

Role of SDF-1 and CXCR4 in the proliferation, migration and invasion of cervical cancer

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Abstract: This study was to investigate the role of stromal cell-derived factor 1 (SDF-1) and its corresponding receptor CXCR4 in the proliferation, migration and invasion of cervical cancer HeLa cells. CXCR4 expression in HeLa cells was measured by flow cytometry and Western Blot. Role of SDF-1 and CXCR4 in the HeLa cells proliferation was measured by MTT. Role of SDF-1 and CXCR4 in the migration and invasion of HeLa cell was measured by Boyden chamber. High expression of CXCR4 was observed on the surface of HeLa cells. Proliferation ability of HeLa cells was significantly increased after SDF-1 stimulation, which showed dose-dependent manner. After knock-down of CXCR4 expression by RNAi, SDF-1-stimulated HeLa cells proliferation was significantly blocked ($P < 0.05$). SDF-1 can induce migration and invasion of HeLa cells, SDF-1-stimulated HeLa cells migration and invasion was significantly blocked ($P < 0.05$) after knock-down of CXCR4 expression by RNAi. High expression of surface CXCR4 plays an important role in the proliferation, migration and invasion of HeLa cells.

Keywords: SDF-1, CXCR4, cervical cancer, HeLa cells.

INTRODUCTION

Cervical cancer is the most common gynecologic malignancy and the second most common cause of death among women in developing countries (Chen *et al.*, 2014; Zhang *et al.*, 2013). The prognosis of cervical cancer patients is closely related to the clinical stage and pathological classification, and the prognosis of cervical cancer patients with lymph node metastasis is worse than that of patients without metastasis (Sun *et al.*, 2012). Studies have shown that stromal cell-derived factor 1 (SDF-1) and its corresponding receptor CXCR4 was closely related to the metastasis of many tumors (Rhee *et al.*, 2014; Liu *et al.*, 2008), while the role of SDF-1 and CXCR4 in cervical cancer is still scarce. In the present study, role of SDF-1 and CXCR4 in the proliferation, migration and invasion of cervical cancer HeLa cells was primarily explored using human cervical cancer HeLa cells as the research model. The results showed that CXCR4 expression was increased on the surface of HeLa cells and SDF-1 stimulation could induce the proliferation, migration and invasion of HeLa cells. When CXCR4 expression was knock-downed, proliferation, migration and invasion of SDF-1-stimulated HeLa cells was significantly blocked. These results indicated that high expression of surface CXCR4 plays an important role in the proliferation, migration and invasion of HeLa cells.

MATERIALS AND METHODS

Materials

Human cervical cancer HeLa cells was purchased from ATCC, CXCR4 staining antibody for flow cytometry was

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purchased from Biolegend, CXCR4 antibody for Western Blot was purchased from R&D, CXCR4 siRNAs was purchased from Santa Cruz, transferring kit was purchased from Lonza, Boyden chamber was purchased from Millipore, CytoBuster protein extraction reagent was purchased from Novagen, protease and phosphatase inhibitors were purchased from Thermo.

Cell Culture

The human cervical cancer HeLa cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum at 37°C in a humid environment with 5% CO₂ atmosphere. HeLa cells were passaged when completed 80% confluence.

Cell cycle analysis by flow cytometry

HeLa cells were washed twice with ice PBS supplemented with 1% BSA and then incubated with corresponding CXCR4 fluorescent antibody in the dark at 4°C for 30 min. Cells were washed again with ice PBS supplemented with 1% BSA and re-suspended in 0.1mL of 1% poly formaldehyde fixed liquid for analysis by flow cytometry.

Total protein extract

HeLa cells were washed twice with ice PBS and centrifuged at 400 ×g for 5min. After removing the supernatant, protease, phosphatase inhibitors and 100μL of Cyto Buster protein extraction reagent were added into the precipitate. After trituration by pipet, the mixture was placed at room temperature for 15min and then centrifuged at 12,000 ×g for 15min at 4°C. The supernatant fraction was the total cell protein, part of specimens was picked up for detecting protein concentration, others were sub-packed in a 15μL tube and stored at -80°C.

