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Original Article Inhibitory Effect of Ginsenoside Rg3 on Ovarian Cancer Metastasis

ABSTRACT

Ginsenosides are main components extracted from ginseng, and ginsenoside Rg3 is one of the most important parts. Ginsenoside Rg3 has been found to inhibit several kinds of tumor growth and metastasis. The present study was undertaken to investigate the effect of ginsenoside Rg3 on human ovarian cancer metastasis and the possible mechanism. The experimental lung metastasis models of ovarian cancer SKOV-3 and the assay of tumor-induced angiogenesis were used to observe the inhibitory effects of Rg3 on tumor metastasis and angiogenesis. The effect of Rg3 on invasive ability of SKOV-3 cells in vitro was detected by Boyden chamber, and immunofluorescence staining was used to recognize the expression of matrix metalloproteinase 9 (MMP-9) in SKOV-3 cells. Ginsenoside Rg3 can significantly inhibit the metastasis of ovarian cancer. The inhibitory effect is partially due to inhibition of tumor-induced angiogenesis and decrease of invasive ability and MMP-9 expression of SKOV-3 cells.

Keywords: *ginseng, neoplasm, metastasis, angiogenesis, ovarian cancer*

1. Introduction

Ginseng is a medicinal herb widely used in Asian countries for its wide spectrum of medicinal effects such as tonic, immunomodulatory, adaptogenic, and anti-aging activities.[1]These effects are attributed to the triterpene glycosides known as ginsenosides and Rg3 is one kind of the ginsenosides. The molecular formula of ginsenoside Rg3 is C42H72O13 and its molecular weight is 784.30. Ginsenoside Rg3 can inhibit catecholamine secretion, protect cultured cortical cells from glutamate-induced neurodegeneration, and anti-contraction of vascular smooth muscle.[2-4] Researchers found that ginsenoside Rg3 can also resist tumor. For example, it inhibited invasion and metastasis of B16 melenoma without impairing cell growth, and proliferation of tumor cells combined with cyclophosphamide.[5-7] However, the effect of ginsenoside Rg3 on human ovarian cancer has not been identified and the mechanism of its anti-tumor is unknown.

The present study was to assess the effects of ginsenoside Rg3 on angiogenesis and metastasis produced by human ovarian cancer and on the invasion and the matrix metalloproteinase 9 (MMP-9) expression of SKOV-3 cells *in vitro*.

2. Methods

2.1. Drug and Reagents

Ginsenoside Rg3 was provided by Department of Organic Chemistry of Preclinical Medicine of Jilin University. Its purity is more than 99.5%. Athymic mice were purchased from the Department of Experimental Animals of Jilin University. Boyden chamber and matrigel were purchased from BD Company, USA.

2.2. Cell Culture

Human ovarian cancer SKOV-3 cell line and mouse NIH3T3 fibroblast cell were obtained from the Tumor Research Department of Jilin Province and maintained in RPMI1640 supplemented with 10% fetal bovine serum (FBS).

Table 1. Inhibitory effect of ginsenoside Rg3 on experimental lung metastasis and angiogenesis caused by ovarian cancer

Group	Dose (mg/kg)	Experimental lung metastasis		Tumor-induced angiogenesis	
		No. of mice (start / end)	No. of metastatic foci/lung (mean±SD)	No. of mice (start / end)	No. of vessels (mean±SD)
Control	0	10/9	36.73±16.21	5/5	16.34±2.2
Ginsenoside Rg3	0.3	10/10	23.41±8.96*	5/5	11.63±2.74*
	1.0	10/10	12.15±14.56**	5/5	10.39±2.31*
	3.0	10/9	11.32±9.47**	5/5	8.33±1.09**

*P <0.01, **P <0.001 compared with control group

2.3. Assay for Experimental Lung Metastasis

In 40 mice, each was injected with SKOV-3 cells (2×10^5) into the lateral tail vein. The mice were randomly divided into 4 groups (n=10 for each group): Rg3 groups (0.3, 1.0 and 3.0 mg/kg) and control group. Ginsenoside Rg3 was injected intraperitoneally at daily doses (0.3, 1.0 and 3.0 mg/kg) to the tumor-bearing mice the next day after tumor inoculation. The mice were killed 20 days after the inoculation. The lungs were fixed in Bouin's solution and the lung tumor colonies were counted under a dissecting microscope.

