RESEARCH ARTICLE

Role of CXCR7 and Effects on CXCL12 in SiHa Cells and Upregulation in Cervical Squamous Cell Carcinomas in Uighur Women

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Abstract

CXCR7 is involved in tumor development and metastasis in multiple malignancies. However, the function and molecular mechanisms of action of CXCR7 in human cervical cancer are still unclear. In the present study a loss of-function approach was used to observe the effects of recombinant CXCR7 specific small interfering RNA pBSilence1.1 plasmids on biological behavior including proliferative activity and invasive potential, as indicated by MTT assays with the cervical cancer SiHa cell line *in vitro*. Reverse transcription polymerase chain reaction and Western blotting revealed that CXCR7 was downregulated in transfected compared with control cells, associated with inhibited cell growth, invasiveness and migration. The expression of CXCR7 and CXCL12 was also determined immunohistochemically in 152 paraffin-embedded, cervical squamous cell carcinoma (CSCC) and cervical intraepithelial neoplasia (CIN), or normal cervical epithelial to assess clinico-pathological pattern and CXCR7 status with respect to cell differentiation and lymph node metastasis in Uighur patients with CSCC. CXCR7 and CXCL12 expression was higher in cervical cancer than CIN and normal cervical mucosa, especially in those with higher stage and lymph node metastasis. CXCL12 appeared to be positively regulated by CXCR7 at the post-transcriptional level in CSCC. We propose that aberrant expression of CXCR7 plays a role in carcinogenesis, differentiation and metastasis of CSCC, implying its use as a potential target for clinical biomarkers in differentiation and lymph node metastasis.

Keywords: Cervical cancer - CXCR7 - CXCL12 - Uighur women

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Introduction

Advanced cervical carcinoma (CC) is one of the most commonly diagnosed cancers in women, especially in developing countries, and it is still remains a major problem in oncology due to treatment failure and distant metastasis. Cervical cancer occurs with high morbidity and mortality in Uyghur women in the Xinjiang region, which is considered a high incidence disease in Xinjiang (Ma et al., 2001; Suzuk et al., 2006). Most patients in Xinjiang, at the time of diagnosis, already have locally advanced or metastatic disease, making surgical resection of the primary tumor of limited therapeutic value (Zhang et al., 2003). Detection of cancer cells in the lymph node is widely-recognized as one of the most important indicators of cervical cancer prognosis and guides the design of appropriate treatment strategies. Yet, the underlying molecular mechanisms that mediate the metastatic process leading to positive lymph node status in cervical cancer patients have yet to be fully elucidated. The formation of metastasis has many rate limiting steps. The expression of metastases initiation genes in primary tumors is driven by the need for cell motility, invasiveness, handling the shear stress in the vasculature and lymphatic circulation, and the survival and persistent growth in the distant organ. However, the expression of the progression genes in the primary tumors has a more complex basis. These metastasis-prone genes support primary tumor growth through one particular effect, whereas they enhance distant metastasis through another effect chemokines were recognized originally for their ability to dictate the migration and activation of leukocytes. Chemokines, small pro-inflammatory chemoattractant cytokines that bind to specific G-protein coupled seven-span transmembrane receptors, are major regulators of cell trafficking and adhesion (Paradkar et al., 2014).

Previous reports have shown that cancer cells now implicate chemokines and their corresponding receptors to stimulate their own growth, invasion, and metastasis. In particular, the stromal cell-derived factor-1 (CXCL12) is a broadly expressed cytokine that plays crucial roles in cellular migration, and are capable of coordinating multiple aspects of the cell migration machinery, which interact with G protein-coupled receptors to activate

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downstream signaling pathways that enhance cancer cell growth, migratory behavior, and cell survival (Sun et al., 2010; Shen et al., 2013), and it is also regulate the chemotactic responses of cells that are essential for organogenesis and immunity through the orchestration of cell movement from one location to another (Heinrich et al., 2012; Roy et al., 2014). CXCR4 and CXCR7 are two receptors of CXCL12 that have been identified. several authors have investigated CXCR4 expression, its function and prognostic impact in cervical cancer, inhibition of the CXCR4 receptor by mac239 has shown an anti-proliferative effect in cervical cancer (Cai et al., 2012; Huang et al., 2013;Wen et al., 2013).