Protein concentration determination by BCA method

A series dilution of BSA standard was prepared according to instructions. The working solution was composed of reagent A and reagent B with a mixture ratio of 50: 1. 200 μ L of working solution was seeded into a 96-well plate, then standards or test samples that diluted by 25 times was added. Each sample was performed in quintuplicate. After incubation at 37°C for 30min, the absorbance at 570 nm was measured. Calibration curve was established according to the absorbance and different concentrations of standard, and concentrations of samples were calculated by the standard curve and measured absorbance.

Western blot analysis

Equivalent protein sample was loaded in 8%~10% of separation gel and 5% of concentration gel (SDS-PAGE), then transferred onto nitrocellulose membrane for electrophoresis and blocked in TBST/5% BSA for 2h. The membranes were exposed to anti-human CXCR4 antibody and incubated overnight at 4°C. The membranes were washed with 0.1% TBST three times for 5 min each, which was followed by incubation with a secondary horseradish peroxidase labelled antibody for 1 h at room temperature. After washed with 0.1% TBST, protein bands were visualized using the Super Signal West Femto chemiluminescent substrate. β -actin was used as internal reference. The experiments were repeated at least three times.

Transfection efficiency assay

Operational procedure was conducted by the method described in instructions.

MTT assay for cell proliferation

Hela cells that cultured in low serum RPMI-1640 medium were seeded into a 96-well plate. After 24 h incubation, SDF-1 at final concentrations of 0, 1, 10, 20, 50, 100 and 200ng/mL were added and the cells were incubated at 37 °C for 72 h. After treatment, 20 μ L of MTT was added to each well and the plates were then incubated for additional 4h. The supernatant was removed and reduced purple-blue MTT formazan crystals were solubilized by adding 150 μ L of DMSO to each well followed by gently shake for 10min. The absorbance at 490nm was measured using a micro plate reader.

Boyden Chamber assay for invasive ability

Role of RNAi on Hela cells was measured by Boyden chamber assay. The cells used in the experiment were counted by trypan blue dyeing and re-suspended. Cells were seeded into the upper chamber embedded with gel at a density of 1×10^5 cells/well, 300 μ L of medium was then supplemented. As for the lower chamber, 500 μ L of RPMI-1640 medium supplemented with 10% fetal bovine serum and SDF-1 at a concentration of 100ng/mL was added. After treatment for 12 h, non-invasion cells were removed from the upper

chamber with sterile cotton swab. And cells in the lower chamber was fixed and dyed with 0.1% crystal violet. Then number of invasive cells was counted.

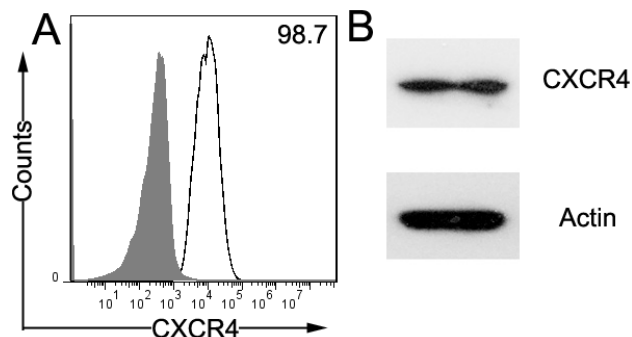
STATISTICAL ANALYSIS

Data analysis was performed using SPSS 16.0 software. For single comparisons, significant differences between the means were determined using T-test. Result of $P < 0.05$ was considered to be statistically significant.

RESULTS

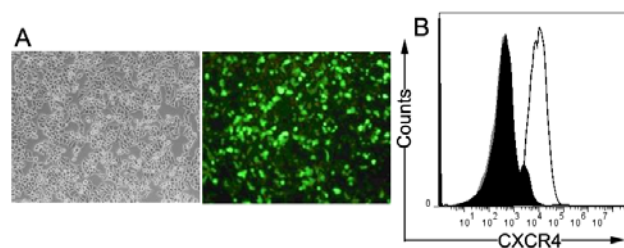
Expression of surface CXCR4 on cervical cancer HeLa cells.