2.4. Cell Invasion Assay

The invasive activity of SKOV-3 cells was assayed in Boyden chambers. The upper surface of the filters in Boyden chambers was precoated with 60 μl of matrigel. The SKOV-3 cells were harvested, washed 3 times and re-suspended to a final concentration of 1×10^6/ml in RPMI1640. Then the cells were divided into 3 groups after pretreatment with ginsenoside Rg3 of different concentrations (0, 2.5 and 5.0 μg/ml) at 37°C for 30 minutes. 200 μl of each cell suspension was added to the upper compartment of the chamber and 200 μl of conditioned medium of NIH3T3 cells to the lower compartment. After 5-hour incubation, the cells on the upper surface of the filters were removed by wiping with cotton swabs and the filters were fixed with FAA solution and stained with hematoxylin and eosin. The cells that had invaded through the matrigel and filters into the lower surface were manually counted under a microscope at a magnification of ×400. Each assay was performed in triplicate. The data were expressed as the number of invaded cells/field.

2.5. Assay for Tumor-induced Angiogenesis

In 20 athymic mice, each was inoculated intradermally with SKOV-3 cells (5×10^5) on the back. They were randomly divided into 4 groups (n=5): Rg3 groups (0.3,

1.0 and 3.0 mg/kg) and control group. Ginsenoside Rg3 at various doses was injected intraperitoneally to the mice 1, 2, 3, 4 and 5 days after tumor inoculation. Two days later, the mice were sacrificed and the skin was separated from the underlying tissues. Angiogenesis was quantified by counting the number of vessels oriented toward the tumor mass under a dissecting microscope.

2.6. MMP-9 Immunofluorescence Studies

The SKOV-3 cells that were seeded onto 24-well plates and treated with 0, 2.5 and 5.0 μg/ml of ginsenoside Rg3 for 24 hours were used for immunofluoresecent staining. First, the cells were washed briefly with phosphate buffered saline three times and then fixed in 4% phosphate buffered paraformaldehyde for 30 minutes. Second, they underwent permabilization with the addition of phosphate-buffered 0.1% Triton X-100 for 10 minutes and then were incubated in 1% bovine serum albumin for 30 minutes all at room temperature. Third, the cells were incubated with mouse anti-human MMP-9 monoclonal antibody (1: 100 dilution) and then with goat anti-mouse fluorescein isothiocyanate (FITC) antibody respectively for 1 hour at 4°C. Finally, the coverslips were mounted and sealed for examination under a confocal microscope.

2.7. Statistical Analysis

Measurement data were evaluated by one-way analysis of variance (ANOVA) for multiple group comparisons, and the LSD test for two-group comparisons. Ranked data were evaluated by the Kruskal-Wallis test for multiple group comparisons and the Nemenyi test for two-group comparisons. P <0.05 was considered statistically significant.

3. Results

3.1. Effect of Ginsenoside Rg3 on Experimental lung Metastasis of SKOV-3 cells

As shown in Figure 1, after intraperitoneal injection of ginsenoside Rg3 at the doses of 0.3, 1.0 and 3.0 mg/kg, the number of tumor colonies in the lung was lower than that of the control group (P <0.01). The results indicate that ginsenoside Rg3 can significantly inhibit the lung metastasis of SKOV-3 cells.

3.2. Inhibitory Effect of Ginsenoside Rg3 on Tumor-induced Angiogenesis

The number of vessels oriented toward the tumor mass in the three groups that the tumor-bearing mice were given

intraperitoneal injection of ginsenoside Rg3 at the doses of 0.3, 1.0 and 3.0 mg/kg was less than that of the control group (Table). This indicates that ginsenoside Rg3 may inhibit ovarian tumor-induced angiogenesis.