It is known that the chemokine receptor CXCR7 can be engaged by both chemokines CXCL12, but the exact expression pattern and function of CXCR7 is controversial. CXCR7 is hypothesized to be involved in cancer invasion and metastasis, and higher levels of this receptor are associated with higher grades and poor prognosis of cervical cancer (Schrevel et al., 2012). While no data are available about the function and prognostic impact of CXCR7 in CC. It is still unclear whether the CXCR7 is able to activate on the biological characteristics of the cervix cancer, such as proliferation, invasion, and migration. Therefore, to gain further insight into the effect of CXCR7 in CC, CXCR7 expression was selectively knocked down in the present study using RNAi in the Siha cervical cancer cell line and the effect of CXCL12/CXCR7 on the metastatic potential of Siha was also observed. Furthermore, we investigate the expression of CXCR7 in a large number of in Uyghur CC specimens and to evaluate its potential role as a prognostic target molecule.

Materials and Methods

Clinical characteristics and tissue samples

For this study, 152 cases of paraffin-embedded (FFPE) cervical tissue specimens were collected from Uighur women with cervical squamous cell carcinoma (CSCC) and cervical intraepithelial neoplasia (CIN), or without cervical diseases, but treated by hysterectomy from June 2009 to March 2010 at Department of Gynecology of the First Affiliated Hospital of Xinjiang Medical University. Gynecological examination was performed in all cervical cancer patients for staging in accordance with the International Federation of Gynecology and Obstetrics (FIGO) criteria. All data including pathologic grade, lymph node metastasis, and disease stage were obtained from a combination of clinical and pathological record review, outpatient clinic medical records and communication with patients and their attending physicians. This study was conducted with the approval of the ethics committee of first affiliated hospital of Xinjiang medical university. Written informed consent was obtained from all patients participating in this study. Of patients with CIN enrolled in this study, were 33 cases with CIN I-II and 31 with CIN III. A total of 63 patients with CSCC were selected for this study, were 21 FIGO stage Ia, 22 FIGO stage Ib, 11 FIGO stage IIa, 9 FIGO stage IIb. Among them, 27 cases were pathologically characterized as well-differentiated, 19 moderately differentiated and 17 poorly differentiated tumors. Lymph node metastasis was documented for 20 tumor patients. The median age of the cervical cancer patients was 48.7 years (IQ range 27-69 years). Control cases (n=25) were obtained from patients without a history of cervical lesions or any form of cancer and planned to undergo a hysterectomy for nonmalignant reasons during the same period.

Cell lines and culture

The SiHa human carcinoma cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were grown in RPMI 1640 media (Invitrogen) supplemented with 10% FBS (FBS fetal bovine serum), L-glutamine, and 1% antibiotics (Sigma-Aldrich).

Transfection

The human CXCR7-homo-960 were designed and provided by Invitrogen. RNA oligonucleotides were transfected by using LipofectamineTM2000. After 24h transfection, cells were used for subsequent experiments including proliferation, migration, and invasion assays.

Detection of cell proliferation by MTT assay

The MTT method was used to estimate SiHa cell viability. The cells were plated at an initial density of 104 cells per well in flat-bottom, 96 well cell culture plate and allowed to grow for 24h. The following eight groups were set up: Group ABC, SiHa cell line plus RPMI 1640 and 10% newborn calf serum (NBS); group DEF, SiHa cell line plus RPMI 1640, 10% NBS and 500ng/ul CXCR7-homo-960; group G, with nothing except RPMI 1640, 10% NBS. After 24h transfection MTT (BIO-BOX) was added to each well followed by at 4h incubation at 37°C. After removing the media, DMSO was added to each well for solubilizing the formosan formed. After 10min at room temperature, the plates were scanned spectrophotometrically with a microplate reader set at 490nm for measuring the absorbance.