As shown in fig. 1, surface CXCR4 expression of cervical cancer HeLa cells as measured by flow cytometry reached above 90%. And high expression of CXCR4 in HeLa cells was also proved by Western Blot.



A. Expression of CXCR4 in Hela cells determined by flow cytometry; B. Expression of CXCR4 in Hela cells determined by Western Blot.

Fig. 1: Expression of CXCR4 in Hela cells



A. Determination of transfection efficiency; B. Determination of interference effect. Dark column shows the effective interference group, black line shows the Mock group.

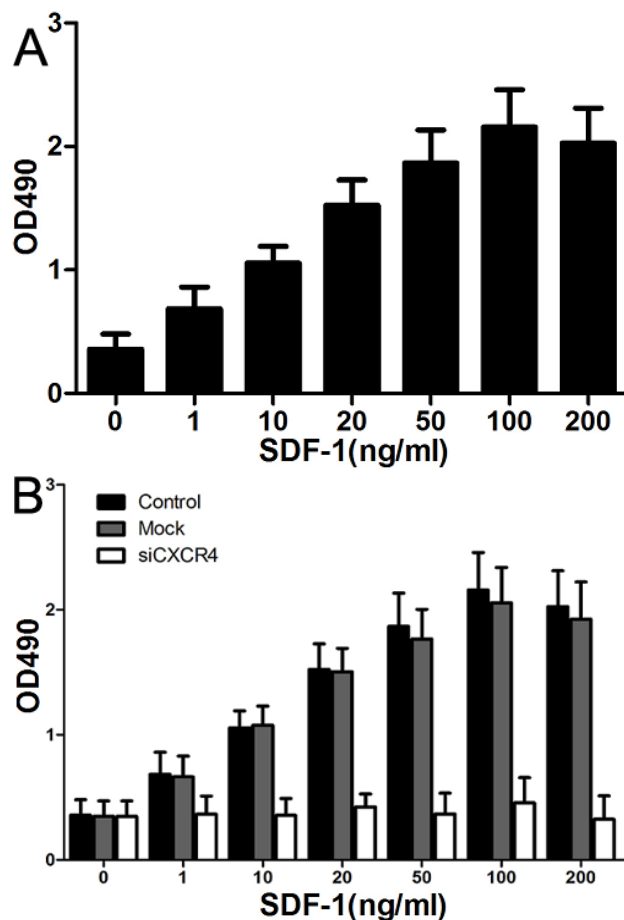
Fig. 2: Role of transfection efficiency and interference effect.

Transfection efficiency and interference effect

As shown in fig. 2A, Hela cells were electroporated with a transfection efficiency of over 90%. After transfection with siRNA CXCR4, the positive rate of CXCR4 expression on the surface of Hela cells was significantly decreased (fig. 2B).

Effect of CXCR4 on HeLa cells proliferation

As shown in fig. 3A, HeLa cells treated with SDF-1 at concentrations of 0, 1, 10, 20, 50, 100 and 200ng/mL exhibited proliferation of 0.358 ± 0.124 , 0.685 ± 0.176 , 1.056 ± 0.135 , 1.524 ± 0.204 , 1.867 ± 0.267 , 2.157 ± 0.301 and 2.025 ± 0.287 , respectively. The proliferation efficiency of HeLa cells increased gradually with the increase of SDF-1 concentration. And the proliferation efficiency reached the highest when the concentration of SDF-1 reached 100 ng/mL. After knock-down of CXCR4 expression by RNAi, SDF-1-stimulated HeLa cells proliferation was significantly blocked ($P<0.05$) (fig. 3B).



A. Proliferation efficiency of HeLa cells induced by SDF-1; B. SDF-1-stimulated HeLa cells proliferation after knock-down of CXCR4.

