Rhein Inhibits the Migration of Ovarian Cancer Cells through Down-Regulation of Matrix Metalloproteinases

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The root of *Rheum officinale* BAILL as a traditional Chinese medicine, which main function is removing heat from the blood, promoting blood circulation and clearing toxins away. Rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid) is one of the most important active components in the root of *Rheum officinale* BAILL, which could inhibit the proliferation of tumor cells. However, the study on the mechanism of anti-cel migration capacity of Rhein on ovarian cancer is not yet clear. Here, we demonstrated that Rhein had dose-dependent effects of ovarian tumors on drugs and could inhibit the proliferations and migration of two typical ovarian cancer cell lines, A2780 and OV2008. Furthermore, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays showed that the survival rate of ovarian cancer cells was significantly decreased when treated with Rhein. Rhein inhibited the proliferation of ovarian cancer cells in dose-dependent manner. Moreover, the wound healing assay and transwell assay indicated that the cell migratory potential and expression of matrix metalloproteinases were markedly inhibited by Rhein. Our findings suggested that Rhein could be a potential candidate to be developed as a drug for the prevention of ovarian cancer cell migration.

**Key words** Rhein; anti-migration effect; ovarian cancer; matrix metalloproteinase

**INTRODUCTION**

Ovarian cancer is one of the most deadly gynecologic tumor. For patients with ovarian cancer, first-line clinical treatment is mainly cytoreductive surgery and paclitaxel-based chemotherapy. Although most of the patients performed well at the beginning of treatment, there are still many difficulties and challenges about treatment. The major clinical issue in the prognosis and management of ovarian cancer is the development of ascites and peritoneal metastases. Therefore, for the ovarian cancer patients, it is urgent to develop novel and effective anti-metastatic drugs.

*Rheum officinale* BAILL is a tradition Chinese medicine, which has been normally used for chronic renal failure, asthma and chronic kidney disease. Rhein is one of the active anthraquinone glycoside separated from *Rheum officinale* BAILL. Rhein has a wide range of bioactivities, such as inhibiting cell proliferation, decreasing hypertrophy, suppressing migration and invasion and inducing tumor cell apoptosis. Nevertheless, the effects of Rhein in resistance to ovarian cancer cell migration and its molecular mechanism are not clear.

Matrix metalloproteinases (MMPs) are a kind of endopeptidases family with the ability to resolve extracellular matrix. There are two important roles of MMPs, one is the function of Pro-angiogenic, another is assistance to transfer cancer cells. These functions of MMPs including MMP-1, MMP-2, MMP-9 make it critical in metastatic disease. MMP-1 plays an important role in the linker region of domain interactions. It has been reported that a kind of bioactivator dryofragin inhibited migration of human osteosarcoma cells by suppressing MMP-2/9. However, little research has been focused on the resistance to ovarian cancer cell migration.

In this study, we found that Rhein could inhibit the proliferation and migration of ovarian cancer cells, and the underlying mechanism was associated with downregulation of MMPs. Rhein might be an effective compound to be developed as a potential drug for treatment of ovarian cancer.

**MATERIALS AND METHODS**

*Materials, Reagents and Chemicals* The root of *Rheum officinale* BAILL were purchased from the drug store of Yichun Tang in Dalian of Liaoning Province, and the materials were identified by associate professor Lin Zhang in the department of Integrative Medicine of Dalian Medical University. A voucher specimen (No. 20171015) was stored in the institute of Integrative Medicine. Chengdu Must Bio-Technology Co., Ltd. (Sichuan, China) applied Rhein. The chemical formula of Rhein is C_{15}H_{8}O_{6}, which purity was >98%.