3.3. Cell invasion Assay

After the SKOV-3 cells were treated with ginsenoside Rg3 at three concentrations of 0, 2.5 and 5.0 μg/ml, the number of the cells invading through matrigel and filters into the lower surface was 157.3±29.4, 110.8±25.6 and 92.5±18.4 respectively. Among the three groups, the group of 0 μg/ml ginsenoside Rg3 was control group. Statistical analysis illustrated that the number of cells invading filters of the ginsenoside groups was less than that of the control group (P <0.001, Figure 1), and that ginsenoside Rg3 can depress the ability of invading of SKOV-3 cells.

3.4. Assay of MMP-9 Immunofluorescence

The SKOV-3 cells were pretreated with ginsenoside Rg3 at doses of 2.5 and 5.0 μg/ml for 24 hours and immunofluorescence staining was used to recognize the expression of MMP-9 in these tumor cells. Figure 2 shows that cytoplasmic fluorescence intensity in the SKOV-3 cells was depressed as the dose of ginsenoside Rg3 increased. This result shows that ginsenoside Rg3 can depress the expression of MMP-9 in the SKOV-3 cells.

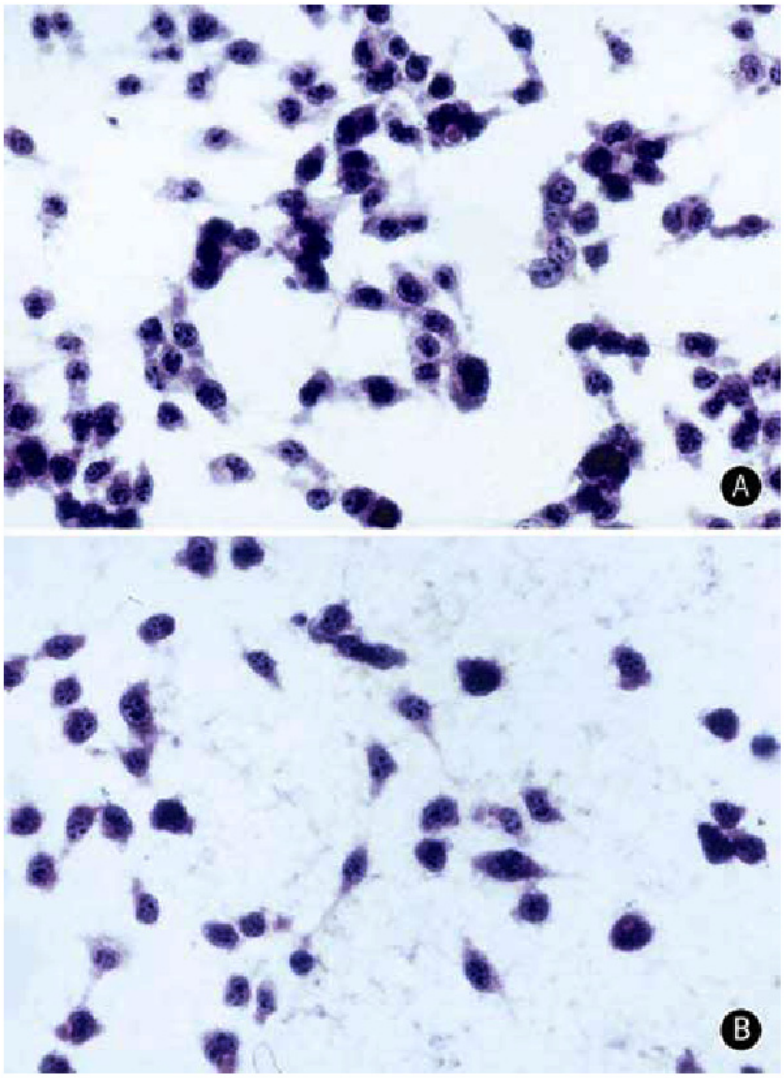

Figure 1. Inhibitory effect of ginsenoside Rg3 on the invasion of SKOV-3 cells. The SKOV-3 cells were pretreated with ginsenoside Rg3 at doses of 2.5 and 5.0 μg/ml for 30 minutes and then were added to Boyden chambers. After 5-hour incubation, the cells that had invaded through the matrigel and filters were manually counted. A: control group; B: 5.0 μg/ml ginsenoside Rg3 group (HE, original magnification ×400)