Cell wound healing assay

106 SiHa cell per well were seeded in a 6 well plate, cultured for 24h to obtain 80% monolayer confluence .three well of this plate were transfected by CXCR7-homo-960 for 24h. A wound was created by scraping the cells using a plastic pipette tip in the bottom of the culture flasks, and the medium was replaced with fresh medium. Images were captured immediately (day 0) and every day for 3 days. Take photos for three consecutive days Cell migration was qualitatively assessed by the size of the wounds at the end of the experiment.

Real-time PCR

CXCR7 mRNA expression was detected by quantitative real-time RT-PCR. Total RNA was extracted from cultured cells using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and treated with TURBODNA-freeTM DNase (Ambion, Austin, TX, USA) to remove the genomic DNA. mRNA was reverse transcribed using a reverse transcription system kit (Thermo). Real-time PCR for CXCR7 was

performed in a 20ul reaction volume using the Platinum SYBR Green qPCR SuperMix-UDG (takara Technologies, Carlsbad, CA, USA) and the Light Cycler 480 system (Roche Diagnostics, Penzberg, Germany). mRNA for GAPDH was used as a normalization control in RT-PCR and as a loading control in conventional PCR. The following CXCR7 and GAPDH (as a reference) primers were used for RT-PCR: CXCR7 forward primer: GCCCAACAAAAGCGTCCTGCTCT; reverse primer: AGGACAACCCACAGGTCGG CAA;GAPDH forward primer: GGCACCCAGCACAATGAAG; GAPDH reverse primer: CCGATCC ACACGGAGTACTTG. The thermal cycle conditions were 95 °C for 60 s for one cycle, followed by 40 cycles of amplification at 95°C for 12s, and 62°C for 40s. The expression level of CXCR7 mRNA was obtained using the 2-DDCT calculation method. All PCR products were analyzed on a 2% agarose gel with ethidium bromide staining.

Protein extraction and western blotting analysis

Total protein from the cultured cells extracted with radio immunoprecipitation assay (RIPA) lysis buffer (Bioteke, Beijing, china) containing protease inhibitor. The proteins were separated by 10% SDS-PAGE (Invitrogen, Carlsbad, CA, USA) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA). The membranes were incubated in blocking buffer (1h with 5% skimed milk in PBST) at room temperature with gently shaking. Next the sample was incubated overnight at 4°C with primary antibody for anti-CXCR7 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After washing with prepared Antibody Wash thrice, the membranes was incubated with Secondary Antibody Solution for 30min. Then wash the membranes thrice with Antibody Wash. The blot was incubate with chromogenic substrate until purple bands develop on the membrane. Western blotting band was quantified using Quantity One software by measuring the band intensity for each group and normalizing to b-tubulin (Sigma) as internal control (Invitrogen). The final results were expressed as fold changes by normalizing date to the control values.

Immunohistochemistry

Immunohistochemistry (IHC) used standard procedures, which performed with primary antibodies recognizing target proteins and IHC Kits containing biotin-labeled secondary antibodies. Briefly, 3-mm-thick sections were cut from the paraffin-embedded tissue blocks. After being dewaxed in xylene and rehydrated in alcohol and distilled water, antigen was retrieved by heating in the microwave oven for 15 min at 95°C in Ethylenediaminetetraacetic acid (EDTA) buffer (pH 8.0). After cooling and rinsing in distilled water, endogenous peroxidase activity was blocked by incubating sections for 15min in 3% H_2O_2, followed by rinsing in 0.01 M phosphate buffered saline (PBS, pH 7.4) for 10min. Samples were preincubated with a protein blocking solution for 10min and the sections were incubated overnight at 4°C with anti-CXCR7 (1:300, SCBT, Santa Cruz, Calif, USA) and anti-CXCL12 (1:400, SCBT, Santa Cruz, Calif, USA), diluted in PBS. Slides were washed three times in PBS and then incubated with a biotinylated secondary antibody (Zhong Shan Goldenbridge Biotechnology Co. Ltd, China) for 15min at room temperature. The reaction products were visualized with diaminobenzidine (DAB Kit; Zhongshan Goldenbridge Biotechnology). PBS was used in place of the primary antibody as a negative control and slides were counterstained with hematoxylin, dehydrated, and evaluated under light microscope subsequently, slides were incubated. Immunohistochemical analysis of the sections was performed without knowledge of the patients' identity or clinical status. Immunoreactivity was scored as negative, weak, moderate or strong staining intensity according to the staining intensity and the fraction of positive tumor cells were scored for each tissue.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 software (Version 17, SPSS Inc, Chicago, IL USA). Data are presented as the means±stan¬dard deviation. All tests were two-sided and *p*-values less than 0.05 were considered statistically significant. The Chi-square test was used to determine whether CXCR7 and CXCL12 expression was associated with clinicopathological characteristics. the Spearman's rank correlation test was performed to assess whether CXCR7 expression was associated with expression of its ligand CXCL12, as the intensity scores for CXCR7 and CXCL12 were ordinal. Student's t test and ANOVA were used to determine quantitative data.

