside-Rh2 plays an important role in strengthening the immune system function and inhibiting carcinoma cell viability. TCM successfully inhibited growth and progress of various types of carcinoma cell lines, including lung, liver, etc. [17]. Ginseng is one of the common components of the TCM which is used for diabetes treatment, inhibition of cancer and reduction of stress [8]. The active
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component present in the ginseng has been identified to be ginsenoside-Rg5 (Figure 1) [9-11]. Screening of the ginsenoside-Rg5 revealed its promising anti-dermatitic, cytotoxic, neuroprotective and anti-inflammatory activity [12-17]. In the present study effect of ginsenoside-Rg5 on the breast carcinoma cell lines was investigated. The results revealed that ginsenoside-Rg5 exhibits inhibitory effect on the proliferation of the breast cancer cells.

Material and methods

Chemicals

Ginsenoside-Rg5 (purity >95%) was a kind of gift from Dr Fk Lin at the Shanghai Institute of Materia Medica of the Chinese Academy of Sciences (Shanghai, China). Dimethyl sulfoxide and other common chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Cell lines and culture

BT-474 and T-47D breast cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA). The cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% streptomycin and penicillin and then incubated in humidified atmosphere of 5% CO₂ and 95% air in an incubator at 37°C.

Cytotoxic activity assay

MTT assay was used for the analysis of the effect of ginsenoside-Rg5 on the viability of BT-474 and T-47D breast cancer cell lines. The cells after washing with phosphate-buffered saline (PBS) were seeded at a density of 2 × 10⁵ cells per ml in flat-bottomed 96-well microtiter plates in 100 µl DMEM medium. The cells were then incubated with various concentrations of ginsenoside-Rg5 for 12, 24, 36, 48 and 72 h. After incubation, medium was removed and 20 µl MTT (Sigma-Aldrich, St. Louis, MO, USA) solutions (5 mg/ml) were added to each well of the plate. Incubation of the plates was continued for 4 h more under the same conditions. For each well of the plate optical density was then recorded three times at 565 nm.

Western blot analysis

Briefly, the breast cancer cells were seeded at a density of 2 × 10⁶ cells per ml in DMEM into each well of a 6 cm dish. The cells were incubated with various concentrations of ginsenoside-Rg5 for 48 h. Following incubation, the cells were harvested lysed in lysis buffer [20 mmol/L Tris-HCl (pH 7.5)], 150 mmol/L NaCl, 2.5 mmol/L sodium pyrophosphate 1 mmol/L h-glycerol phosphate, 1 mmol/L EGTA, 1% Triton, and complete protease inhibitor mixture inhibitors (Roche, Mannheim, Germany). The cell lysates were electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gel and then transferred to a nitrocellulose membrane (Millipore, Bedford, MA, USA). The primary antibodies used were: anti-phospho-specific (Thr172) AMPK α and anti-AMPKα, anti-phospho-p70S6K (Thr389), anti-p70S6K, anti-S6 and anti-β-actin (Cell Signaling Technology, Beverly, MA, USA). The secondary antibody used was the anti-rabbit IgG horseradish peroxidase-conjugated antibody (7074) (Cell Signaling Technology). ImmunobLOTS were analyzed by enhanced chemiluminescence.

Administration of siRNA

siRNA for AMPKa1 or negative control siRNA Oligofectamine (Invitrogen) was administered into the BT-474 breast carcinoma cells according to the manufacturer’s guideline. The cells after administration of siRNA were cultured for 48 h with various concentrations of ginsenoside-Rg5.

Statistical analysis

The data obtained were processed using the Statistical Package for Social Sciences (SPSS for Windows, version 17.0; SPSS, Inc., Chicago, IL, USA). In addition, the monofactorial analysis of variance was used. All the data presented are the mean ± standard deviation. The differ-

Figure 1. Chemical structure of ginsenoside-Rg5.
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Results

Ginsenoside-Rg5 inhibits cell proliferation and p7OS6K activation in BT-474 and T-47D breast carcinoma cells

Ginsenoside-Rg5 treatment induced a concentration and time dependent reduction in the growth of MCF-7 cells (Figure 2). Cell viability of BT-474 was reduced to 27.7% compared to 100% in control on treatment with 10 mg/ml concentration of ginsenoside-Rg5 for 48 h. For T-47D cell line, the viability was decreased to 23.5% by exposure to 10 mg/ml concentration of ginsenoside-Rg5.

