RESEARCH ARTICLE

Inhibition of Metastasis and Invasion of Ovarian Cancer Cells by Crude Polysaccharides from *Rosa Roxburghii* Tratt *in Vitro*

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Abstract

Background: *Rosa Roxburghii* Tratt is a promising wild fruit crop in Southwest China. Its extracts have been used as traditional Chinese medicine, which benefit immune responses and cure various health disorders. However, whether *Rosa Roxburghii* Tratt polysaccharides could inhibit metastasis and invasion of ovarian cancer cells remains unknown. Materials and Methods: Effects of crude polysaccharides from *Rosa Roxburghii* Tratt on the viability of ovarian cancer A2780 cells were detected by MTT assay. Ovarian carcinoma cell migration and invasion after exposure to *Rosa Roxburghii* Tratt polysaccharides were quantified by wound healing and Transwell assays, respectively. Western blotting was applied to assess protein levels of MMP-9. Results: The results indicated that *Rosa Roxburghii* Tratt polysaccharides significantly reduced wound closure rate of A2780 cells, inhibited their migration and invasion, and suppressed the expression of MMP-9. Conclusions: Our findings indicated that *Rosa Roxburghii* Tratt polysaccharides have potential for develop as anti-metastatic cancer drug preparations for ovarian cancer patients.

Keywords: *Rosa roxburghii* tratt polysaccharides - ovarian cancer - metastasis - MMP-9

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Introduction

Epithelial ovarian carcinoma is one of the most common gynecological malignancies, and the fifth most frequent cause of cancer death in women (Siegel et al., 2014). The current standard treatments for ovarian cancer patients rely on surgery following by chemotherapy. However, chemotherapeutical drugs resistance and early metastasis caused its poor prognosis (Sun et al., 2013). The survival rate of ovarian cancer patients is only 40% in all stages and 15%-30% patients with widespread metastatic disease survive 5 years after the initial treatment (Vaughan et al., 2011). Mortality risk increased 3.44-fold in patients over 50 years of age, 2.75 times in the presence of lymph node invasion (Arikan et al., 2014).

The metastasis and invasion of ovarian cancer is a complex and multistage process which depend on the activity of many mediators. Previous studies have shown that extracellular matrix (ECM) plays an important role in tumor invasion and metastasis (Stallings-Mann and Radisky, 2007; Kessenbrock et al., 2010). The MMPs are a family of structurally conserved, zinc-dependent endopeptidases, which are involved in proteolytic modeling of the ECM. MMP-9 can degrade components of the ECM, which constitutes an important barrier to tumor cell invasion (Liotta and Stetler-Stevenson, 1990; Himelstein et al., 1994). Therefore, MMP-9 is commonly suggested as a potential prognostic factor for ovarian cancer (Hu et al., 2012).

Chinese herbal medicine had been confirmed to have anti-tumor activities in recent years (Sengupta et al., 2001; Kanazawa et al., 2003; Hsu et al., 2005). *Rosa roxburghii* Tratt, which grown in Southwest China, contains high percentage of polysaccharides and vitamin C. Studies showed that *Rosa roxburghii* Tratt extracts displayed anti-tumor effects and induced apoptosis of cancer cells (Ma et al., 1997; Liu et al., 2012). However, the effects and mechanisms of *Rosa roxburghii* Tratt polysaccharides on metastasis and invasion of ovarian cancer are still not clear. The present study was performed to evaluate anti-metastatic effects of *Rosa roxburghii* Tratt polysaccharides for ovarian carcinoma and explore molecular mechanisms *in vitro*.

Materials and Methods

Regents and chemicals

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco, American. Matrigel and transwells were purchased from BD Biosciences, American. Antibodies against MMP-9 and β-actin were obtained from Ptoteintech, China. The enhanced chemiluminescence (ECL) kit was from Amersham Life Science, UK.