Results

siRNA expressing vector inhibits CXCR7 mRNA expression in cervical cancer SiHa cells

We measured the expression of CXCR7 in SiHa cell transfected with or not, using quantitative RT-PCR. Following PCR, recombinants were digested with the SacI restriction enzyme. All plasmids, CXCR7 1, 2 and 3, produced ~179 bp DNA fragments, indicating that the target fragment had been successfully inserted into the pBSilence1.1 plasmid and in the right direction.

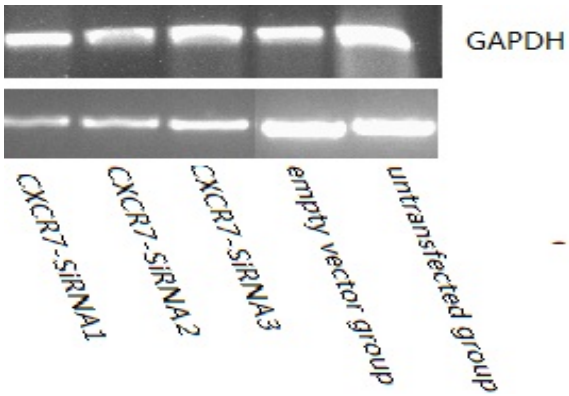

Figure 1. mRNA levels in SiHa Cells Treated with siRNA. expressing vectors were assessed by real time polymerase chain reaction analysis. CXCR7. siRNA 1, 2 and 3 inhibited CXCR7 mRNA at 48h following transfection of siRNA. expressing vectors in human cervical cancer cells. Control cells were treated with empty vector and untransfected groups. siRNA, small interfering RNA

The quantitative real-time PCR analysis displayed that the mRNA expression levels of CXCR7 in the SiHa human carcinoma cells line were down regulated in the CXCR7 siRNA transfected group compared with the untransfected group (Figure 1). Of the three CXCR7 siRNAs, the strongest interference efficiency siRNA was pBSilence1.1 CXCR7 1. However, CXCR7 1 exhib¬ited the most significant inhibitory effects on the cervical cancer SiHa cells (P<0.05). No significant difference was identified between the untransfected and empty vector groups (P>0.05).