Ginsenoside-Rg5 promotes activation of AMP kinase in BT-474 breast cancer cells

Effect of ginsenoside-Rg5 treatment on the activation of AMP kinase in BT-474 cells was also analyzed. The results from western blot analysis showed a significant increase in the activation of AMPK with increase in the concentration of ginsenoside-Rg5 from 2 to 10 mg/ml (Figure 3). Analysis of p7OS6K and mTOR activation revealed a significant decrease in BT-474 cells on treatment with ginsenoside-Rg5 for 48 h compared to the untreated control cells (Figure 3).

Effect of the administration of silencer RNA for AMP kinase on BT-474 cells

Administration of the silencer RNA for AMP kinase prevented the BT-474 cells from inhibition in viability induced by ginsenoside-Rg5 (Figure 4A). The cells administered with siRNA prior to ginsenoside-Rg5 treatment showed viability similar to those of the untreated control cells. Ginsenoside-Rg5 treatment induced increase in the expression of AMPKα1 in MCF-7 cells.
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Figure 4. Administration of siRNA for AMPK inhibits AMPK activation and reduction in BT-474 cell viability induced by ginsenoside-Rg5. A. The BT-474 cells were administered siRNA for AMPK (50 nmol/l) or only control siRNA and cultured for 24 h. Following culture, the cells were treated with ginsenoside-Rg5 for 48 hours and analyzed for proliferation using MTT assay. B. BT-474 cells were administered siRNA for AMPK (50 nmol/l) or only control siRNA and cultured for 24 h. The medium was then replaced by FBS containing ginsenoside-Rg5 and the cells were incubated for 48 h. The cells were subjected to western blot analysis using β-actin as the internal loading control. Rg5 and siRNA are the ginsenoside-Rg5 and siRNA for AMP kinase.

Figure 5. Ginsenoside-Rg5 treatment for 48 h inhibited the production of proteins in BT-474 cells. The cells were incubated for 48 h with ginsenoside-Rg5 followed by treatment with 35S methionine-protein labeling mixture. After 1 h cells were incorporation of radioactivity was analyzed. *P<0.05 compared to the control.

Discussion

The present study was aimed to investigate the effect of ginsenoside-Rg5 on the rate of proliferation in BT-474 and T-47D breast cancer cells. Ginsenoside-Rg5 has been shown to exhibit a promising cytotoxic effect by inhibiting the viability of cancer cells [14, 15]. Inhibition of the carcinoma cell proliferation by various chemotherapeutic agents is a promising strategy for the cancer treatment. The present study demonstrated that treatment of the breast cancer cells with ginsenoside-Rg5 inhibited cell viability in dose and time dependent manner. Viability of T-47D and BT-474 cell lines was reduced to 23.5 and 27.7%, respectively after 48 h by exposure to 10 mg/ml concentration of ginsenoside-Rg5. The underlying mechanism of the ginsenoside-Rg5 induced reduction in the breast cancer cell viability was also investigated. There are reports that activation of the LKB1/AMP pathway in various cells is associated with the initiation of cellular processes which regulate cell growth and proliferation [18]. The reduced expression of the tumor suppressor gene, LKB1 is associated with the enhancement in susceptibility of the cells to several types of cancers.
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[4, 5]. In various types of the cells LKB1 exhibits its effect through increase in the activation of AMP kinase. Results from the present study revealed that ginsenoside-Rg5 treatment enhanced the activation of AMPK pathway in BT-474 cells. The activation of p70S6K and S6 was reduced significantly in the cells treated with ginsenoside-Rg5. However, when BT-474 cells were administered with siRNA for AMPK the ginsenoside-Rg5 induced activation of AMP was inhibited. This provided a strong evidences that ginsenoside-Rg5 exhibited its effect through promoting the activation of AMPK. This was further confirmed by the administration of silencer RNA for AMP kinase in BT-474 cells. The results revealed that siRNA AMP kinase prevented the inhibition in viability induced by ginsenoside-Rg5. Ginsenoside-Rg5 treatment induced increase in the expression of AMPKa1 in BT-474 cells, however, siRNA for AMP kinase inhibited the expression. Treatment of the BT-474 cells with ginsenoside-Rg5 reduced the activation of p70S6K and S6 significantly (P<0.05) compared to the control cells. However, administration of the siRNA for AMP kinase inhibited the ginsenoside-Rg5 induced reduction in p70S6K and S6 activation. Thus ginsenoside-Rg5 inhibits proliferation of breast cancer cells through promotion of protein involved in the AMPK pathway. Therefore, ginsenoside-Rg5 can be used for the treatment of breast cancer.

Disclosure of conflict of interest

None.

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