**RESEARCH ARTICLE**

Momordica cochinchinensis Seed Extracts Suppress Migration and Invasion of Human Breast Cancer ZR-75-30 Cells Via Down-regulating MMP-2 and MMP-9

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Abstract  

**Objective:** Metastases and invasion are the main reasons for oncotherapy failure. *Momordica cochinchinensis* (Mu Bie Zi in Chinese) had been used for a variety of purposes, and shown anti-cancer action. In this article, we focused on effects on regulation of breast cancer cell ZR-75-30 metastases and invasion by extracts of *Momordica cochinchinensis* seeds (ESMCs). **Methods:** Effect of ESMCs on ZR-75-30 human breast cancer cells proliferation were evaluated by MTT assay and on invasion and migration by wound-healing and matrigel invasion chamber assays. Expression and protease activity of two matrix metalloproteinases (MMPs), MMP-2 and MMP-9, were analyzed by Western blotting and gelatin zymography, respectively. **Results:** ESMC revealed strong growth inhibitory effects on ZR-75-30 cells, and effectively inhibited ZR-75-30 cell invasion in a dose-dependent manner. Western blot and gelatin zymography analysis showed that ESMC significantly inhibited the expression and secretion of MMP-2 and MMP-9 in ZR-75-30 cells. **Conclusions:** ESMC has the potential to suppress the migration and invasion of ZR-75-30 cancer cells, and it might prove to of interest in the development of novel inhibitors for breast cancer.

**Keywords:** *Momordica cochinchinensis* - breast cancer - invasion - MMP-2 - MMP-9

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Introduction

Breast cancer is one of the leading causes of cancer death in women worldwide. More than 90% of lethality in cancer patients is caused by metastasis (Mueller et al., 2001; Weigelt et al., 2005; Fang et al., 2013). Lungs, bone and brain are the most common sites for breast cancer metastasis and the occurrence of distant metastases severely limits the prognosis of breast cancer patients. Factors, such as neoplastic cell molecular, genetic characteristics and biological environment, are thought to be determinant in the metastatic process (Chambers et al., 2002; Mendes et al., 2005).

To form metastases, neoplastic cells must invade through the basement membrane, enter lymphatic and blood vessels for dissemination into the circulation, and establish a new tumor in distant organs (Friedl and Wolf, 2003). To migrate, the cell body must modify its shape and stiffness to interact with the surrounding tissue structures. Hereby, the extracellular matrix (ECM) provides the substrate, as well as a barrier towards the advancing cell body (Yilmaz et al., 2007). Matrix metalloproteinases (MMPs), a family of highly homologous, zinc- and calcium-dependent extracellular enzymes, were capable of degrading essentially all of the components of the extracellular matrix and classified into 5 groups (collagenases, gelatinases, stromelysin, matrilysin and the membrane-type MMP) based on substrate specificity, protein domain structure, sequence homology and ability/inability to be secreted. MMP2 and MMP9 (gelatinases A and B or 72- and 92-kD type IV collagenases) are of particular interest because of their role in early cancer development and progression. They are implicated in tumor invasion and metastasis. Therefore, inhibition of the function of MMPs, especially MMP-2 and MMP-9, in the ECM is being most actively pursued for anticancer therapy (Hidalgo and Eckhardt, 2001; Somiari et al., 2006).

Now, cancer chemotherapy drugs are research hotspot. And nature plant is one of important sources to discover new therapeutic drugs (Gordon M. Cragg and Newman, 2005). *Momordica cochinchinensis* Spreng., a member of the Cucurbitaceae family, has been highly valued for its nutritional and medicinal qualities and wide range of adaptability. It has been used as an indigenous food and traditional medicine throughout East and Southeast Asia for a long time (Ishida et al., 2004; Tsoi et al., 2006; Sanwal et al., 2011; Parks et al., 2012). As a medicinal plant, the seeds of the fruit, used as traditional Chinese medicine
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powder in water for 1 h,

were

Spreng. seed

with a

inverted microscope. Cells were incubated in medium with or without concentrations of ESMC. Cell migration into the wound surface and the average distance of migrating cells were observed in different times using a phase contrast microscope.