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Preparation of crude polysaccharide from Rosa roxburghii Tratt (CLP)

The dried fruits of *Rosa roxburghii* Tratt were obtained from Guizhou, China. The crude polysaccharides were isolated as previously described (Yang et al., 2010). Briefly, the dried powder of *Rosa roxburghii* Tratt (100g) were extracted in a Soxhlet's apparatus with ligroin and 95% alcohol. After the mixture was filtered, the residues were dried and then were extracted with hot water (1:15, w/v) at 100°C for three times (1h each time). The combined extracts were pooled, concentrated to 40% of the original volume. The concentrated solution was dialyzed for 1 day against distilled water and twice volumes of 95% alcohol were added to precipitate the polysaccharides, and then at 4°C overnight. Finally polysaccharide pellets were obtained by centrifugation at 4000 rpm for 15 min and repeatedly washed sequentially with anhydrous ethanol, acetone and diethyl ether. Finally, the filtrate was lyophilised to yield the crude polysaccharides (CLP) which dissolved in DMEM with 10% FBS for further use. The yield of crude polysaccharides was 6.8% and the percentage of total sugar was about 76%. There was no absorption at 260 and 280nm in the crude polysaccharides, suggesting no contamination of nucleic acid and protein.

Cell culture

The epithelial ovarian cancer A2780 cell line was obtained from ATCC. A2780 cells were maintained in DMEM media (Gibco, USA) supplemented with 10% FBS at 37°C in with 5% CO_2. Cells were stored with 10% DMSO (Tianjin, China).

MTT assay

A2780 cells were planted into 96-well plates and attached for 24h. The cells were then treated with different concentrations of CLP for 48h. The MTT solution (5mg/ml) was added to each well and incubated 4 h at 37ºC. Then, 150μl of DMSO was added into each well and mixed for 10 minute at 37ºC. The optical density (OD) was determined by spectrophotometer (Shanghai, China) at 490 nm. Cells without CLP were used as the negative control. Each assay was performed triplicate.

Wound healing assay

5×10^5 cells were seeded into 6 well plates and grown to 70-80% confluence. The cell monolayer was scratched using a 200 μl sterile pipette tip. Cells were then washed twice with PBS. Ovarian cancer cells were treated with 0, 100 μg/ml and 200 μg/ml CLP solution for 24h. Cell migration of ovarian cancer cells was observed under a phase-contrast microscope at 100× magnification field at 0 and 24h post-induction of injury. Migrated cells into the denuded area in each of six random fields were measured and quantified with computer-assisted microscope.

Invasion assays

Cell invasion were detected by the Transwell assay. Ovarian cancer cells were treated with 0, 100 μg/ml and 200 μg/ml CLP solution for 24h. Ovrian cancer cells were added to the upper chamber with serum-free DMEM medium. The cells migrated to the lower chamber whiched filled with 10% FBS DMEM medium as a chemoattactant. After 24h treatment, cells on the upper surface of membrane were cleared and the cells on the underside of membrane were stained with 0.1% crystal violet for 10min. The migrated cells of radom six fields of each Transwell membrane were counted under a microscope at 100× magnification field.

Western blot analysis

The total cell lysates were separated by 10% SDS-PAGE and then transferred to nitrocellulose membrane by semi-dry apparatus for 45 min. The membrane was blocked with 5% non-fat milk for 1.5h and then incubated with the specific primary antibody solution overnight at 4°C. After incubation with secondary antibody for 1 h, the protein bands were visualized by ECL kit.

Data analysis

Data are presented as mean±SED for three independent experiments and then analyzed Data using one-way analysis of variance (ANOVA) by SPSS 13.0 software. The value of $p<0.05$ was considered statistically significant.

Results

The effects of CLP on ovarian cancer proliferation

The growth curves of ovarian cancer cells following various concentrations of CLP for 48h were shown in Figure 1. CLP showed potent cytotoxic activity in ovarian cancer A2780 cells. There was a significantly, dose-dependent decrease in proliferation of cells incubated with CLP ($p<0.05$). CLP with the concentration higher than 200μg/ml has cytotoxic effective on A2780 cells (Figure 1).

Effects of CLP on ovarian cancer cell movement

To investigate whether CLP could affect ovarian cancer cell movement, wound healing assay were performed. In the wound healing assay, CLP solution dose dependently decreased the movement of A2780 cells contrast with control group (Figure 2A). The wound closure rates of CLP (100 μg/ml and 200 μg/ml) treated cells were 10.5% and 8.5% respectively which were significant lower than that of non-treated cells ($p<0.05$) (Figure 2B).