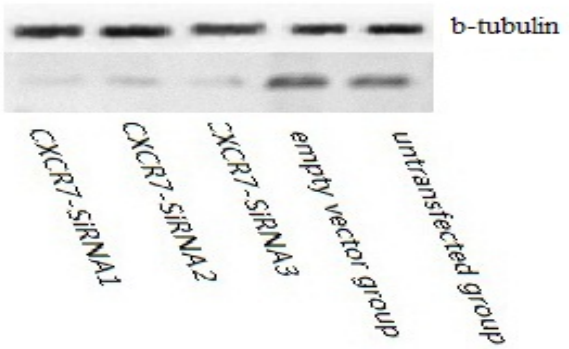

Figure 2. Effect of CXCR7. siRNA on CXCR7 protein expression in SiHa cells

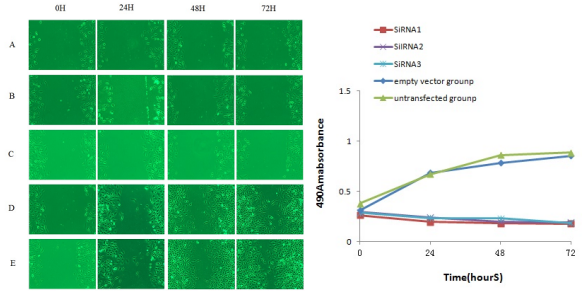

Figure 3. Effect of siRNA expressing vectors on cell invasion and migration in each group. (A) SiHa cells in Cxcr7siRNA1; **(B)** SiHa cells in Cxcr7siRNA2; **(C)** SiHa cells in Cxcr7siRNA3; **(D)** normal SiHa; **(E)** empty vector. following transfection. Cells were cultured in 96.well plates and cell viability was determined by MTT assay at 0, 24, 48 and 72h, respectively, following transfection. siRNA, small interfering. RNA

Effect of siRNA expressing vectors on CXCR7 protein expres¬sion in cervical cancer SiHa cells

The effect of siRNA expressing vectors on target protein CXCR7 was examined by western blotting (Figure 2). Compared with the untransfected group, protein expression levels of CXCR7 were down regulated in the CXCR7 siRNA trans¬fected group (P<0.05). In addition, results showed that siRNA targeting CXCR7 1, 2 and 3 decreased CXCR7 expression significantly at the protein level when compared with that of scrambled siRNA. However, CXCR7 1 exhibited the most significant inhibitory effects on the cervical cancer cells (P<0.05). No significant difference was identified between the untrans¬fected and empty vector groups (P>0.05).

CXCR7 promote the proliferation of SiHa cells

The growth activity of SiHa cell was determined by MTT assay, which results showed that following cultivation for 24h, the cell proliferative activity in groupsA (CXCR7 siRNA 1 SiHa), B (CXCR7 siRNA 2

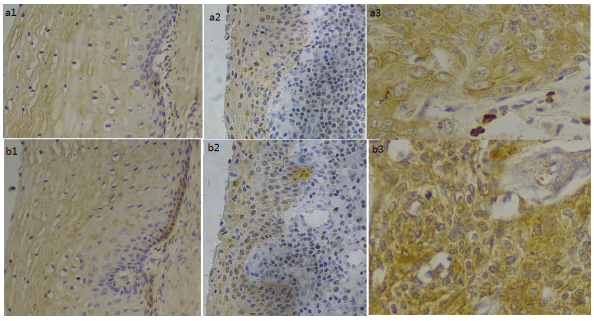

Figure 4. Representative Examples of CXCR7 and CXCL12 Staining in the Epithelial Compartment of Squamous Cell Carcinoma of the Cervix, CIN and Normal Cervical Mucosa

Table 1. Effect of siRNA Expression Vector on the Cell Proliferation of SiHa Cells Following Transfection

Group	OD(24H)	OD(24H)	OD(48H)	OD(72H)
Untransfectedgroup	0.384	0.673	0.863	0.887
Empty vector group	0.313	0.688	0.786	0.856
Cxcr7siRNA1	0.263	0.195	0.182	0.178
Cxcr7siRNA2				
Cxcr7siRNA3	0.294	0.243	0.197	0.194
	0.287	0.235	0.231	0.184

Table 2. CXCR7 and CXCL12 Expression in Cervical Cancer Patients, CIN and Normal Control and with Clinicopathological Parameters

		N	CXCR7				P	CXCL12				P
			negative	weak	moderate	strong		negative	weak	moderate	strong	
Normal control	25	12	7	5	1	-0.854	16	6	1	2	-0.905	
CIN	64	28	13	15	8	0.393	36	14	8	6	0.366	
	CIN I-II	33	14	7	8	4		21	8	3	1	0.052
	CIN III	31	14	6	7	4	-2.039	15	6	5	5	
CSCC	63	19	10	20	14	0.041	27	10	16	10		
	WD	27	7	5	9	6	-0.332	10	7	6	4	-0.917
	M/P D	36	12	5	11	8	0.74	17	3	10	6	0.356
FIGO stage												
	≤I b	43	18	9	10	6	-3.706	23	5	10	5	-2.456
	≥IIa	20	1	1	10	8	0	4	5	6	5	0.014
LN metastasis												
	negative	43	17	9	10	7	-3.163	22	8	10	3	-2.767
	positive	20	2	1	10	7	0.002	5	2	6	7	0.006