Migration and invasion assays

In vitro cell migration assays were performed using transwell chambers (8 μM pore size; Millipore, USA). Cells were allowed to grow to sub-confluency (75–80%) and were serum-starved for 24 h. After detachment with trypsin, cells were washed with PBS, and resuspended in serum-free medium. Cell suspension (2×10^5 cells/ml) was added to the upper chamber with ESMC (0, 30, 60 and 120 μg/ml) in 400 μl of 1 % BSA RPMI 1640 medium. The bottom chamber contained medium with 10 % FBS RPMI 1640 medium to serve as a chemoattractant to induce invasion. For the screen, after 24 h the cells that had not migrated were removed from the upper face of the filters using cotton swabs. To determine the number of migratory cells, the invaded cells were fixed with 100 µl (1 mg/ml) matrigel (Becton–Dickinson, USA) were used to determine invasive potential in the invasion assay.

Zymography

Secreted metalloproteinase were detected and characterized by zymography. Conditioned media were obtained by a 24 h incubation of ZR-75-30, which were treated overnight with ESMC (0, 30, 60 and 120 μg/ml) in serum-free media. The media were collected, centrifuged for 10 min at 4 °C at 2000 rpm. Conditioned media (20 μl) were loaded on 8 % SDS-PAGE gels that had been copolymerized with 1 mg/ml gelatin. The samples were not activated before running which allowed the latent and active forms of each enzyme to be visualized. Electrophoresis was performed under nonreducing conditions at 100 V for 2 h at 4°C. The gels were immersed in Triton X-100 (2.5% in water) for 3 h to deactivate the enzymes by removing the SDS, and were incubated in collagenase buffer (50 mM Tris-HCl pH 7.6, 10 mM CaCl_2) for 40 h at 37°C. Gels were stained with 0.5% Coomassie blue for 30 min at room temperature and destained until revealing clear bands containing proteolytic activity on a dark blue background. The presence of metalloproteinases was indicated by an
Momordica cochinchinensis and Migration and Invasion of ZR-75-30 Cells - Effects on MMP-2 and MMP-9

Figure 1. The Effects of ESMC on Viability of ZR-75-30 Cells. a The plant about Momordica cochinchinensis b The seed of Momordica cochinchinensis c The effects of ESMC on viability, ZR-75-30 cells were treated with various concentrations of ESMC for 24, 48 and 72 h. Data were represented as means ± SEM at least three independent experiments. Statistically significant changes at *p < 0.05, **p < 0.01 vs. the control group.

Figure 2. Effect of ESMC on ZR-75-30 Cell Migration in Vitro. Photographs of wound of cells treated with 0, 30, 60 and 120 μg/ml of ESMC for 24, 48 and 72 h.

Figure 3. Effect of ESMC on ZR-75-30 Cell Migration in Vitro. a Photographs showed the cell migration through the polycarbonate membrane treated with concentrations of ESMC stained by 0.2 % crystal violet. The inhibitory effect of ESMC on the migration of the cells was in a concentration-dependent manner. b Quantification of the number of cells migrating through the polycarbonate membrane. Data were represented as means ± SEM at least three independent experiments. Statistically significant changes at *p < 0.05, **p < 0.01 vs. the control group.

Results

ESMC suppressed ZR-75-30 cell growth

We investigated the effect of ESMC on cell viability. ESMC showed significant anti-proliferative effect on human breast cancer ZR-75-30 cells in a dose- and time-dependent manner, as shown in Figure 1 C, and the 50 %-growth inhibitory concentrations (IC50) of ESMC at 24, 48 and 72 h were 93.24, 34.04 and 53.43 μg/ml. A significant inhibitory effect was noted, compared with the control group.
ESMC inhibited the migration of ZR-75-30 cells

