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Research Article

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Ginsenoside Rg3 Inhibit The Proliferation And Metastasis of Cervical Cancer Cells in Vitro By Regulating NF-κB Signaling Pathway

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Abstract

Objective: *To investigate the effect and mechanism of ginsenoside Rg3 on the proliferation and metastasis of cervical.*

Methods: *Cervical cancer cells HeLa were treated with different concentrations (0, 0.12, 0.24, 0.48 mmol/L) of ginsenoside Rg3, and then the survival rate of HeLa cells was detected by CCK-8 method, and the migration and invasion of HeLa cells were assessed using Transwell test, and expression of E-cadherin, N-cadherin, vimentin, Toll receptor 4 (TLR4), myeloid differentiation factor 88 (MyD88), phosphorylated nuclear transcription factor κB p65 (P-NF-κB p65) proteins were calculated by Western blot.*

Results: *After ginsenoside Rg3 (0.12, 0.24, 0.48 mmol/L) treatment, the survival rate, migration number, invasion number, and N-cadherer number, and N-cadherin, Vimentin, TLR4, MyD88, p-NF-κB p65 protein expression of HeLa cells were significantly reduced (P<0.05) Ginsenoside protein expression was significantly increased (P<0.05), and showed a concentration-dependent relationship. Conclusion: Ginsenoside Rg3 could inhibit the proliferation and metastasis of cervical cancer cells in vitro, and its mechanism might be related to the inhibition of NF-κB signaling pathway.*

Keywords: Ginsenoside Rg3, Cervical cancer, Cell proliferation, Migration, Invasion, NF-κB signaling pathway

Cervical cancer is a common gynecologic malignancy and more important cause of cancer-related deaths in women worldwide [1]. With the combination of surgery, radiation and chemotherapy, the survival rate of early-staged cervical cancer improved significantly, but the 1-year survival rate of patients with recurrent and metastatic cancer was still low [2]. Therefore, there is an urgent need to find a therapeutic method that effectively inhibits the proliferation and metastasis of cervical cancer cells. Ginsenoside Rg3 is an active ingredient extracted from the roots of *Panax ginseng C.A. Mey. of* the family Ginseng, which plays an important role in cancer prevention and treatment by inducing apoptosis, inhibiting proliferation, angiogenesis and metastasis, and enhancing immune function [3]. Nuclear factor-κB (NF-κB) is considered as one of the key signaling pathways for tumor metastasis, and NF-κB is abnormally activated in cervical cancer and promotes malignant proliferation and metastasis of tumor cells [4]. In this study, the NF-κB signaling pathway was used as an entry point to investigate the anticancer effects of ginsenoside Rg3 on cervical cancer, aiming to provide a theoretical basis for the use of ginsenoside Rg3 in the treatment of cervical cancer.

Materials and Methods

Materials

Cells

Human cervical cancer cells HeLa were purchased from Shanghai Suer Biotechnology Co.

Reagents

RPMI-1640 medium was purchased from Gibco, USA; ginsenoside Rg3 (batch number 110804-201504, purity 99.5%, first with dimethyl sulfoxide solution, diluted to the desired concentration with culture solution during the experiment) was purchased from

China Academy of Food and Drug Administration; cell counting kit (CCK-8) was purchased from Beijing Biolab Biological Company; Transwell was purchased from Beijing Unicon Biological Company, goat anti-rabbit IgG (ab205718) secondary antibody, rabbit-derived β-actin polyclonal antibody (ab8227), rabbit-derived vimentin polyclonal antibody (ab137321), rabbit-derived Toll-like receptor 4 (TLR4) polyclonal antibody (ab13556), and rabbit-derived MyD88 polyclonal antibody (ab2064), goat anti-mouse IgG secondary antibody (ab6789) purchased from Abcam, USA; mouse-derived E-cadherin monoclonal antibody (sc-71008), mouse-derived N-cadherin monoclonal antibody (sc-8424), mouse-derived NF-κB p65 monoclonal antibody (sc-8008), and mouse-derived p-NF-κB p65 monoclonal antibody (sc-166748) were purchased from Santa Cruz, USA.