SiHa) , C (CXCR7 siRNA 3SiHa) D (normal SiHa), E (empty vector), The results show in Table 1. Following SiHa cell interference with CXCR7, the proliferative activity was significantly lower when compared with that of the normal SiHa cells. Cell proliferation was verified by MTT assay and for 48 and 72h, the results were the same. The analysis confirmed that the CXCL12/CXCR7 biological axis can induce cervical cancer cell proliferation.

CXCL12 induces CXCR7-dependent migration and invasion of cervical cancer SiHa cells

The increased migratory ability of tumor cells determines their metastatic phenotype and it is an important biological characteristic of malignant cancer cells. The cervical cancer SiHa cells expressing CXCR7 was first evaluated for the role of CXCR7 in CXCL12-induced chemotaxis. We found that downregulation of CXCR7 using sh-RNA significantly (P<0.05) reduced CXCL12-induced migration and wound healing of SiHa cells. Moreover, CXCL12-dependent increase in migration or wound-healing capability of SiHa cells was significantly reduced in the presence of CXCR7 inhibitor. These results suggest that CXCR7 enhanced CXCL12-induced migration and invasion of cervical cancer SiHa cells.

CXCR7 is over expressed in cervical cancer patients and is associated with worse clinical outcome

Immunohistochemical staining of CXCR7 and CXCL12 was observed to be both cytoplasmic and membranous in cervical specimens. Representative examples of positive and negative CXCR7 and CXCL12 staining in cervical cancer, CIN and normal cervical epithelium specimens are shown in Figure 4. The distribution of staining intensities is shown in Table 2. Normal cervical epithelium stained weakly for CXCR7 and CXCL12, with moderate CXCL12 expression in cells of the basal layer and both were confined to the basal layers of the CIN I-II. Whereas strong positives were observed in cervical cancer and in CIN3. A significantly increased in intensity and distribution of CXCL12 or CXCR7 were noted as the lesions progressed from CIN and to CC (P<0.05), but no significant from normal cervical mucosa to CIN. In addition, the expression of CXCR7 associated with tumor stage and lymph node status in Uyghur cervical cancer patients (P<0.05). To assess whether CXCR7 expression was associated with expression of its ligand CXCL12, the Spearman's rank correlation coefficient was determined. CXCR7expression was significantly correlated with CXCL12 expression (r=6.076, P=0.001).

Discussion

Metastatic cervical cancer is the leading cause of cancer-related death in women worldwide, especially in the Xinjiang Uygur women. Therefore, to understanding of the mechanism that facilitates metastatic tumor progression is of great importance. Chemokines and their receptors are widely influences the development of primary tumors and metastases (Balkwill, 2004). The significance of the CXCL12/CXCR4 axis in cervical cancer invasion and metastasis has been widely investigated (Huang et al., 2013). In addition to CXCR4, CXCR7 was a recently identified and as a second, high-affinity receptor for CXCL12, which was originally cloned on the basis of its homology with conserved domains of G-protein coupled seven-span transmembrane receptors (Sun et al., 2010; Zabel et al., 2011). This receptor is highly expressed by a variety of cancers, including breast, liver, and lung (Heinrich et al., 2012; Xue et al., 2013; Roy et al., 2014), as well as cervical cancer (Huang et al., 2013).

However, conflicting reports with respect to role of CXCR7 have been made. Some support the role of CXCR7 in cancer growth (Tachezy et al., 2013), whereas another study highlights the role of CXCR7 in inhibiting invasion and metastasis of cancer (Schrevel et al., 2012). The differences in the results observed with regard to CXCR7 by different groups might be due to different cell types used or differences in experimental.