To investigate the effect of ESMC on cell migration, wound-healing (scratch motility) and transwell invasion assays were used. Confluent monolayers of cells were scratched to form wounds, then cultured in the absence or presence of various concentrations of ESMC (30, 60, 120 μg/ml), and observed at a different time after cell monolayers had been wounded. As shown in Figure 2, ESMC-induced cells moved slowly compared with the control group in a dose-dependent manner. Similar results were obtained from the transwell invasion assays. Millicell was also used to determine the inhibitory effect of ESMC on ZR-75-30 cell migration. Results showed that, after 24 h of treatment with various concentrations of ESMC (30, 60, 120 μg/ml), the cell number on the lower surface of the membrane decreased in a dose-dependent manner (Figure 3). Taken together, our data suggested that ESMC could impair breast cancer cell ZR-75-30 migration.

ESMC inhibited the invasion of ZR-75-30 cells

The possible effect of ESMC on cell invasion was examined using matrigel-coated chambers. Cells were treated with various concentrations of ESMC (30, 60, 120 μg/ml) or vehicle for 24 h in the upper side, and then allowed to migrate through a membrane coated with matrigel. As shown in Figure 4, ESMC inhibited the invasion ability of ZR-75-30 cells in a dose-dependent manner. These data were consistent with results we found above.
found that ethanol extract of Saussurea involucrata have anti-metastatic potential against hepatic cancer, Waraporn, Y. found that Phyllanthus emblica extract suppressed metastasis on human fibrosarcoma cell, and Song, F.Q. et al focused on the anticancer mechanisms of medicinal mushroom. Traditional medicine has its boundedness that was due to active ingredients being unclear in cancer treatment. However, with the development of analysis technology, nature extracts provide leads for the potential novel agents (Munkhzaya et al., 2013; Waraporn et al., 2013; Song et al., 2013).

In this study, the authors demonstrated, for the first time, that *Momordica cochinchinensis* seed played a remarkable role in inhibiting metastasis via down regulation of MMP-2 and MMP-9 in breast cancer treatment.

In the pre-experiment, we have found that extracts of *Momordica cochinchinensis* seed indicated anti-cancer effect. The mechanism of this effect was not expounded yet. In this article, we explore the therapeutic mechanism of this plant by relevant experiment research.

The effect of ESMC on ZR-75-30 breast cancer cell invasion was investigated in this study by a matrigel chamber invasion assay. ESMC displayed obvious inhibition of invasion in a dose-dependent manner.

We examined whether the effect of ESMC on ZR-75-30 cells influenced cell motility. The scratch motility (wound healing) and millicell assays indicated that ESMC significantly reduced the migration of ZR-75-30 cells in a dose- and time-dependent. The ability of cells to migrate through uncoated porous filters in response to a chemotactic stimulus was examined in a Transwell migration assay. Treatment of ESMC displayed obvious inhibition of invasion in a dose-dependent.

Then, we examined whether the mechanism was linked to elevated levels of MMPs, which are well documented ECM-degrading enzymes and whose activity is associated with tumor invasiveness. MMPs activities were measured by a zymography assay of conditioned media from ESMC and control ZR-75-30 cells.

The expression and activity of MMPs against matrix macromolecules have been associated with the development of malignant phenotypes and the promotion of cell invasiveness and metastasis. ESMC’s anti-invasive action is also reflected by its suppressive effects on the expression of MMP-2 and MMP-9, two major MMPs mediating the degradation of the ECM. In our study, ESMC treatment not only reduced the protein expression but also repressed the enzymatic activity of MMP-2 and MMP-9. These results suggest that ESMC’s anti-invasive action is mediated, at least in part, by diminishing the ability of breast cancer cells to degrade the components of ECM by modulating MMP-2 and MMP-9 expression and activity.

Taken together, our results demonstrated that ESMC was able to inhibit breast cancer cell adhesion, migration and invasion. The mechanism underlying the above effects was attributed to attenuation of the activity and expression of MMP-2 and MMP-9. This study suggests ESMC is a potential candidate for interventions against breast cancer metastases.

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