Instrument

EL10A automatic microplate reader, Shandong Brocade Instrument Company; IX73 type Olympus inverted microscope, Shanghai Puhe Optoelectronics Technology Company.

Methods

Cell Culture

HeLa cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin double antibodies and incubated in an incubator at 37^{O}C with 5% CO2 and 95% humidity. The cells were trypsinized when they spread 80% of the bottom of the flask and then subjected to 1:4 passaging culture, and the fluid was changed twice a week, and the 4th generation of log phase cells were selected for the experiment.

Grouping

5 × 103 HeLa cells were inoculated in 96-well plates, after cell attachment, the cells were incubated with culture medium containing 0, 0.12, 0.24, and 0.48 mmol/L ginsenoside Rg3, which were recorded as Control group, 0.12 mmol/L Rg3 group, 0.24 mmol/L Rg3 group, and 0.48 mmol/L Rg3 group [5].

CCK-8 assay for HeLa cell survival [6]

The cells were incubated with ginsenoside Rg3 for 24h according to the grouping of method "1.2.2", and the survival rate of HeLa cells was detected according to the literature method.

Transwell assay to detect HeLa cell migration and invasion [7]

Cells were incubated with ginsenoside Rg3 for 24h according to method "1.2.2", and the number of migrating and invading HeLa cells was detected according to the literature method.

Western blot detection of E-cadherin, N-cadherin, Vimentin, TLR4, MyD88, NF-κB p65, p-NF-κB p65 protein expression [6]
After incubation of HeLa cells with ginsenoside Rg3 for 24h, total cellular protein was extracted by RIPA lysis and protein concentration was determined by BCA kit. The proteins were denatured and subjected to SDS-PAGE, followed by membrane transfer. After membrane closure, primary and secondary antibodies were incubated separately. The proteins were detected by chemiluminescence chromogenic kit. The grayscale values of the electrophoretic bands were analyzed using Image-Lab software, and the expression of the target protein was expressed as the ratio of the target protein to the grayscale value of β-actin. 1:800 dilution for E-cadherin and MyD88 antibodies, 1:500 dilution for N-cadherin, TLR4, NF-κB p65, p-NF-κB p65 antibodies, and Vimentin antibody at 1:1500 dilution, β-actin antibody (1:3000 dilution, goat anti-rabbit IgG secondary antibody at 1:2500 dilution, and goat anti-mouse IgG secondary antibody at 1:3000 dilution.

Statistical methods

SPSS 22.0 was used for statistical analysis. Data satisfied normal distribution and met chi-square, and were expressed as mean ± standard deviation (`X±s). One-way ANOVA and SNK-q test were used between multiple groups. $p<0.05$ was considered a statistically significant difference.

Results

Ginsenoside Rg3 Inhibits The Proliferation of Cervical Cancer Cells

The cell proliferation was detected by CCK-8 after incubation of HeLa cells with ginsenoside Rg3 for 24 h. The results showed that the survival rate of HeLa cells in the 0.12 mmol/L Rg3, 0.24 mmol/L Rg3 and 0.48 mmol/L Rg3 groups was significantly lower compared with the Control group ($P<0.05$). The inhibitory effect of ginsenoside Rg3 on the survival of HeLa cells was gradually increased with the increase of its concentration. See Figure 1.