*Extraction and Isolation of Rhein* The dried and powdered root of *Rheum officinale* BAILL (4kg) were extracted with 10 times 75% ethanol and heated for two times (3 h each time). The combined filtrate was decompressed and evaporated to obtain the extract (467 g). The extracts were then dispersed in water and extracted with petroleum ether, dichloromethane and n-butanol, respectively. The dichloromethane extraction (36g) was separated by silica gel chromatography and eluted with different ratio of ethyl acetate and petroleum ether. Rhein (12.4 mg) was obtained by recrystallization from the forth fraction eluted (1:4), the structure of Rhein was shown...
Rhein (as orange-yellow needle crystal; $^1$H-NMR (Dimethyl sulfoxide ((DMSO), 600 MHz) $\delta$: 13.8 (1H, s), 11.9 (2H, s), 8.10 (1H, br s), 7.82 (IH, t, $J = 7.8, 7.8$ Hz), 7.74 (1H, br s), 7.71 (1H, d, $J = 7.8$ Hz), 7.40 (1H, d, $J = 7.84$ Hz). $^{13}$C-NMR (DMSO, 150 MHz) $\delta$: 161.9 (s), 124.6 (d), 138.5 (s), 119.2 (d), 119.9 (d), 138.1 (d), 125.1 (d), 161.5 (s), 191.8 (s), 181.4 (s), 134.2 (s), 133.6 (s), 116.6 (s), 119.1 (s), 165.9 (s). The structures of Rhein were identified by comparing the NMR data with those in published paper.\textsuperscript{18}

Cell Lines and Cell Culture American Type Culture Collection (ATCC) supplied A2780, OV2008 and IOSE80 cell lines. The cells were maintained in Dulbecco’s modified eagle’s medium (DMEM) with 10% Fetal bovine serum (FBS) at 5% CO$_2$ in a incubator with 37°C.

Cell Proliferation Assays 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to examined the effect of Rhein on cell viability. The cells (1 $\times$ 10$^4$/well) with Rhein (0, 50, 100, 150, 200 or 250 $\mu$M) were incubated for 24h. After MTT solution incubation for another 4h, 150 $\mu$L of DMSO was added to each well. 490nm wavelength was used to measure the absorbance.

Colony Formation Assay The cells (500/dish) were culture with Rhein (0, 100, 200 or 300 $\mu$M) for 14d. Afterwards, 70% ethanol and crystal violet (Sigma, St. Louis, MO, U.S.A.) was used to fix and stain cells. Numbers of colonies was counted by Image J software.

Wound Healing Assays A 200 $\mu$L pipette tip was used to scrape the cells in 24 well plate. The cells were incubated with 0, 30, 60 or 90 $\mu$M Rhein solution for 24h. Cell migration was detected by a phase-contrast microscope after 24h. The Image J software was used to measure and quantify the denuded area.

Transwell Migration Assays Ovarian cancer cells were treated with 0, 30, 60 or 90 $\mu$M Rhein for 24h. 2 $\times$ 10$^4$ cells were added to each upper compartment containing serum-free DMEM, while medium with 10% FBS used as a chemical inducer was added to the lower compartment. After 24h, 0.1% crystal violet was used to stain the cells on the underside of cell membrane. The cells on the underside of transwell mem-

Fig. 1. Effect of Rhein on the Proliferation of Ovarian Cancer Cell Lines and Normal Ovarian Cell Line

(A) Chemical structure of Rhein. (B) Ovarian cancer cells A2780, OV2008 and normal ovariann cells IOSE80 were treated with Rhein (0, 50, 100, 150, 200 or 250 $\mu$M) for 24h and 48h. MTT assay was used to verify cell viability. (C) Rhein inhibited colony formation in a dose dependent manner. $^* p < 0.01$ and $^{**} p < 0.001$ as compared to the control group.
brane were measured and counted by a microscope.

**Western Blot** Total protein was extracted from the cells samples by RIPA lysis buffer (Beyotime, China). The same amount of protein was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to the polyvinylidene fluoride (PVDF) membrane. After incubation with the specific primary and secondary antibody, protein expression were detected by ECL kit and measured by Image J software.

**Statistical Analysis** The data was collected from three independent assay. The Student’s t-test was used to compare two groups by GraphPad Prism 5.0 (San Diego, CA, U.S.A.). The difference was considered statistically significance when \( p < 0.05 \).