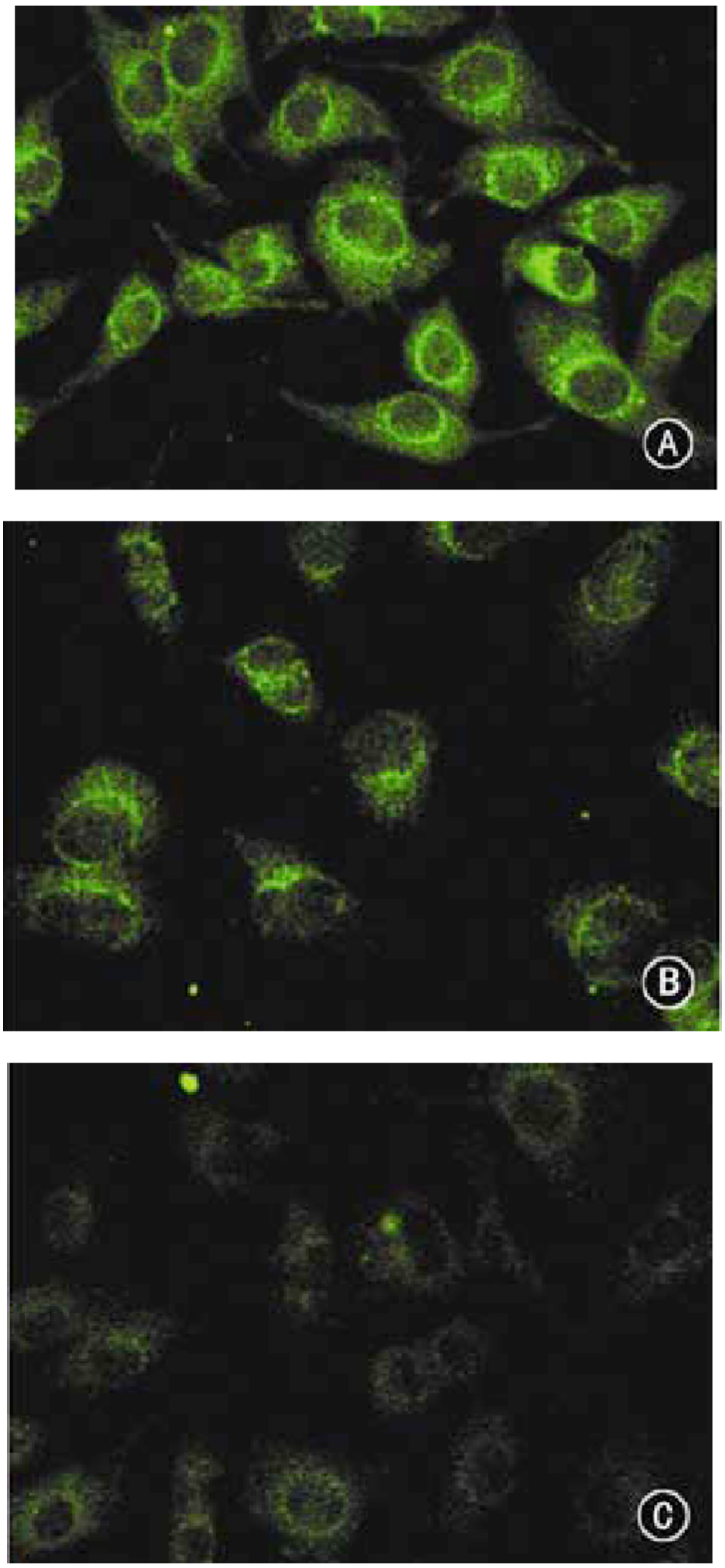

Figure 2. Immunofluorescence staining of MMP-9 antibody in SKOV-3 cells. The SKOV-3 cells were pretreated with ginsenoside Rg3 at doses of 2.5 and 5.0 μg/ml for 24 hours and immunofluorescence staining was used to recognize the expression of MMP-9. A: control group; B: 2.5 μg/ml ginsenoside Rg3 group; C: 5.0 μg/ml ginsenoside Rg3 group (original magnification ×400)