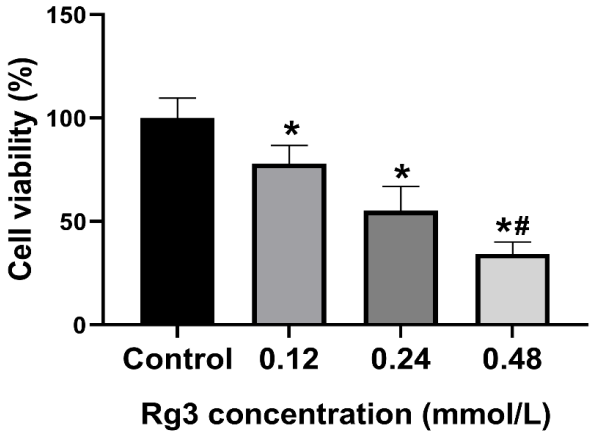

Figure 1: Effect of ginsenoside Rg3 on the survival rate of HeLa cells by CCK-8 assay

Note: *Compared with Control group, $P<0.05$; #Compared with 0.12 mmol/L Rg3 group, $P<0.05$

Ginsenoside Rg3 Inhibits The Migration Of Cervical Cancer Cells

Ginsenoside Rg3 incubation of HeLa cells for 24 h followed by Transwell assay to detect cell migration showed that the number of HeLa cell migration was significantly reduced in the 0.12 mmol/L Rg3 group, 0.24 mmol/L Rg3 group and 0.48 mmol/L Rg3 group compared with the Control group ($P<0.05$). As the concentration of ginsenoside Rg3 increased its inhibitory effect on HeLa cell migration was gradually enhanced. See Figure 2.

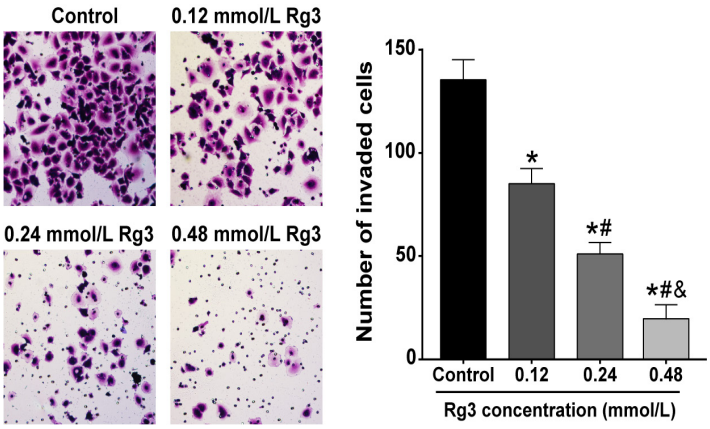

Figure 2: Effect of ginsenoside Rg3 on the migration of HeLa cells by Transwell assay

Note: *Compared with Control group, P<0.05; #Compared with 0.12 mmol/L Rg3 group, P<0.05; & Compared with 0.24 mmol/L Rg3 group, P<0.05.

Ginsenoside Rg3 Inhibits The Invasion Of Cervical Cancer Cells

Ginsenoside Rg3 incubation of HeLa cells for 24 h followed by Transwell assay to detect cell invasion showed that the number of HeLa cell invasion was significantly reduced in the 0.12 mmol/L Rg3 group, 0.24 mmol/L Rg3 group, and 0.48 mmol/L Rg3 group compared with the Control group (P<0.05). The inhibitory effect of ginsenoside Rg3 on HeLa cell invasion was gradually increased with the increase of its concentration. See Figure 3.

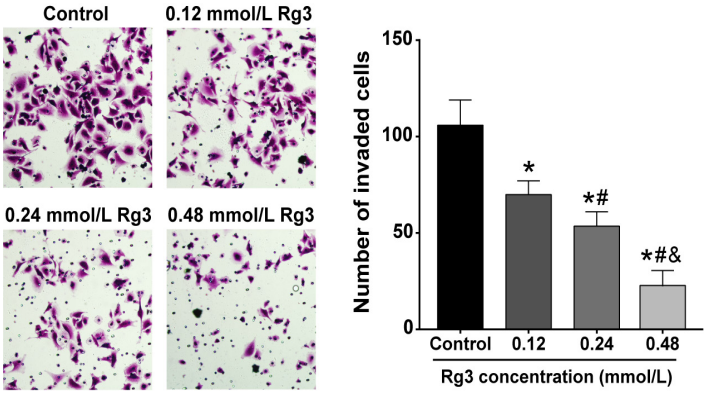

Figure 3: Transwell method to detect the effect of ginsenoside Rg3 on HeLa cell invasion

Note: *Compared with Control group, P<0.05; #Compared with 0.12 mmol/L Rg3 group, P<0.05; & Compared with 0.24 mmol/L Rg3 group, P<0.05.