RESULTS

**Inhibiting Proliferation of Ovarian Cancer Cells by Rhein** For purpose of investigating the anti-proliferation activity of Rhein on ovarian cancer, the ovarian cancer cell lines A2780, OV2008 and the normal ovarian cell line IOSE80 were treated with Rhein in different concentrations (0, 50, 100, 150, 200 or 250 \( \mu \text{M} \)) for 24h and 48h (Fig. 1B). The activity of ovarian cancer cells was examined at the suitable dose. The result showed that the ability to proliferation and growth of the ovarian cells treated with Rhein was reduced in time-dependent manner (\( p < 0.001 \)). Nevertheless, the ability to proliferate and grow of the normal ovarian cell line was not significantly inhibited (\( p > 0.05 \)). In addition, the cytotoxic effects of Rhein were used to examine and verify by colony formation assay. The colony formation was proportional to the treatment concentrations. As shown in Fig. 1C, the colony formation capacity of both the A2780 and OV2008 cells were inhibited by Rhein in dose-dependent manner. As our results showed, Rhein could inhibit the proliferation and had a long-term cytostatic effect on colony formation in ovarian cancer cells.

**Suppressing the Migration of Ovarian Cancer Cells by Rhein** The wound healing assay and the transwell assay were performed to evaluate the anti-migration capacity of Rhein, and the inhibition effects of Rhein on proliferation of ovarian cancer cells was not shown at low concentrations (0, 30, 60 and 90 \( \mu \text{M} \)). Rhein dose-dependently reduced the migration of A2780 and OV2008 cells in the wound healing assay (Fig. 2). The wound closure rates of Rhein-treated cells were lower than of non-treated cells (\( p < 0.0001 \)). Furthermore, in the transwell assay, it was shown that Rhein significantly inhibited the migration of ovarian cancer cells (Fig. 3A), and the migration cells of Rhein-treated group were lower than that of control group (Fig. 3B). The results from wound healing assay and transwell assay all indicated that the migration of ovarian cancer cells was suppressed by Rhein.

**The Effects of Rhein on MMPs Expression** Based on the effects of Rhein on ovarian cancer cell migration, the mechanisms were further investigated. Now that MMPs act important roles in cancer cell migration, the MMP-1, MMP-2 and MMP-9 protein expression were detected in A2780 cells by Western blot (Fig. 4). As our results showed, the inhibitory activity of Rhein on the migration of ovarian cancer may be related to the down regulation of MMP-1, MMP-2 and MMP-9 expression.

DISCUSSION

Since the chemotherapy resistance and unexpected side effects are the critical challenges for cancer treatments, traditional herbal medicines are increasingly being used to treat a wide variety of cancer.\(^{19,20}\) The recurrence rate of ovarian cancer caused by drug resistance is one of the main causes of tumor death. Chemotherapy drugs have obvious killing effects on cancer, but they also produce drug resistance for a long time, leading to the re-proliferation of tumor cells. Our results are based on the potential drug candidate Rhein, which has a sustained killing effect on tumor cells treated with cisplatin in many other tumors, such as breast cancer, but the mechanism of anti-migration in ovarian cancer still uncertain.\(^{21}\) Rhein,
an important chemical component of *Rheum officinale* has been reported to possess anti-inflammatory,\(^{22}\) anti-oxidation\(^{23}\) and anti-tumor effects.\(^{24}\) Studies have showed that MMPs are expressed as proenzymes that are activated in the extracellular space.\(^{25}\) The first step for cancer cell migration was degradation of extracellular matrix by MMPs, MMPs acts an important role in cancer cell migration.\(^{26}\) Moreover, it was difficult to work out prognoses in ovarian cancer patients due to the abnormal MMPs expression in ovarian cancer cells.\(^{27}\) Our results indicated that Rhein could inhibit the expression of MMP-1, MMP-2 and MMP-9 compared with the control groups. These data suggested that Rhein may suppress ovarian cancer migration through down regulation of MMPs expression. However, Further studies *in vivo* will be done to verify the conclusions.

In conclusion, Rhein could effectively suppress the proliferation and migration of ovarian cancer cells, and the underlying mechanism may be related to the expression of MMPs. The results revealed that Rhein could be a kind of novel and effective drug for ovarian cancer patients.

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design, experiment performing, data analysis, or manuscript preparation.

Conflict of Interest The authors declare no conflict of interest.

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