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EXPERIMENTAL STUDY

Ginsenoside Rg3 inhibits colon cancer cell migration by suppressing nuclear factor kappa B activity

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Abstract

OBJECTIVE: To study the mechanism of the inhibitory effect of ginsenoside Rg3 on colon cancer cell migration.

METHODS: Transwell migration assays were performed to investigate the inhibitory effect of ginsenoside Rg3 on SW480 cell migration. Electrophoretic mobility shift assays (EMSAs) and dual luciferase reporter assays were used to study the suppression capability of Rg3 on nuclear factor kappa B (NF-κB) activity. Western blotting was adopted to determine protein levels.

RESULTS: Two-hundred micromolar ginsenoside Rg3 significantly inhibited SW480 cell migration ($P < 0.05$). EMSA showed that Rg3 suppressed the DNA binding ability of NF-κB. Dual luciferase reporter assay showed that Rg3 decreased NF-κB-regulated gene transcription ($P < 0.01$). Western blots indicated that Rg3 down-regulated expression of the NF-κB-regulated matrix metalloproteinase 9, cyclooxygenase-2 and C-Myc. An NF-κB inhibitor, pyrrolidine dithiocarbamate, enhanced the inhibitory effect of Rg3 on SW480 cell migration.

CONCLUSION: Ginsenoside Rg3 has a strong antitumor migration capability by suppressing NF-κB activity and expression of NF-κB-regulated gene products. It could be a good adjuvant for colon cancer patients during the course of chemotherapy.

Key words: Colonic neoplasms; Ginsenoside Rg3; NF-kappa B; Cell movement

INTRODUCTION

Colorectal cancers are the third most common type of cancers worldwide, and is the second leading cause of mortality in developed countries.[1-3] Although surgical resection may prevent mortality before tumor cell metastasis, metastasis occurs in about three-fifths of the patients before the diagnosis is made, and 90% of them only survive for 5 years.[4-6] At present, for metastatic colon cancer patients, median survival can only be prolonged for 14 months by adjuvant chemotherapy.[7] Thus, it is necessary to develop rationally designed, targeted therapies to manage the metastatic process in colorectal cancer patients. In recent years, new therapeutic methods for colon cancer patients have substantially increased,[8] among which new adjuvant chemotherapy has proved effective.[3] In the past several decades, a large number of substances derived from edible plants have been studied in the antitumor research field. Some of them have been proven to exhibit chemo-preventive properties,[9-12] and can be used as an adjuvant in chemotherapy.

Ginsenoside Rg3, derived from the Chinese herb ginseng and used as a medicine for more than 2000 years,[13] has been demonstrated to have antitumor effects.[14,15] Ginsenoside Rg3 can inhibit tumor growth by down-regulation of Wnt/β-catenin signaling,[14] and may induce cancer cell apoptosis via the mitogen-activated protein kinase and adenosine 5'-monophosphate-activated protein kinase signaling pathways.[15,16] It can also sensitize cancer cells to chemotherapy.[17,18] However, the molecular mechanisms for ginsenoside Rg3 activity have not yet been clearly elucidated. The present study is designed to explore the molecular mechanisms of ginsenoside Rg3 inhibiting colon cancer cell migration.

MATERIALS AND METHODS

Cell culture

Human colon cancer cell line SW480 was purchased from the American Type Culture Collection (ATCC, MD, USA) and grown in McCoy's 5A medium (Life Technology, Madison, WI, USA) supplemented with 10% fetal bovine serum and 5 units of penicillin/streptomycin, under 5% CO_2, at 37 ℃.

Chemicals

Ginsenoside Rg3 was purchased from the Delta Information Center for Natural Organic Compounds (Xuancheng, Anhui, China), dissolved in DMSO to make a stock solution (15 mM) and stored in aliquots at −80 ℃. Other chemicals were purchased from Sigma (St. Louis, MO, USA) unless otherwise indicated.

In vitro migration assay

The in vitro migration assay was performed as previously described with some modifications[19,20] using transwells (8 μm pore size; BD Corporation, Franklin Lake, NJ, USA). SW480 cells treated with 200 μM ginsenoside Rg3 were added to the upper compartment of the chamber (in 200 μL serum-free medium containing 2×10^4 cells), and 600 μL serum free medium was added to the lower chamber. After 24 h of incubations, cells were removed from the upper surface of the filter with a cotton swab; cells that had migrated to the bottom surface of the filter were fixed with methanol and stained with crystal violet. The migrated cells were counted and the migration ability of the control group was set as 100%. The other groups' migration ability was determined with the formula: [migrated cell count of the test group/migrated cell count of the control group] × 100%. Each experiment was performed in triplicate.

Gel electro-mobility shift assay (EMSA)

Protein samples were prepared, stored and quantified by methods described previously.[21] No reducing agents were added to the electrophoretic mobility shift assays (EMSAs). EMSA for nuclear factor kappa B (NF-κB) DNA binding was performed using a DNA – protein binding detection kit (Viagene, Changzhou, China) according to the manufacturer's protocol. Briefly, the NF-κB oligonucleotide probe (5'-AGTT-GAGGGGACTTTCCCAGGC-3') was labeled with biotin. The binding reaction was carried out in 10 μL of mixture containing 1 μL of 10× incubation buffer, 1 μL of Poly (deoxyinosinic-deoxycytidylic) acid (Poly (dI-dC)), and nuclear extracts. After 20 min incubation at room temperature, 0.5 μL biotin-labeled probe (Sangon, Shanghai, China) were added. After 20 min incubation at room temperature, samples were electrophoresed through a 6% non-denaturing polyacrylamide gel at 150 V in a cold room for 2 h. Finally, the gel was dried and exposed to X-ray film.

Dual-luciferase reporter assay

SW480 cells (2.5×10^5 cells/cm^2) were seeded in 24-well plates (BD Corporation) and transiently transfected with pNF-κB-TA-Luc (Beyotime, Shanghai, China) and plasmid pRL-TK (Promega, Madison, WI, USA) using Effectene reagent (Qiagen, Düsseldorf, Germany). The transfected cells were treated with 200 μM ginsenoside Rg3 for 4 h. Then, the luciferase activity was determined using a dual luciferase reporter assay kit (Promega) according to the manufacturer's instructions.

Western blotting

As previously described,[20] whole cell lysates were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, electrotransferred to polyvinylidene fluoride membranes and probed with appropriate antibody. Then, the blots were probed with appropriate horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and were visualized using an enhanced chemiluminescence assay kit (ECL kit; Applygen Technology, Beijing, China). The membranes were also probed with anti-actin antibody (Santa Cruz Biotechnology) to monitor sampling differences.

Statistics

Statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± standard deviation and were analyzed by one-way analysis of variance. $P < 0.05$ were considered statistically significant.

RESULTS

Ginsenoside Rg3 inhibits colon cancer cell (SW480) migration in vitro

To determine whether ginsenoside Rg3 can be used as an adjuvant chemotherapy for colon cancer, transwell assays were performed to examine the effects of ginsenoside Rg3 on colon cancer cell SW480 migration *in vitro*. Figure 1 shows that the migration of SW480 cells