Effect of ginsenoside Rg3 on the expression levels of E-cadherin, N-cadherin, and Vimentin proteins

The western blot detection of migration and invasion-related protein expression in HeLa cells after 24 h incubation with ginsenoside Rg3 showed that, compared with the Control group, E-cadherin in HeLa cells in the 0.12 mmol/L Rg3 group, 0.24 mmol/L Rg3 group and 0.48 mmol/L Rg3 group protein expression was significantly higher and N-cadherin and Vimentin protein expression was significantly lower in HeLa cells in the 0.48 mmol/L Rg3 group (P<0.05). As the concentration of ginsenoside Rg3 increased, its promoting effect on E-cadherin protein expression and its inhibiting effect on N-cadherin and Vimentin protein expression gradually increased. See Figure 3.

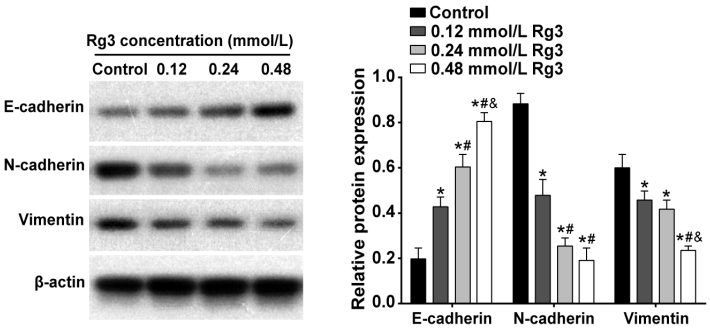

Figure 4: Effect of ginsenoside Rg3 on E-cadherin, N-cadherin and Vimentin protein expression levels in HeLa cells by Western blot.

Note: *Compared with Control group, P<0.05; #Compared with 0.12 mmol/L Rg3 group, P<0.05; & Compared with 0.24 mmol/L Rg3 group, P<0.05.

Effect of ginsenoside Rg3 on the expression of NF-κB pathway-related proteins

The western blot detection of NF-κB pathway-related protein expression in HeLa cells after 24 h incubation with ginsenoside Rg3 showed that, compared with the Control group, the expression of TLR4, TLR4 in HeLa cells in the 0.12 mmol/L Rg3 group, 0.24 mmol/L Rg3 group and 0.48 mmol/L Rg3 group. MyD88, p-NF-κB p65 protein expression were significantly reduced (P<0.05). The inhibitory effect of ginsenoside Rg3 on TLR4, MyD88, and p-NF-κB p65 protein expression was gradually increased with the increase of ginsenoside Rg3 concentration.

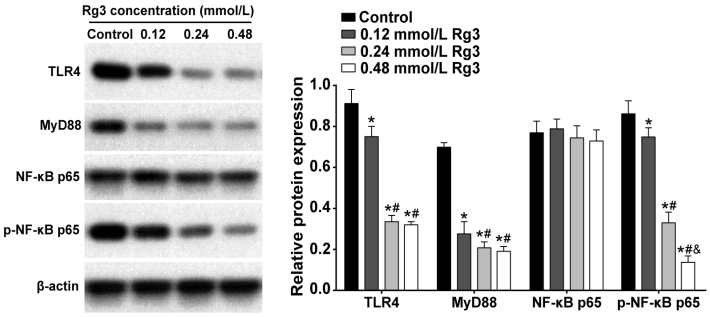

Figure 5: Effect of ginsenoside Rg3 on the expression of NF-κB pathway-related proteins in HeLa cells by Western blot.