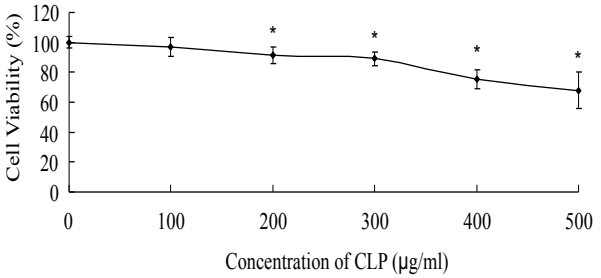

Figure 1. Effects of CLP on the Growth of A2780 Cell. MTT assay determined cytotoxicity of CLP after 48h incubation with A2780 cells at varied concentrations (sample size N=3). *$p<0.05$

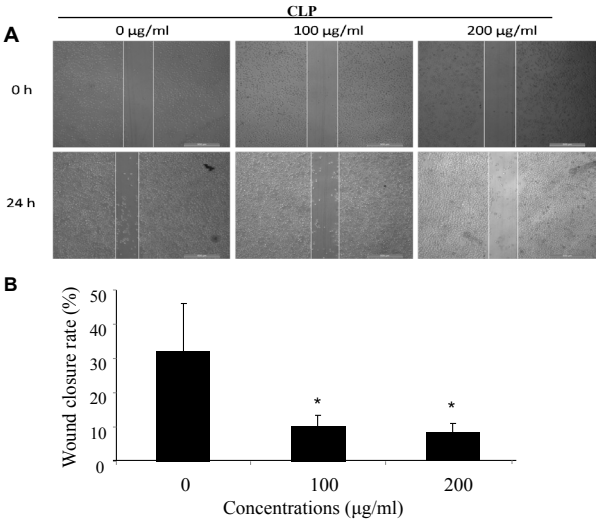

Figure 2. Effect of CLP on Wound Healing in Ovarian Cancer Cells A2780. A2780 Cell was scraped with pipette tip and treated with CLP (0, 100, 200 μg/ml) for 24h. A. The photos represented cell migration under microscope at 100× magnification field before and after injury. B. The migration of A2780 cells was quantified by measuring wound closure areas before and after injury. The experiments were repeated three times. *p<0.05 compared to the control group

Effects of CLP on ovarian cancer invasion

In the transwell assay, CLP significantly inhibited ovarian cancer cells migration and invasion in a dose dependent manner for 24h (Figure 3A). Treatment with 100 μg/ml and 200 μg/ml CLP inhibited cell migration by 37.56% and 30.85% respectively (Figure 3B). Transwell chamber assay suggested that CLP suppressed the invasion and metastasis of ovarian cancer.

Effects of CLP on the expression of MMP-9 in A2780 cell

To further understand the mechanisms of CLP induced surpression of invasion in ovarian cancer cells, the expression of metastasis related gene MMP-9 was examined with Western blotting. As compared to untreated cells, the expression of MMP-9 decreased after treatment with CLP for 24 h (Figure 4).

Discussion

Ovarian cancer is the most lethal of the gynecologic cancer due to the late diagnose. For the ovarian cancer patients at metastatic stage, there were limited effective therapies (Goff et al., 2007; Dinh et al., 2008; Jemal et al., 2008). In addition, both patients and gynecologists had to face the serious side effect of chemotherapy (Shan et al., 2012). Therefore, finding some new agents which had better anti-tumor effect without toxicities is an urgent for ovarian cancer patients.