4. Discussion

Ovarian cancer is a severe disease threatening the health and life of women. The 5-year survival rate of patients with such disease remains low after conventional treatment.[8] Thus it is necessary to develop new agents and methods for a cure.

With the development of Chinese herbal medicine, researchers are increasingly interested in detecting anti-tumor components in Chinese herbal medicine. Ginsenoside Rg3, a saponin extracted from ginseng, was found to have a high anti-tumor effect. It inhibited angiogenesis of Lewis lung carcinoma,[9] invasion and metastasis of intestinal adenocarcinoma and B16 melenoma,[5,6] and proliferation of prostate cancer cell.[10,11] The present study demonstrated that ginsenoside Rg3 can also inhibit the metastasis caused by ovarian cancer, which will benefit patients with ovarian cancer.

The ability to invade tissues and establish colonies at remote sites is a definite characteristic of malignant neoplasms. As a complex cascade it is affected by many factors. Angiogenesis is a key step in tumor invasion and metastasis.[12] Since massive formation of blood vessels at the tumor site increases the opportunity for tumor cells to enter the circulation, the suppression of angiogenesis will decrease the metastasis of malignant tumor. In this study, the number of vessels oriented toward the tumor mass after intraperitoneal injection of ginsenoside Rg3 at the doses of 0.3, 1.0 and 3.0 mg/kg was less than that of the untreated group, indicating that ginsenoside Rg3 may inhibit ovarian tumor-induced angiogenesis, and subsequently decrease the metastasis of ovarian cancer.

In addition, tumor invasion and adhesion to an extracellular matrix and basement membrane components are important events in the process of tumor metastasis. In this study, matrigel, an analogue of basement membrane, was used to determine the effect of ginsenoside Rg3 on invasion of ovarian cancer cells *in vitro*. Because the tumor cells adhere to matrigel, then invade and cross the matrigel, we can evaluate the invasion of tumor cells by the number of cells passing the matrigel. We observed that the number of the SKOV-3 cells invading through the matrigel and filters into the lower surface decreased after they were treated with ginsenoside Rg3. This indicates that ginsenoside Rg3 can prevent the invasion of ovarian cancer cells.

However, angiogenesis as well as tumor invasion and metastasis depend heavily on the controlled interactions between the cells and the extracellular matrix (ECM).[13] These interactions are mediated by integral membrane proteins and extracellular proteinases. Extracellular proteolysis plays an important role in many aspects, including basement degradation and cell migration/ extracellular matric invasion, and is mediated by metalloproteinases (MMPs) and serine proteinases.[14,15]

MMPs belong to a family of zinc-dependent endopeptides.

They secrete as inactive pro-enzymes and are activated by partial proteolytic cleavage.[16] MMP-9 or the dominant MMPs released by most endothelial cells and tumor cells appear to play an important role in degradation of basement membrane type VI collage and other matrixproteins.[17-19] Therefore, MMP-9 is regarded as marker of tumor invasion and metastasis, and the suppression of its expression may inhibit malignant tumor invasion and metastasis.[20] The results of the present study demonstrated that ginsenoside Rg3 depressed the secretion of ovarian cancer cells, which may be the channel by which ginsenoside Rg3 inhibits the metastasis of ovarian cancer.

In conclusion, ginsenoside Rg3 can significantly inhibit the metastasis of ovarian cancer. The inhibitory effect is partially due to the inhibition of tumor-induced angiogenesis and decrease of the invasive ability, which may be related to the depression of MMP-9 expression of ovarian cancer cells.

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