Note: *Compared with Control group, P<0.05; #Compared with 0.12 mmol/L Rg3 group, P<0.05; & Compared with 0.24 mmol/L Rg3 group, P<0.05.

Discussion

Ginsenoside Rg3 has been shown to exhibit anticancer activity in a variety of tumors. Shi Yanyan et al. reported that ginsenoside Rg3

inhibited gastric cancer cell proliferation and induced apoptosis by blocking the PI3K/AKT signaling pathway [8]. Liu Jun et al. indicated that ginsenoside Rg3 may inhibit bladder cancer cell invasion, proliferation and angiogenesis by reducing epidermal growth factor receptor tyrosine kinase (EGFR-TPK) and DNA topoisomerase I (DNA TOP I) activities [9]. Han Ping et al. demonstrated that ginsenoside Rg3 also induced apoptosis and inhibited the proliferation and migration of colon cancer cells. In this study, we found that the survival rate, migration number and invasion number of HeLa cells were significantly reduced after ginsenoside Rg3 treatment, and the inhibitory effects on proliferation, migration and invasion of HeLa cells were gradually increased with the increase of ginsenoside Rg3 concentration, indicating that ginsenoside Rg3 has anti-proliferative, anti-migration and anti-invasive effects on cervical cancer cells in vitro.EMT is an important event in the process of tumor metastasis , from manifesting as epithelial to mesenchymal cell phenotype transformation, accompanied by loss of cell polarity and increased cell adhesion, migration and invasiveness [10, 11]. In this study, we found that ginsenoside Rg3 significantly decreased the expression of mesenchymal markers N-cadherin and Vimentin and increased the expression of epithelial marker E-cadherin protein in HeLa cells with a concentration-dependent effect, suggesting that ginsenoside Rg3 inhibits cervical cancer cell migration and invasion by inhibiting EMT and thus.

NF-κB, an important transcription factor, has a wide range of cellular functions. It has been shown that NF-κB is associated with the progression of cervical cancer , and aberrant NF-κB activation can regulate apoptosis-related genes, cell cycle proteins, and cell adhesion gene expression, affecting cell malignant behavior [12-15]. Ying Xia et al. showed that low expression of miR-224-5p in cervical cancer could activate NF-κB signaling pathway to induce cervical cancer cell proliferation, migration and invasion [13]. Chen Jiancui et al. reported that interleukin 1 receptor-associated kinase 1 (IRAK1) inhibited cervical cancer cell apoptosis by mediating NF-κB activation and thereby [14]. However, inhibition of NF-κB activation induces cervical cancer cell cycle arrest and apoptosis, inhibiting their malignant potential [15]. In this study, we found that ginsenoside Rg3 decreased the expression levels of TLR4, MyD88, and p-NF-κB p65 proteins, important components of the NF-κB signaling pathway, in HeLa cells in a dose-dependent manner, indicating that ginsenoside Rg3 can inhibit the activation of NF-κB signaling pathway in cervical cancer, which suggests that the anti-proliferative, anti-migratory, and anti-invasive effects of ginsenoside Rg3 on cervical cancer cells This suggests that the anti-proliferative, anti-migration and anti-invasive effects of ginsenoside Rg3 on cervical cancer cells may be achieved by inhibiting the NF-κB signaling pathway.

In conclusion, ginsenoside Rg3 can inhibit the proliferation and metastatic ability of cervical cancer cells in vitro, and its mechanism may be related to the inhibition of NF-κB signaling pathway, which initially elucidates the antitumor effect and possible mechanism of ginsenoside Rg3 on cervical cancer and provides an experimental basis for the application of ginsenoside Rg3 in the clinical treatment of cervical cancer.

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