Previously, CXCR7 has been shown to be associated with tumor size, lymph node metastasis , disease recurrence and strongly associated with poor disease-specific survival in cervical squamous cell carcinoma that detected by immunohistochemically (Kodama et al., 2007). Although their data supported the hypothesis that CXCR7 higher expression was associated with cervical squamous cell carcinomas aggressive, functional studies on cervical cancer cell lines are required to determine whether CXCR7 to be involved in cancer invasion and metastasis. In the present study, siRNA mediated downregulation of CXCR7 expression in human cervical cancer cells led to a significant decrease in SiHa cell proliferation and invasion. This result is consistent with previous breast cancer studies showing that CXCR7 mediates the invasive and metastatic potential of breast cancer cells (Wani et al., 2014). The direct effect of CXCL12/CXCR7 in breast cancer metastasis is that CXCR7 recruits tumor-promoting macrophages to the tumor site through regulation of the macrophage colony-stimulating factor/macrophage colony-stimulating factor receptor signaling pathway. In addition, CXCR7 regulated breast cancer metastasis by enhancing expression of metalloproteinases (MMP-9, MMP-2) and vascular cell-adhesion molecule-1 (Miao et al., 2007).

RNAi is characterized by high efficiency, high specificity and low toxicity of post transcriptional gene silencing, mediated by ds siRNAs. In the present study, three pairs of ds siRNA oligonucleotides were designed and constructed against CXCR7. the suppressed expression of CXCR7 was confirmed by western blotting and RT-PCR at protein and mRNA levels, respectively. The results revealed that the RNAi constructs induced the selective degradation of CXCR7 mRNA and thereby decreased CXCR7 protein expression levels in cervical cancer SiHa cells.

The proliferation of the SiHa cervical cancer cell line in response to CXCL12 was found to be reduced by the downregulation of CXCR7 expression by the pBSilence1.1 siRNA CXCR7 vector, as determined by MTT assay. The results revealed that the reduction in cell absorbance in the CXCR7 siRNA group was greater compared with that of

the untransfected and empty vector groups at 24, 48 and 72h following transfection, respectively. This indicated that CXCR7 functions as a posi¬tive regulator in the growth and proliferation of SiHa cells. By contrast, the proliferation of SiHa cells was significantly reduced by CXCR7 siRNA, indi¬cating that the downregulation of CXCR7 impaired the ability of the cervical cancer cells to grow. The inhibitory effect was not time dependent, as no differences in CXCR7 inhibition were identified at 24, 48and 72h, respectively. CXCL12 promoted the colony forming capacity of SiHa cells and CXCR7 positive cells were highly viable in response to CXCL12. This result indicated that the CXCL12/CXCR7signaling pathway promotes tumor cell proliferation. It is is consistent with previous breast cancer studies showing that the CXCL12 /CXCR7 axis supports cancer cell growth (Wani et al., 2014). The mechanisms and signaling pathways involved in CXCL12/CXCR7 have been reported in pancreatic cancer and the results indicated that CXCR4 and CXCR7 signaling is b-arrestin-2-dependent and controls CXCL12 signals to the MAPK/AKT pathway (Miao et al., 2007).

The current study also investigated the expression of CXCR7 and CXCL12 in Uyghur cervical cancer, CIN and normal cervical mucosa with IHC and observed that its expression was higher in patients with cervical cancer than CIN and normal cervical mucosa. Its expression was higher in patients with higher stage and lymph node metastasis as compared with lower stage and negative lymph node metastasis. Taken together, our data indicate that CXCR7 expression in the human cervical tumors predicts worse outcomes, and its expression is higher in invasive and metastatic cervical cancer patients. These results consistent with previous cervical cancer studies showing that the CXCR7 expression is predict poor disease-free and disease-specific survival in cervical cancer patients (Heinrich et al., 2012).

In conclusion, CXCR7 siRNA treatment may significantly inhibit the growth, invasion and metastasis of cervical cancer cells and CXCR7 is highly expressed in invasive and metastatic tumors. Importantly, the overexpression of CXCR7 in human cervical tumors correlates with worse clinical outcome. Thus, the results suggested that CXCR7 expression leads to a biologically more aggressive tumor, which may represent a therapeutic target for cervical cancer patients, and may serve as a potential therapeutic target.

Acknowledgements

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