Regents with natural origin are the preferred priority in the drug screening programs. Several medicinal plants had been used traditionally for the prevention and treatment of cancer (Alabsi et al., 2013). *Rosa Roxburghii* Tratt is a rare fruit crop in Southwest China which has been used to improve immune response, anti-aging and enhancing digestive ability in Chinese medicine (Zhang et al., 2001; Huang et al., 2014). In this study, we explored the anti-

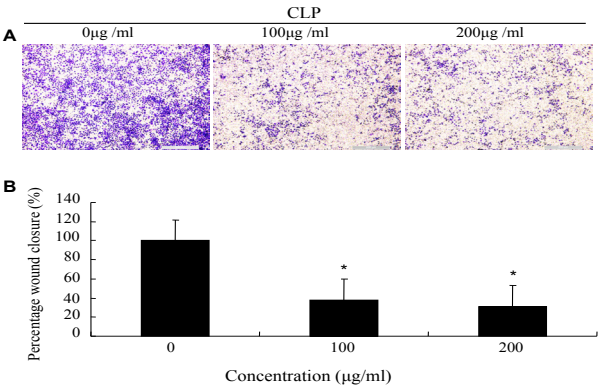

Figure 3. Effect of CLP on Migration and Invasion Ability in Ovarian Cancer Cells by Treanwell Assay. The cells were treated with CLP (0, 100, 200 μg/ml) for 24h. **A).** The photos represented cell migration or invasion onto the underside of transwell membrane under microscope at 200×magnification field. **B).** Cell number of each field was counted and averaged. Migrated cells were expressed relative to that of control group. The experiments were repeated three times. *p<0.05 compared to the control group

Figure 4. MMP-9 Protein Expression in A2780 Cells at Various Concentrations of CLP by Western Blotting. Total lysates of A2780 cells (lane 1: Control), A2780 cells co-cultured with varied concentrations of CLP (lane 2: 100 μg/ml, lane 3: 200 μg/ml, and lane 4: 300 μg/ml). β-actin was used a internal control. Data was representative of at least three independent experiments

metastatic effect of CLP on ovarian cancer cells *in vitro* and the mechanisms underlying this process.

Rose Roxburghii Tratt extracts combined with Fagopyrum Cymosum has been reported to inhibit tumor growth and induce apoptosis effects on human esophageal squamous carcinoma CaEs-17, human gastric carcinoma SGC-7901 and pulmonary carcinoma A549 cells (Liu et al., 2012). Our data showed that CLP also significantly inhibited cell proliferation of ovarian cancer cells from 200μg/ml and the cell survival rate decreased in dose dependent manner.

Moreover, we firstly found that CLP can effectively prevent the migration and invasion of ovarian cancer cells. Insight into molecular mechanisms indicated that CLP decreased MMP-9 expression. MMP-9 were reported to be related with tumor invasion because it can degrade the basement membrane collagen (Parmo-Cabanas et al., 2006). Increased expression of MMP-9 was associated with poor prognosis in ovarian cancer patients. Down regulation of MMP-9 may contribute to improve outcome of ovarian cancer (Li et al., 2013). Our data suggested that CLP suppressed ovarian cancer A2780 cells invasion through down-regulated MMP-9 expression. The exact substances in the crude CLP responsible for this process were not be elucidated in this study and need further investigation.

In the conclusion, our results demonstrated that CLP could inhibit proliferation of ovarian cancer. In addition, CLP prevent migration and invasion of ovarian cancer by decreasing MMP-9 expression. Therefore, we believed that CLP which did not have serious side-effects may develop as novel alterative drug for ovarian cancer patients especially at metastatic stage.

Acknowledgements

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References

Alabsi AM, Ali R, Ali AM, et al (2013). Induction of caspase-9, biochemical assessment and morphological changes caused by apoptosis in cancer cells treated with goniothalamin extracted from *Goniothalamus macrophyllus*. *Asian Pac J Cancer Prev*, **14**, 6273-80.

Arikan SK, Kasap B, Yetimalar H, et al (2014). Impact of prognostic factors on survival rates in patients with ovarian carcinoma. *Asian Pac J Cancer Prev*, **15**, 6087-94.

Dinh P, Harnett P, Piccart-Gebhart MJ, et al (2008). New therapies for ovarian cancer: cytotoxics and molecularly targeted agents. *Crit Rev Oncol Hematol*, **67**, 103-12.

Goff BA, Mandel LS, Drescher CW, et al (2007). Development of an ovarian cancer symptom index: possibilities for earlier detection. *Cancer*, **109**, 221-7.