Fig. 3: Proliferation efficiency of HeLa cells.

Effect of SDF-1 and CXCR4 on HeLa cells invasion by Transwell

As shown in fig. 4, number of invasive cells in untreated HeLa cells was 100.25 ± 19.46 , while that of SDF-1-stimulated HeLa cells was 304.25 ± 33.24 . Number of invasive cells in HeLa cells that knock-downed CXCR4 expression was 103.24 ± 22.34 , while the number was 114.45 ± 24.24 after knock-down of CXCR4 expression by RNAi. These results indicate that SDF-1-stimulated HeLa

cells migration and invasion was significantly blocked after knock-down of CXCR4 expression by RNAi ($P<0.05$).

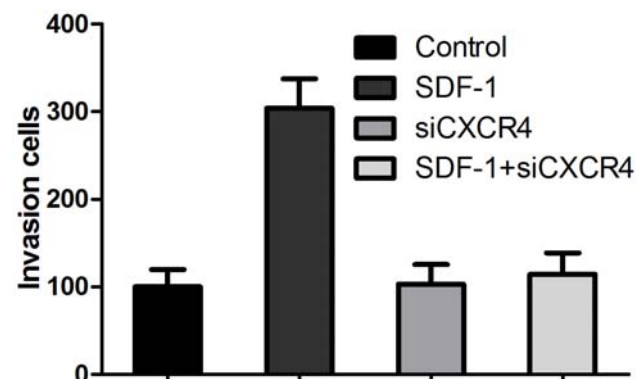


Fig. 4: Invasion and metastasis activity of cervical cancer HeLa cells

DISCUSSION

Cervical cancer is the most common gynecologic malignancy and the second most common cause of death among women in developing countries (Chen *et al.*, 2014; Zhang *et al.*, 2013). The prognosis of cervical cancer patients is closely related to the clinical stage and pathological classification, and the prognosis of cervical cancer patients with lymph node metastasis is worse than that of patients without metastasis (Sun *et al.*, 2012). Chemokines contributes to the migration of chemotaxis cells. Cells migrate to high levels of chemokines following increased level signals. Several studies showed that a variety of chemokines and related receptors was involved in the invasion and metastasis of tumor cells, which play an important role in the occurrence and development of tumors. CXCR4 is an important member of the chemokine receptor family. It is closely related to the growth, invasion and metastasis of breast cancer, gastric cancer, colorectal cancer, pancreatic ductal cell carcinoma and liver cancer (Chen *et al.*, 2013; Satomura *et al.*, 2014; Heckmann *et al.*, 2014; Arora *et al.*, 2013; Ghanem *et al.*, 2014). Our results found that higher CXCR4 expression in cervical cancer HeLa cells, as well as the corresponding chemokine SDF-1 (Jin *et al.*, 2012). These results indicated that CXCR4 played an important role in the occurrence and development of cervical cancer.

Effects of CXCR4 and SDF-1 on the proliferation and invasion of cervical cancer HeLa cells were observed in order to further explore the mechanism of CXCR4 in the occurrence and development of cervical cancer. Results showed that proliferation ability of HeLa cells was significantly increased after stimulated by different concentration of SDF-1 ($P<0.05$). After knock-down of CXCR4 expression by RNAi, SDF-1-stimulated HeLa cells proliferation was significantly blocked ($P<0.05$). SDF-1 could induce migration and invasion of

HeLa cells, and SDF-1-stimulated HeLa cells migration and invasion was significantly blocked after knock-down of CXCR4 expression by RNAi ($P < 0.05$).

In summary, our result suggests that high expression of CXCR4 on the surface of HeLa cells plays an important role in the proliferation, migration and invasion of HeLa cells. CXCR4 may participate in the occurrence and development of cervical cancer by promoting the proliferation, invasion and metastasis of HeLa cells. However, the mechanism of CXCR4 and its corresponding chemokine induced proliferation, invasion and metastasis of HeLa cell remains to be further studied.

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