Himelstein BP, Canete-Soler R, Bernhard EJ, et al (1994). Metalloproteinases in tumor progression: the contribution of MMP-9. *Invasion Metastasis*, **14**, 246-58.

Hsu YL, Kuo PL, Lin LT, et al (2005). Isoliquiritigenin inhibits cell proliferation and induces apoptosis in human hepatoma cells. *Planta Med*, **71**, 130-4.

Hu X, Li D, Zhang W, et al (2012). Matrix metalloproteinase-9 expression correlates with prognosis and involved in ovarian cancer cell invasion. *Arch Gynecol Obstet*, **286**, 1537-43.

Huang M, Xu Q, Deng XX (2014). l-Ascorbic acid metabolism during fruit development in an ascorbate-rich fruit crop chestnut rose (*Rosa roxburghii* Tratt). *J Plant Physiol*, **171**, 1205-16.

Jemal A, Siegel R, Ward E, et al (2008). Cancer statistics, 2008. *CA Cancer J Clin*, **58**, 71-96.

Kanazawa M, Satomi Y, Mizutani Y, et al (2003). Isoliquiritigenin inhibits the growth of prostate cancer. *Eur Urol*, **43**, 580-6.

Kessenbrock K, Plaks V, Werb Z (2010). Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell*, **141**, 52-67.

Li LN, Zhou X, Gu Y, et al (2013). Prognostic value of MMP-9 in ovarian cancer: a meta-analysis. *Asian Pac J Cancer Prev*, **14**, 4107-13.

Liotta LA, Stetler-Stevenson WG (1990). Metalloproteinases and cancer invasion. *Semin Cancer Biol*, **1**, 99-106.

Liu W, Li SY, Huang XE, et al (2012). Inhibition of tumor growth *in vitro* by a combination of extracts from *Rosa roxburghii* Tratt and *Fagopyrum cymosum*. *Asian Pac J Cancer Prev*, **13**, 2409-14.

Ma YX, Zhu Y, Wang CF, et al (1997). The aging retarding effect of 'Long-Life CiLi'. *Mech Ageing Dev*, **96**, 171-80.

Parmo-Cabanas M, Molina-Ortiz I, Matias-Roman S, et al (2006). Role of metalloproteinases MMP-9 and MT1-MMP in CXCL12-promoted myeloma cell invasion across basement membranes. *J Pathol*, **208**, 108-18.

Sengupta A, Ghosh S, Das S (2001). Modulation of DMBA induced genotoxicity in bone marrow by quercetin during skin carcinogenesis. *J Exp Clin Cancer Res*, **20**, 131-4.

Shan Z, Feng-Nian R, Jie G, et al (2012). Effects of valproic acid on proliferation, apoptosis, angiogenesis and metastasis of ovarian cancer *in vitro* and *in vivo*. *Asian Pac J Cancer Prev*, 13, 3977-82.

Siegel R, Ma J, Zou Z, et al (2014). Cancer statistics, 2014. *CA Cancer J Clin*, **64**, 9-29.

Stallings-Mann M, Radisky D (2007). Matrix metalloproteinase-induced malignancy in mammary epithelial cells. *Cells Tissues Organs*, **185**, 104-10.

Sun ZL, Tang YJ, Wu WG, et al (2013). AZD1480 can inhibit the biological behavior of ovarian cancer SKOV3 cells *in vitro*. *Asian Pac J Cancer Prev*, **14**, 4823-7.

Vaughan S, Coward JI, Bast RC, et al (2011). Rethinking ovarian cancer: recommendations for improving outcomes. *Nat Rev Cancer*, **11**, 719-25.

Yang X, Lv Y, Tian L, et al (2010). Composition and systemic immune activity of the polysaccharides from an herbal tea (Lycopus lucidus Turcz). *J Agric Food Chem*, **58**, 6075-80.

Zhang C, Liu X, Qiang H, et al (2001). Inhibitory effects of *Rosa roxburghii* tratt juice on *in vitro* oxidative modification of low density lipoprotein and on the macrophage growth and cellular cholesteryl ester accumulation induced by oxidized low density lipoprotein. *Clin Chim Acta*, **313**, 37-43.